Does the effectiveness of interventions depend on the influenza pandemic profile?

S. Kernéis, Elisabeta Vergu, R.F. Grais, L. Coudeville

Friday, February 23, 2007

Saturday, February 24, 2007

Sunday, February 25, 2007

SESSION 1 (Plenary Session)
Surveillance of Emerging Diseases in the 21st Century

Friday, February 23, 2007
Room: Park Congress
14:30–15:15

1.001 **Surveillance of Emerging Diseases in the 21st Century**

J.M. Hughes. Emory University, Atlanta, GA, USA

Infectious diseases remain a major threat to public health, claiming more than 15 million lives each year. Global killers such as acute lower respiratory disease, HIV/AIDS, diarrhea, tuberculosis, and malaria continue to affect millions, while new diseases such as SARS have emerged with far-reaching public health, social, political, and economic consequences. More recently, outbreaks of avian influenza in parts of Asia have brought concerns of pandemic influenza and its potentially catastrophic effects to the forefront of public health efforts. Experiences with these outbreaks have uncovered both strengths and weaknesses in local, national, and global public health efforts, providing important lessons for improving our ability to detect and respond to infectious diseases. A particularly important lesson has been the need to increase collaborations across a broad range of specialties, including the clinical, research, and public health communities involved in human health as well as their veterinary counterparts. Such collaborative activities across multiple disciplines will expand our ability to monitor infectious diseases and implement the revised International Health Regulations, enabling rapid recognition and prompt communication of unusual symptoms, signs, or laboratory results that can signal an outbreak. These networks and partnerships can also facilitate proactive communication of critical information across a wide spectrum of audiences, extending beyond the professionals working to address these threats to include policymakers, the media, and members of the public in countries around the world. We must strengthen public health systems and increase laboratory capacity to optimize early detection and response and to monitor trends. Finally, we must continue to strive to understand the many complex factors that contribute to the emergence and spread of disease and to encourage the global commitment and actions necessary to address these issues.

SESSION 2 (Parallel Session)
Animal Reservoirs for Emerging Pathogens

Friday, February 23, 2007
Room: Park Congress
15:30–17:00

2.001 **Bats: Host, Vector and Reservoir of Emerging Viral Diseases**

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More than fifty millions years ago Bats adapted to a changing environment, early occupied a variety of ecological niche and, after having etch their natural history, humans came across their trail. They probably had brief encounters in a forested environment where tribes were hunting and gathering food from trees, or human clans find shelter in the entrance of a bats inhabited cave. If, for a long time, people perceived Bats with beliefs and superstitions, it is only in the late twenty century, that scientists have produce a better understanding of such unfamiliar mammals.

In the light of recent emergence of several Bat Borne Zoonoses, we will analyze Bat Borne Virus transmission, on the ecological ground including specific environmental natural and humans factors, in order to understand fundamentals and domains of Bat Borne Viral Diseases.

At first one have to consider the specificities of Chiropteran representing more than twenty percent of mammal species, the only one with a powered flight, and having a sophisticated laryngeal echolocation. Bats also have an original life traits including feeding habits from plant nectar to animal preys. They migrate and hibernate, roost and have wild and peridomestic habits.

More than sixty types of viruses has been find associated with bats belonging to height virus families.

Putative and established natural cycle of several exemplary Bat Borne Viral Diseases are presented.

Although Bat borne Lyssavirus are known for a long time, human infection are increasing in number and space and the association of rabies virus and bats provide a unique model of coevolution.

The Severe Acute Respiratory Syndrome first emerged in the 21 Century from forest to human with a yet not entirely understood complex virus natural cycle including other carnivorous potential bridge-host like Civet.

Out of the heart of darkness, putative Ebola fever virus natural cycle start to be unveiled after more than a two decade long quest, when only bats has been identified as a potential and efficient natural host.

The one time emerging Nipah encephalitis in Malaysia and its following emergences and re-emergences year after and far away in Bangladesh, India and Cambodia or Thailand address the question of a spread and emergence of an entirely new Bat Borne virus group.

Reducing the risk of disease transmission to human and animals lies primarily in minimizing direct or indirect contact with bats, improving disease recognition, epidemiology and farming biosecurity and an integrated knowledge of bats in their natural habitat, conservation policy, environmental factors and climate tendencies.

Bats are apart in the world of life, they fly when only insects and birds do, they make colonies of thousands like, among mammals, only human do, they interact with animals, plants and environment and, their importance for human health needs largely to be discovered.

2.002 **Ebola—The Search for the Animal Host**

R. Swanepoel. National Institute for Communicable Diseases, Johannesburg, South Africa

On behalf of: The International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the CongoAn outbreak of Marburg hemorrhagic fever (MHF) was observed in a gold mining village in northeastern Democratic Republic of the Congo (DRC) from October, 1998, to September, 2000. A total of 154 cases, 48 laboratory-confirmed and 106 suspected, were identified, with a case-fatality rate of 83%. Most primary infections arose in young male miners who worked in an underground mine as opposed to surface diggings in the village. The primary infections were followed by short chains of transmission involving mainly family members of the miners and occasionally health care workers. The pattern suggested the occurrence of multiple introductions of infection into the human population, and this was substantiated by the detection of at least 9 genetically distinct virus lineages in circulation during the outbreak. Cessation of the outbreak coincided with flooding of the underground mine. The fauna of the underground mine included bats, rodents, shrews, frogs, snakes, cockroaches, crickets, spiders, wasps and moth flies. Positive RT-PCR results for Marburg virus nucleic acid were obtained on tissue samples from 12 bats from the underground mine, including an extra 6 lineages of virus, and antibody to the virus found in 13% of bat sera tested.

2.003 Simian Reservoirs for Yellow Fever

J.P. Woodall. Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

In both Africa and South America, monkeys have been found to be the most important amplifying hosts for the yellow fever (YF) virus in nature, while the true reservoir is the various species of vector mosquitoes, in which the virus is a life-long infection transmitted to their progeny. In Africa, the virus appears to have adapted to become non-fatal to monkeys, whereas infected New World monkeys have a variable fatality rate, suggesting that the virus has had a shorter time in South America to evolve to a less virulent relationship with its hosts. Muzo, Colombia, is the only known locality in the world where a rural YF outbreak occurred in the absence of monkeys.

Specimens of most subfamilies of African and South America monkeys have been tested and found to produce sufficiently high level viremias to infect mosquitoes and ensure transmission. In many cases, cyclic transmission by A. aegypti and other vector mosquitoes between monkeys has been demonstrated in the laboratory. Survivors become immune to further infection.

Some authorities have questioned whether enzootic YF in Africa, or in small islands like Trinidad, West Indies, can find sufficient non-immune monkeys on a regular basis to maintain a natural cycle, and have postulated the involvement of another non-human primate, such as a species of African galago in Kenya and marsupials in Muzo, Colombia, both of which have been found naturally infected

In Central and South America, YF virus occurs in the jungle where, unlike the situation in Africa, there are no forest-dwelling A. aegypti or other aedines to maintain it. But it has found suitable vectors in canopy-dwelling Haemagogus and Sabethes mosquitoes, and is maintained in a cycle involving them and tree-living monkeys. This cycle in the absence of A. aegypti is known as sylvatic yellow fever.

2.004 Pets: Contagious Companions

D.H. Lloyd. Royal Veterinary College, North Mymms, United Kingdom

It is well recognised that infected pet animals can serve as sources of infection for humans. These infections vary from common, relatively low-grade diseases, such as dermatophytosis caused by Microsporum canis, to rare but fatal diseases such as rabies. Recently, the risks posed by commensal organisms that can be carried by dogs and cats and passed to in-contact people have become more apparent. These risks relate not only to their potential pathogenicity to humans, particularly those with increased susceptibility to infection, but also to their possession of multi-resistance to systemic antimicrobials. Particular attention has been focused on Eschericia coli, Enterococcus spp. and the pathogenic staphylococci, especially methicillin-resistant Staphylococcus aureus (MRSA). Recent data show that MRSA is readily transferred between companion animals and man, including both owners and veterinary staff, and indicate that transfer occurs more readily when the pet is infected. Prevalences varying from 14 to 27% carriage of MRSA have been reported amongst staff in veterinary hospitals in Europe and North America. Chronic and recurrent human infections have been reported when susceptible humans have been in contact with carrier pets, with resolution of infection when the pet animal source has been removed. In a risk factor study of owners of pets with S. aureus infection, MRSA was found in the nasal mucosae of 11% of owners with MRSA-infected pets but in none of the owners with methicillin-susceptible S. aureus infected pets. Molecular studies indicate that the MRSA isolates found infecting or carried by pets are in most cases epidemic human hospital strains and there is concern that such strains may be becoming established in domestic pets. There is a particular need for further studies of the occurrence of MRSA in healthy dogs and cats.

SESSION 3 (Parallel Session)
Agents of Bioterrorism

Friday, February 23, 2007
Room: Brahms/Mahler/Bruckner
15:30–17:00

3.001 Where Will Anthrax Strike Next? Generating and Validating Prediction Maps of Anthrax Oubreaks in North America and Central Asia

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Anthrax, caused by the bacterium Bacillus anthracis, threatens public health as a zoonosis and a biological weapon, and can lead to massive economic losses in livestock and wildlife, with two of the largest North American outbreaks in several years in the 2005/2006 summers. This same threat exists throughout Central Asia. Despite this, our understanding of anthrax natural ecology is weak, yet necessary for cost effective control within its endemic range, and to distinguish natural outbreaks from bioterrorism events.

While the traditional control response of annual vaccination, outbreak site management and herd treatment is effective it needs to be made more efficient, rapid, and make better use of recent scientific advances. Improving our understanding of complex disease systems and distributions across international borders requires a multi-disciplinary, collaborative international approach. Ecological modeling and GIS-based analyses can provide methodologies for predicting the spatial distribution of anthrax, while at the same time GIS-based surveillance systems can be employed to track disease and response in (near) real-time. Reliable spatial predictions of endemicity allow control programs to target and monitor specific farms and their livestock, not counties or municipalities, which pays off in greater efficiency and quicker recognition of unusual outbreaks.

This paper will review current ecological modeling approaches used in anthrax prediction. Model predictions for the distribution of B. anthracis will be presented for both the contiguous United States and the Kazakhstan, where similar outbreak patterns and control efforts are present. These modeling approaches provide insight into disease agent-specific biogeographic affinities across multiple continents and landscapes.

Additionally, these predictions allow for an evaluation of the species success within an evolutionary ecology framework. Integrating disease predictions and GIS-based tools also provides a framework for identifying gaps in vaccination and carcass disposal efforts, exploring the ecology of natural outbreaks, and improving response time through carefully monitored democratization of data and farmer education programs.

3.002 Genotyping Bacillus anthracis: From Global Diversity to Epidemiological and Bioforensic Investigations

Matthew N. Van Ert[1], W.R. Easterday[2], L.Y. Huynh[3], M.E. Hugh-Jones[4], T. Hadfield[1], J. Ravel[5], T. Pearson[2], T.S. Simonson[2], J.M. U'Ren[2], P.R. Coker[4], K.L. Smith[6], B. Wang[7], L.J. Kenefic[2], D.M. Wagner[2], C.M. Fraser-Liggett[5], and P. Keim[2]. [1]Midwest Research Institute, Palm Bay, Florida, United States of America; [2]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona United States of America; [3]Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, Georgia, United States of America; [4]Department of Environmental Studies, Louisiana State University, Baton Rouge, Louisiana, United States of America; [5]The Institute for

Genomic Research, Rockville, Maryland, United States of America; [6]Office of Research and Development, Science and Technology Directorate, Department of Homeland Security, Washington, District of Columbia, United States of America; [7]Lanzhou Institute of Biological Products, Lanzhou, China

Anthrax is a disease of historical and current importance that is found throughout the world. In the past, the genetic homogeneity of B. anthracis severely compromised efforts to reconstruct its natural history and the basis of its global genetic population structure has remained largely cryptic. Recently, whole genome sequencing efforts identified rare genomic variation in B. anthracis that can be used to examine relationships among worldwide isolates. We used single nucleotide polymorphism (SNP) and variable number of tandem repeat (VNTR) markers to examine a worldwide collection of B. anthracis isolates. This analysis described the worldwide diversity of the pathogen and provides insight on the transmission of clonal lineages across a global landscape. Understanding of the evolutionary history of the pathogen also facilitates the identification of highly precise diagnostic signatures for epidemiological and forensic investigations. For example we describe SNPs that can be used to: 1) separate B. anthracis from closely related Bacillus species; 2) identify the major genetic lineages within B. anthracis and; 3) to define specific B. anthracis strains, such as the 2001 attack strain (Ames). We also demonstrate the value of our genetic-geographic database for distinguishing between natural and bioterrorist-mediated anthrax outbreaks.

3.003 The Agroterrorism Threat

K.L. Smith, M. Urlaub. Department of Homeland Security, Washington, DC, USA

Globalization of international trade in agriculture has placed an increased emphasis on the early detection, identification and eradication of animal and plant diseases. As a key component of the economies of both industrialized and developing nations, agriculture security is a shared national interest and an integral component to successful international trade across the spectrum of animal and plant commodities.

The agriculture sector has and continues to be targeted by various extremist elements as well as lone wolf individuals as a means to protest use of GMOs (genetically modified organisms) in finished food products, for reasons of personal disgruntlement or for purposes related to political causes. Consequence management protocols which can aid in distinguishing intentional vs natural disease introduction will aid first responder communities in characterizing disease outbreaks earlier and facilitate implementation of protective measures which are more efficacious and cost effective.

3.004 The Threat Agent Detection and Response Network

S. Cali[1], S. Mayer[2], **R. Breeze[2]**. [1]Defense Threat Reduction Agency, Fort Belvoir, VA, USA; [2]SAIC, Alexandria, VA, USA

The U.S. Department of Defense through the Threat Reduction Agency (DTRA) is establishing the Threat Agent Detection and Response (TADR) system to integrate and enhance existing veterinary and public health surveillance, reporting, detection and response activities in Ukraine, Georgia, Azerbaijan, Kazakhstan and Uzbekistan. TADR is focused on a specific list of viruses and bacteria that pose a potential biological weapons threat to people and animal agriculture and on emerging infections that cause sudden illness and unexplained death in humans. TADR's goal is close to real time disease reporting and detection so that suspicious cases can be quickly investigated and then confirmed or denied. TADR provides a nationwide case definition-based electronic integrated disease surveillance system (EIDSS) that provides immediate, geographically-defined reporting of suspicious disease outbreaks from the local level through the regional (oblast) level to national authorities at the republican level in the Ministries of Health, Agriculture, Emergency Situations, and Defense. Regional veterinary, military or public health authorities can make informed decisions and respond immediately to the report by sending experts to the site of the outbreak in dedicated transport vehicles to examine patients, conduct an epidemiological investigation, collect samples, and transport these samples back to the regional lab for analysis under optimum conditions for detection of live microorganisms. In each country, a network of strategically located regional laboratories at biological safety level 2 is being equipped with modern molecular diagnostic devices for rapid ELISA and PCR testing. At the national level, a central reference laboratory will provide biological safety level 2 and 3 space for research and diagnosis and a secure repository of especially dangerous pathogens with electronic inventory control. Two national rapid response teams will augment regional surveillance and detection capacity should this be needed. DTRA is engaged in a comprehensive training program, including: facilities operations and maintenance; equipment operations and maintenance; biological safety; biological security; bio-ethics; laboratory diagnostic procedures; epidemiology and surveillance; quality control and quality assurance systems and proficiency testing; EIDSS; computer and information technology skills; and statistics and data analysis.

SESSION 4 (Plenary Session)
Marburg: The Angola Experience

Saturday, February 24, 2007
Room: Park Congress
08:30–09:15

4.001 Marburg: The Angola Experience

A. Duse. NHLS and School of Pathology of the University of Witwatersrand, Johannesburg, South Africa

SESSION 5 (Parallel Session)
Emerging Zoonoses

Saturday, February 24, 2007
Room: Park Congress
09:45–11:15

5.001 Transmissible Spongiform Encephalopathies

D. Heim. Swiss Federal Veterinary Office, Bern, Switzerland

The family of Transmissible spongiform encephalopathies (TSE) comprises several diseases of animals and humans. The longest known TSE is scrapie in sheep and goats, which was first described in 1772. TSE in humans were first described in the beginning of the 20th century. The most common is the sporadic form of Creutzfeldt-Jakob Disease (CJD).

The occurrence of a new TSE in cattle, Bovine spongiform encephalopathy (BSE) in 1986 in the United Kingdom and later in several other countries caused concern in the veterinary community. However, initially, BSE was considered to be a purely British issue. Greater attention was paid after the occurrence of BSE in cattle in other European countries. Though, it was until 2000 seen as a problem of some specific countries in Europe. As a result of the introduction of targeted surveillance systems in risk populations in 2000, countries that for years considered themselves as BSE-free have subsequently detected BSE. The first indigenous case outside Europe was reported in Japan in 2001 and has been followed by cases in Israel and North America.

The suggested link that cases of a variant form of CJD (vCJD) which were detected in the UK in 1996 might have resulted from exposure to

BSE caused a public crisis. Subsequently, much more rigorous measures have been implemented in several countries to eradicate BSE and to minimize the human exposure risk. The success of the measures implemented is reflected in the decreasing trend in BSE cases in most countries and the and the falling incidence of vCJD.

Although it seems that the situation is under control in most countries, it is still necessary to control the effective implementation of the most important measures and to monitor the situation. Moreover, from a lot of countries in the world the BSE situation is not known. Therefore, risk assessments for to evaluate the BSE-situation in these countries are needed.

5.002 Nipah and Hendra Viruses and Pteropid Bats: More Outbreaks, New Observations, Future Challenges

P. Daniels. CSIRO Australian Animal Health Laboratory, Geelong, Australia

In Australia sporadic cases of Hendra virus disease in horses continue to occur, with new human exposures. The geographic range over which cases have been diagnosed has expanded. The chain of transmission of the virus appears to have remained as first identified, from free living Pteropid bats to horses, and then on occasion to people exposed to body fluids of infected horses. In Bangladesh and India the pattern of emergence of Nipah virus disease in people is described as quite different from that in the original outbreak in Malaysia. Rather than massive amplification of the virus in a domestic animal host, the pig, prior to human infection as seen in Malaysia, in Bangladesh transmission appears to have been directly from the Pteropid wildlife reservoir, with the possible new dimension of human to human transmission. However experimental studies of henipavirus infections in Pteropid species do not indicate productive infections or florid host responses, but rather a well contained host parasite relationship that is far from being clearly understood. Serological and virological studies of Pteropid spp bats across their geographical range show that infection in these species is widespread. Companion animals such as cats, dogs and horses have been shown to be susceptible to one or other of the currently known viruses. New patterns of zoonotic disease emergence in new locations might be expected. From the perspective of response to naturally occurring disease in humans, a post exposure therapy rather than a prophylactic vaccination would appear more cost effective. Current studies of receptors are potentially an important contribution.

5.003 Rift Valley Fever Virus: Identification of interactions between Viral and Cellular Proteins as an Approach to Unravel Pathogenesis

M. Bouloy. Institut Pasteur, Unité de Génétique Moléculaire des Bunyavirus, Paris, France

Rift Valley fever virus (RVFV) is an arbovirus transmitted by many species of mosquitoes, which infects humans and ruminants and causes dramatic epidemics and epizootics. The disease is associated with fatal hemorrhagic fevers in humans and infection of ruminants, sheep in particular, leads to a high rate of mortality and teratogenesis. The virus circulates in Africa and has recently been introduced in Yemen and in Saudi Arabia, demonstrating its capacity to emerge into new regions.

RVFV is a trisegmented negative stranded RNA virus belonging to the Phlebovirus genus in the Bunyaviridae family. In cells infected with RVFV, like with the other bunyaviruses, all the steps of the viral cycle occur in the cytoplasm, but unexpectedly, the nonstructural protein coded by the small segment, NSs, is localized in the nucleus where it forms filamentous structures. This protein is not essential for the viral cycle since there exits a variant, Clone 13 which has a large deletion in NSs but replicates in all types of cells. Inoculation of RVFV kills mice within a few days, except Clone 13 which is avirulent. By genetic analyses and transient expression, we showed that NSs is a virulence factor which antagonizes the type I interferon response. To better understand the molecular mechanisms involved in pathogenesis, we identified cellular proteins interacting with NSs. Recent data, which will be discussed, indicate that, through interactions with cellular partners, NSs inhibits cell gene transcription and thus, counteracts the antiviral host defense.

5.004 Food-borne Zoonoses

P.J. Fedorka-Cray. USDA-ARS Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Athens, GA, USA

Background: The awareness of food borne illness has shifted over the years as international agribusiness and transportation have steadily increased. At least 30 food borne agents have been identified, with one-third emerging in the last 3 decades. Despite an increased emphasis on control measures, the estimated annual burden of illness remains high in both developed and developing countries.

Methods: Control measures have offered only limited success and no single reliable intervention has been successful in eliminating any zoonotic illness. Surveillance is central for the control of food borne disease. Globally, this requires an integrated farm-to-fork approach including harmonization of program goals, methodology and reporting. Risk assessments have been undertaken in a number of countries to quantify the burden of food borne related illness. However, these estimates are often hampered by a lack of data as well as a continued inability to link outbreaks to specific food sources.

Results: An increased awareness of the complexity of the problem has resulted in greater coordination and communication between and within countries. While a farm-to-fork approach must be maintained, increasing efforts must also be applied to understand the impact imparted by other ecological factors including waterways, wildlife, and migratory bird populations.

Conclusions: Food borne illness is a complex issue and the burden is spread across the farm-to-fork continuum. Vigilant efforts, new paradigms, and sensitive methods are required to provide more precise estimates of the burden associated with food borne disease. Additionally, enhanced efforts must also be directed to accurately determine the source (food, animal, human and/or ecologic) of food borne illness through attribution studies.

SESSION 6 (Parallel Session)

Challenges in Establishing and Coordinating Outbreak Surveillance and Response: The European Experience—Follow-up to the Epidemic Intelligence Meeting in Stockholm January 2006

Saturday, February 24, 2007
Room: Klimt Ballroom 2 & 3
09:45–11:15

6.001 The EU Role

G. Thinus. European Commission, Luxembourg, Luxembourg

In the context of Health crisis the surveillance and risk assessment activities are covered by ECDC, while risk management is dealt with by the European Commission which can advice on measures and co-ordinate the response of Member States. It can propose actions but has not the monopoly of proposition, nor does the European Council decide; this remains Member States responsibility. The Commission position on risk management will be a matter for political validation in-house, even if, at the end, the Commission position only constitutes a suggestion to Member States.

In the context of communicable diseases, Decision 2119/98/EC, applies and describes the risk management part. This decision does not cover CBNR events.

In order to increase its potential of management and response, the Commission has developed an Health Emergency Operations Facility, (HEOF) which consist of dedicated premises, and includes a Medical Intelligence tool (MedISys) [http://medusa.jrc.it/], dedicated rapid alert systems (EWRS Early Warning and Response System for communicable diseases; RAS BICHAT Rapid Alert System for Biological and Chemical agents and threats for the coverage of CBRN alerts and RAS CHEM for chemical events) as well as a situational awareness and interface tool with Member States (HEDIS). The presentation will tackle all the relevant networks and systems in place.

6.002 The ECDC Role

D. Coulombier. Stockholm, Sweden

6.003 Emerging Diseases in a Changing European Environment—the EDEN Project; Organization, Structure and Aims

S. de La Rocque[1], D.J. Roger[2], G. Hendrickx[3]. [1]CIRAD, Montpellier, France; [2]Oxford University, Oxford, United Kingdom; [3]Avia-GIS, Zoersel, Belgium

In recent years, several vector-borne, parasitic or zoonotic diseases have (re)-emerged and spread in Europe and elsewhere with major health, ecological, socio-economical and political consequences. At present little is known about the causes of such changes and the relative contributions to them of human-induced landscape changes, changing activity patterns, the breakdown of traditional control methods and global and local changes in climate. The EDEN (Emerging Diseases in a changing European Environment) project explores the impact of environmental and other changes on human health. Its aims are to identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial and temporal distribution and dynamics of human pathogenic agents. The project develops and co-coordinates at the European level a set of generic methods, tools and skills such as predictive emergence and spread models, early warning, surveillance and monitoring tools and scenarios, which can be used by decision makers for risk assessment, decision support for intervention and public health policies. Part of EDENs innovation is to combine spatial data (earth observation data, GIS etc) with epidemiological data. EDEN has selected for study a range of indicator human diseases that are especially sensitive to environmental changes and will be studied within a common scientific framework (involving Landscapes, Vector and Parasite bionomics, Public Health, and Animal Reservoirs). Some of these diseases are already present in Europe (tick- and rodent-borne diseases, leishmaniasis, West Nile fever), others were present historically (malaria) and so may re-emerge, whilst others are on the fringes of Europe (Rift Valley fever) in endemic regions of West and Northern Africa. EDEN integrates research in 47 leading institutes from 24 countries with the combined experience and skills to reach the projects common goals. The eco-geographical diversity of the project area covers all relevant European eco-systems from the polar circle in the North to the Mediterranean basin and its link with West Africa in the South, and from Portugal in the West to the Danube delta in the East. EDEN is organised into a series of vertical Sub-Projects linked together by a series of Integrative Activities that include biodiversity monitoring, environmental change detection, disease modelling, remote sensing and image interpretation, information and communication.

6.004 Climate, Environment, Economy: Complex Relationships in Variation of Entomological Malaria Risk in Europe

D. Fontenille. Montpellier, France

6.005 Land-Use/Land-Cover Change and Vector-Borne Diseases: Remote Sensing and Multi-Agent Simulations

E.F. Lambin, C. Linard, S. Vanwambeke. University of Louvain, Louvain-la-Neuve, Belgium

The transmission of vector-borne and zoonotic diseases, resulting from contacts between people and vectors or hosts, depends on the density of vectors and on the exposure of people to vectors, and thus on landscape patterns and land-use change. Earth observation satellites allow estimation of disease transmission risk by predicting the habitat suitability for vectors and the likelihood of contact between people and vectors.

We illustrate these concepts with results from empirical studies in Europe. Various environmental variables extracted form high-resolution remote-sensing data explain the abundance of bank voles and the hantavirus prevalence in Belgium. Statistical results show that winter temperature and drainage of the soil have a significant impact on the persistence of the virus in the soil. Regression analysis shows that Lyme borreliosis risk is much higher in periurban zones with high-income residential areas with a forest cover. Puumala hantavirus infection risk is higher in remote forested areas with low incomes and no urbanization.

Results of statistical analyses can be used to build agent-based simulation models (ABM) to predict the risk of disease emergence. ABM represent complex interactions between a diverse set of agents in a spatially explicit environment. We model with ABM contacts between humans and mosquitoes to assess the risk of re-emergence of malaria in Camargue (southern France). Results indicate that land-use changes have a detectable impact on mosquito populations and on infection risk. This impact varies according to the local environment but can be counteracted by adoption of preventive measures.

Environmental and spatial factors play an important role in the transmission of vector-borne and zoonotic diseases. The combination of remote sensing, geographic information systems and agent-based simulation models is promising for spatial epidemiology. The complexity of vector-borne disease transmission calls for an integrated approach considering ecological, biological and human aspects.

SESSION 7 (Poster Session)

Surveillance of Emerging Diseases in the 21st Century

Saturday, February 24, 2007
Room: Klimt Ballroom 1
11:15–12:30

7.001 SurvNet—Electronic Surveillance System for Infectious Disease Outbreaks in Germany

G. Krause[1], D. Altmann[1], D. Faensen[1], J. Benzler[1], T. Pfoch[2], A. Ammon[1], M.K. Kramer[3], H. Claus[1]. [1]Robert Koch Institute, Berlin, Germany; [2]picoware, Berlin, Germany; [3]Ministry of Health, Berlin, Germany

In 2001, the Robert-Koch-Institute (RKI) implemented a new electronic system for reporting infectious disease outbreaks in Germany (SurvNet). This system is based on linking case reports of nationally notifiable diseases resulting in 30,578 outbreak-reports in 2001–2005. Median duration from notification of the first case to the local health department (LHD) until receipt of the outbreak-report at RKI was 7 days. Most commonly reported location settings to which the outbreaks were associated among 10,008 entries for 9,946 outbreaks in 2004/05 were: household (5,262; 53%), nursing home (1,218;12%), hospital (1,248;12%) and kindergarten (783; 8%). Median outbreak duration ranged from 1 day for outbreaks caused by Campylobacter and 2 days by Salmonella up to 73 days for

outbreaks caused by M. tuberculosis. Among the 2,662 listed items of food-borne connection (among 2,554 outbreaks) outbreaks in 2004/05, 762 (29%) were associated with egg-consumption and 500 (19%) with meat-consumption. SurvNet may minimize work load of LHD for outbreak documentation, enhancing compliance with outbreak reporting and may be a useful tool for other national or international outbreak surveillance systems.

7.002 Identification of External Virulence Factor from Leishmania Major by a Specific Monoclonal Antibody

A.R. Khabiri, F. Bagheri, Z. Aghighi, M. Assmar. Pasteur Institute of Iran, Tehran, Iran

Leishmania Spp. are digenic parasitic protozoans that survive in hostile environments—the midgut of the insect vector and the phagolysosome of the mammalian macrophage. These are many virulence factors that determine the broad spectrum of clinical forms and severity of leishmaniniasis. Two different groups of internal parasite molecules are defined as virulence factor, one group consist of largely surface and secretory products, and the second group includes intracellular molecular referred to as pathoantigens. In this study we defined an external virulence factor from surface of leishmania major (L.major) that induce infection in susceptible BALB/c mice. Our study shown that monoclonal antibody species-specific L.major recognizes a component on surface and culture supernatant of L.major. Since mAb, cannot reacted with component of medium, it is questionable that the parasite borrowing this component from the growth medium with simple absorbsion. The result also shown that following fragmentation and modification via specific mechanism unknown component transfer to parasite surface. We also shown that mAb inhibited transfer of modified component to parasite surface and the uncoated parasite could not induce infection in susceptible BALB/c mice. These observations explain a novel option that parasite acquire pathogenesis from the external medium via novel mechanism.

7.003 Surveillance Among Influenza Viruses Isolated Early During the 2006 in Taiwan

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Background: Monitoring influenza activity has been coordinated by the Center for Diseases Control, Taiwan since 1999. Surveillance of influenza in Taiwan is mainly based on laboratory isolation of influenza viruses and sentinel reports of influenza-like illness. Analyses of epidemic influenza virus isolates have chiefly focused on antigenic and genetic characterization of the hemagglutinin (HA) glycoprotein in order to detect new variants of each epidemic strain.

Methods: Data from sentinel physician networks and other sources, mainly hospitals were collected. Respiratory specimens were tested for influenza and mainly by virus culture and polymerase chain reaction amplification. Phylogenetic analysis was carried out for genes encoding hemagglutinin of influenza A and B viruses isolates obtained from January to September 2006.

Results: A total of 834 cases of laboratory-confirmed influenza were reported, which was 55.5 percent lower compare to the same period in 2005. Clinical influenza activity has only reached moderate levels and has mainly been associated with influenza A viruses (H1N1). The results showed that there has been co-circulation of influenza A and B viruses in Taiwan, and influenza A (H1N1) has become predominant in 2006. Two antigenically and genetically distinct HA lineages of influenza B virus are presently known to exist, however, there were very few Yamagata lineage viruses where approximately 90 percent of all B strains isolated were of the Victoria lineage in 2006 which were different from previous years.

Conclusions: Preparation for an influenza pandemic is presently a high priority in Taiwan. Laboratory-based surveillance systems must be timely in order to be effective. This study indicates that multiple viruses cocirculate in Taiwan and it seems likely that both influenza A and B viruses evolve through antigenic change of surface glycoproteins of recent circulating strains. In addition, we provide update information for predicting newly emerging influenza viruses in human.

7.004 Rift Valley Fever Virus Seroprevalence in Sheep and Goats Born Before and After the 1997–98 Epizootic in Nakuru District, Kenya

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Background: Rift Valley fever virus (RVFV) is an emerging zoonotic hemorrhagic virus, with an unknown reservoir. In livestock, the disease causes severe economic losses by decimating herds with high mortality and abortion rates. In this study, we investigated the seroprevalence of RVFV antibodies in two breeding herds of sheep and goats in the Nakuru district of Kenya, where an epizootic occurred in 1997–98. We hypothesized that only animals alive and exposed during that outbreak would be seropositive for RVFV.

Methods: Blood was collected from 229 sheep and 70 goats ranging in age from 4 months to 11 years. RVFV IgG was measured in sera by ELISA and seropositivity defined by a titer >0.200.

Results: Seroprevalence of RVFV was significantly higher in goats (17% or 12/70) than sheep (3% or 6/229). Both sheep and goats born in 1998 or earlier (6/26) were more likely to be seropositive than animals born in 1999 or later (12/270). Sheep born in 1998 or earlier had a seropositivity rate of 19% (3/16), 1999–2001 had 2% (1/60), 2002–2004 had 1% (1/75), and 2005–2006 had 1% (1/78). Goats born in 1998 or earlier had a seropositivity rate of 30% (3/10), 1999–2001 had 18% (4/22), 2002–2004 had 13% (4/31), and 2005–2006 had 14% (1/7).

Conclusion: Sheep are the preferred animal for RVFV sentinel herds; this should be reconsidered due to the higher seropositivity in goats. The detection of three seropositive sheep and nine seropositive goats born after the 1997–98 outbreak clearly indicates more recent circulation of RVFV.

7.005 Verifying the Preparedness of the Serbian Health System for Avian Flu in Humans

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Background: It is considered that the world is, more than ever, close to influenza pandemic caused by the virus similar to H5N1, the so-called Avian (influenza of birds). All states are obliged to define, verify and update their respective national plans to fight the influenza outbreaks and a possible pandemic. To show whether the questionnaire is applicable and the data showing preparedness of the Serbian health system in case of case appearance of the Avian pandemic type strain in humans.

Method: Descriptive method was used. Data from questionnaire with 39 variables, were collected and estimated by special team

Results: The realization of National Plan according to the institutions and Guidelines was uneven, ranging from complete 8% to superficial 72%. National documents have been distributed, elaborated and studied in all health care facilities. However, they have not been submitted to all health care workers covered by the initial plan. Knowledge of the procedures and their realization were best in facilities dealing with public health. The exchange of information between human and veterinary health services was mostly done by telephone and during occasional meetings. The system of informing and inviting of the team members, special medical team and health managers has been established. It has been 100% functional. In all districts of Serbia, through special features on radio and TV stations, lectures and magazine articles we have managed to get the audience informed about the issue. About 90% of the facilities have allocated space for isolation/observation of suspect cases. However, only 4% of facilities do have proper respiratory isolation. In all districts we have provided personal protection equipment and other items for team members, except vehicles. Most frequently observed drawbacks are related to insufficiently develop written instructions on the local level (to carry out certain stages within "Measures").

Conclusion: The used questionnaire allows verification of readiness of the Serbian health care system to realize Plan of Activities in case of Avian Pandemic in human populations. Generally, the Serbian system of health care has been prepared to apply plan of activities in case of outbreak of avian flu in human populations. There are significant differences among health care facilities.

7.006 The Complications in Generalized Tetanus in Adults: Clinical Study in Albania

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Objective: Our aim was to describe the complications of generalized tetanus and its association with age.

Methods: We performed a case-series study that included 60 patients aged 14 years and over with generalized tetanus. All cases were enrolled from October 1984 to May 2004. Patients were divided into two groups: 18 were intubated, whereas 42 were not intubated. Clinical data were obtained from clinical charts and personal experience. Binary logistic regression was used to assess the relationship between complications with age, mechanic ventilation and mortality.

Results: Our findings indicate that the prevalence of complications of cardio-circulator system was 35%, the prevalence of complications of respiratory system was 38%, and the prevalence of complications of the other systems (such as gastrointestinal, sepsis, or skin breakdown) was 27%. We have seen an increase likehood of complication with increase of age. Intubated patients were more prone to represent complications.

Conclusions: In cases of generalized tetanus, respiratory and cardiovascular complications are the most frequent life-threatening complications with the same average of occurrence. The likelihood of occurrence of complications increased with age. Prompt recognition and treatment of generalized tetanus complications are important in reducing the mortality in this disease.

7.007 A Study of a Potential Escape Route in the BSE Surveillance

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BSE (Bovine Spongiform Encephalopathy) is a progressive neurodegenerative disease occurring in cattle. It belongs to the group of Transmissible Spongiform Encephalopathies (TSEs), that includes diseases associated with the accumulation of a pathological isoform of a host-encoded glycoprotein, named PrPsc. In the mid-nineties, epidemiological and experimental studies strongly suggested that a new human disease, the variant of Creutzfeldt-Jakob Disease (vCJD) was caused by the same agent of BSE. In order to verify the actual epidemiological BSE status of member states, the European Commission (EU) set up an Active Surveillance system for BSE, which started on 1st January 2001 and is based on rapid and easily applicable post mortem tests. In the frame of Active Surveillance for BSE, emergency slaughtered animals, animals with non-specific clinical signs at ante mortem examination and fallen stock represent 'risk categories for BSE'. It is assumed that bovines which test positive for BSE are more likely to be found in those groups than in regularly slaughtered animals. Due to huge costs, surveillance is going to be targeted on these categories. Since February 2001, Italy tested for BSE all fallen stock ageing equal or greater than 30 months. Since July 2001, this age limit was moved to equal or greater than 24 months. EU legislation enforced testing of just a sample of this subpopulation till august 2002, finally, since September 2002 all EU Member States are testing the whole subpopulation of fallen stock. Testing on the field the overall fallen stock subpopulation can be very difficult and may result in an escape route for affected animals.

Aim of this study is to evaluate the size of the gap between the number of dead animals recorded in the National Bovine Population Database (NBPD) and the number of tested dead animals as resulting in the National Active BSE Surveillance Database (NASD) in the first six months of 2006. 9,258 dead on farm were recorded in the NBPD but did not appear in the NASD. A cross-sectional investigation was carried out on those animals: a stratified random sampling was used to select 370 dead animals and a questionnaire was administrated to the veterinary officers in charge of BSE control. The accepted error was established to be ±5% around the estimated percentage of the not tested dead animals, at the 95% level of confidence. On the basis of the results, we can conclude that actually the surveillance on the fallen stock, may represent a real problem but probably it does not modify significantly the Italian prevalence of the disease.

7.008 Nephropathia Epidemica in Oppland and Hedmark Counties, Norway, 1981–November 15, 2006, Vectors, Virology and Epidemiology

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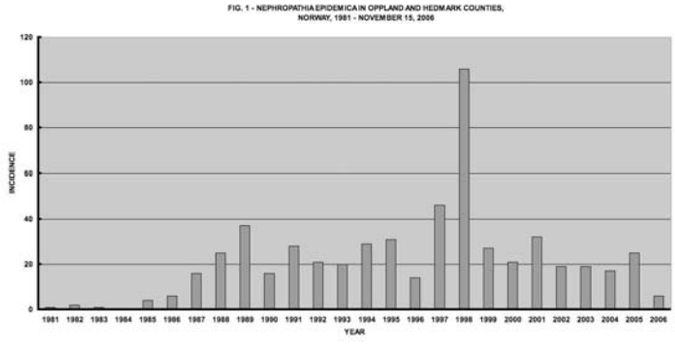
Background: Oppland and Hedmark Counties are located in Central Eastern Norway. The area of the counties is 53 200 square kilometres i.e. approx. that of Denmark (43 094 square kilometres). The total population is 371 500 (data as of the end of 2004). Thes two counties account for a large number of cases of nephropathia epidemica (NE) diagnosed and notified in Norway.

In Norway the main rodent vectors are Clethrionomys glareolus and Apodemus sylvaticus. Our counties have both. The Norwegian, indigenous strain of Hantavirus is the Puumala virus.

Method: Our Department diagnoses antibodies against the Puumala virus of IgG and IgM classes with ELISA as a routine. All cases of NE in Norway are notified to the Norwegian Notification System for Infectious Diseases (MSIS).

Results: In the period 1981–November 15, 2006. The Norwegian total up to November 15, 2006 was 1239 cases of NE. Our two counties accounted for 569 cases (46%) notified to MSIS, Fig. 1:



The high incidence recorded in 1998—106 cases (19%) co-incided with a large rodent population that year. This yearly incidence has never been reached that level before or after 1998. Of the cases 181 (32%) were females and 388 (68%) males. A total of 331 cases (58.1%) was among persons aged 30–59 years. The clinical picture has been mild. No deaths from NE were recorded.

Conclusion: Although no other areas in Norway have similar high incidences as Hedmark and Oppland counties there still probably is some degree of underreporting due to this infection being less severe in Scandinavia compared to Hantavirus infections caused by the e.g. Belgrade, Sin Nombre viruses.

7.009 Antigenic and Genetic Surveillance of Influenza A/H3N2 Viruses in Austria

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Background: Antigenic characterisation of influenza viruses with hemagglutination-inhibition-test is an established and non-replaceable technique. Nevertheless in the light of the high sensitive tool of genotyping it becomes more and more evident that strain characterisation only by HI may be too ineffective for the rapid strain identification and for tracing the dynamic of genetically changes of the fast evolving influenza-viruses.

Methods: Influenza A/H3N2 viruses from samples, obtained during three seasons (2002/03 to 2004/05) in Austria were analysed by genotyping. Data obtained were compared to those of antigenic characterisation by HI test. Additionally, sequencing of H3N2 viruses directly derived from swabs as well as from isolates was performed to find out whether genotyping directly from clinical material is feasible and provides reliable data.

Results: Tracing the changes of the HA-gene by genotyping revealed that with increasing epidemic activity viruses started to evolve with decreasing homology to the dominant circulating strain. These emerging strains showed a close relationship to the dominant strain of the following influenza-season. Further our results demonstrate that strain identification directly from clinical material is possible and produces reliable and rapid information on the current circulating strains.

Conclusion: Monitoring of isolates by genotyping, during an epidemic outbreak, provides valuable and prompt information on the dynamics of influenza-virus genetic drift. Enhanced and more rapid availability of molecular data of recent isolates, not only of local surveillance systems, but also from different European countries, as suggested by EISS, most probably will enable a better prediction of the dominant strain for the forthcoming season.

7.010 A Bacteriology Study of Infectious Diabetic Foot Ulcer Among Patients Admitted in Razi Hospital, Ahvaz—Iran (2004–2005)

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Background: Diabetic foot Infection is caused by gram positive and gram negative aerobic and anaerobic bacteria and most of them are resistant to antibiotics.

Methods and Patients: In this cross sectional study 32 patients with diabetic foot ulcer that referred to Razi hospital were admitted. All patients were evaluated by punch biopsy and samples were sent to microbiology laboratory of medical faculty of Ahvaz Jondishapur medical university for aerobic and anaerobic cultures and determining antibiotic susceptibility pattern.

Results: Twenty five samples (78%) had polymicrobial positive cultures (aerobic and anaerobic bacteria). Most of them are multidrug resistant.

Conclusion: Foot infection is one of the most important limb threatening complication of diabet and due to polymicrobial and antibiotic resistant pattern, surgical intervention most be concerned.

7.011 Aetiology of Encephalitis: A Multi-Centre Prospective Study

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Background: Many gaps exist in our knowledge of encephalitis despite the morbidity and mortality of those affected. The aetiology of these infections, as well as the risk factors and associated prognoses, remain unclear. With growing evidence that an increasing number of viruses are able to cause encephalitis in human hosts, there is concern that new and emerging infections may be contributing to the 60% of cases currently of unknown aetiology in the UK.

Methods: The Health Protection Agency, alongside colleagues in the National Health Service, has embarked on a prospective cohort study of encephalitis. Patients are being recruited over a 2-year period from 26 hospitals located in the South West, North West and London areas of England. Samples, collected as part of good clinical practice, are being tested for an array of different organisms, beyond those screened routinely. Innovative pathogen discovery techniques are being developed and will be used on study samples of unknown aetiology to assess the possibility of new and emerging infections. In parallel, research nurses are collecting extensive clinical information about the patients who participate.

Results: The study commenced in October 2005 in the South West region, and was extended to London in January 2006 and North West in November 2006. We are currently recruiting from 11 centres. Forty patients have so far consented to take part, including 21 (53%) females and 19 (27%) males. Sixty-two percent are adults, 28% are children aged between one and 16 years, and 10% are infants below the age of one. Of the first 25 cases, 19 have been classified as encephalitis, three as meningo-encephalitis, and three as non-encephalitis. The predominant causes of encephalitis thus far are HSV (37%) and those of unknown aetiology (37%). Other causes include VZV, HHV-6 and post-infectious encephalitis.

Conclusion: The findings of this study so far are in accordance with other studies of encephalitis published in the literature, HSV and encephalitis of unknown aetiology being the most common categories. The rigorous diagnostic approach and developed methods for pathogen discovery may identify new viral causes of encephalitis. Also, they will have applicability for other enigmatic infections and for emerging diseases.

7.012 Measures Undertaken In Serbia in Case of Suspected Avian Influenza In Humans

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Introduction: Adhering to the recommendations of WHO and OIE, we have prepared national plan of activities (to be applied before the pandemic and afterwards). It was followed by creation of the Working Group, which also defined the Guidelines of Good Practice. Monitoring of epizootologic and epidemiologic situation in the country, during March 2006,

has detected two foci of highly pathogenic H5N1 virus of avian influenza in the two dead swans. In one of the foci, domestic animals have also been contaminated and suspicion was defined for avian influenza in people.

Aim: To show timely application of veterinary measures in the zone of contagion and the measures undertaken by health care services and procedure in case of suspicion of avian influenza in people. This was done on the local and central levels.

Method: The paper used descriptive epidemiologic method. The source of our data was epidemiologic questionnaires, patient histories and results of laboratory investigations.

Results: On March 9, 2006, veterinary services isolated highly pathogenic virus of avian influenza, H5N1 in one dead swan and, later on, in one rooster. The results were confirmed in the Waybridge reference laboratory. The area was proclaimed contagious zone. All veterinary measures were undertaken to cull the poultry in individual farms. Simultaneously, information was dispatched to human health care services in order to initiate epidemiologic surveillance of the exposed populations. During this surveillance, between March 14 and 19, 2006, we have established that there were 9 families (33 persons) living in the focus of outbreak (i.e. the territory with infected poultry). They were all placed under surveillance: daily medical examinations and taking of their temperature at home. Epidemiologic surveillance (carried out through the questionnaire, which was filled by all persons with respiratory problems and these who had been in contact with infected poultry) encompassed 35 families (with 111 individuals). During health surveillance, on March 16, 2006, in four persons (aged 5, 6 and two aged 18) were defined suspicious for avian influenza in the humans. According to the Guide, they were taken to hospital in a special vehicle and placed under respiratory isolation (at the Department for Infectious diseases of the Uzice Hospital). Biologic samples were taken from these four cases (throat swab and blood) for viral and serologic analyses. The samples were then sent to the Torlak- Belgrade Laboratory. On March 19, 2006, viral findings were negative for influenza A (H1, H3 and H5). They removed suspicion of avian influenza in humans. In accordance with the Guide, local management (human and animal health care services) have undertaken defined procedures and measures and informed relevant authorities about the findings. After that, press conference was held and the general public was informed that there were no reasons for fear and panic in the respected community. The public was also called to participate in all activities provided by the Plan and show high level of personal motivation.

Conclusions: It was estimated that all actions and procedures were carried out in accordance with the Guide (stages 1, 2 and 3); that excellent cooperation was established among human (epidemiologic mobile teams and HC teams) and animal health services. Consultations were held on a daily basis, confirming an active approach toward the issue of influenza pandemic.

7.013 Increasing Incidence of Antiviral-Resistant Among Influenza A Viruses Isolated in Taiwan during 2004 to 2006

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Background: Both of M2 ion channel inhibitor and neuraminidase inhibitor have been used to treat influenza A virus infection for many years. These two classes of drugs have been approved by the Department of Health, Taiwan, in 1986 and 2001, respectively. We screen influenza A viruses isolated during January 2004 to June 2006 in Taiwan for the incidence of resistance to antivirals.

Material and Methods: All viruses isolated were collected from clinical specimens in Taiwan. Viral RNA was extracted from supernatant of virus culture. Pyrosequencing was performed and full sequence data of MP and NA genes were aligned and analyzed by Mega 3 programs.

Results: An increased incidence of amantadine-resistant was observed in our result, 15 of 67 H1N1 and 86 of 173 H3N2 isolates contained an amino acid substitution at position 31 (S31N). Yearly analysis showed a substantially rising percentage of drug-resistant viruses in Taiwan, significantly form 0% to 26.9% for H1N1 and 28% to 100% for H3N2 during 2004–2006.

Several oseltamivir resistance in clinical isolates has been associated with substitution in the NA active site were not been observed in our sequence data of influenza A viruses.

Discussion: Influenza viruses constitute pandemic threats, the increasing resistance to adamantine is a problem for concern. The incidence of resistance dramatically increased in Taiwan possibly owing to increased use of over-the-counter amantadine after the emergence of severe acute respiratory syndrome (SARS) in 2003. However, none of the isolates were oseltamivir resistant in our sequence analysis, this preliminary finding indicates that a very low frequency of oseltamivir resistance was present in community isolates in Taiwan. It is unknown whether these infections represent low-level transmission of resistant variants to contacts or spontaneous emergence of resistance. Such circumstances highlight the need for rapid methods of ascertaining the susceptibility profiles of epidemic strains that can be used for quick detection of resistance mutations.

7.014 Syndromic Surveillance for New and Old Rickettsiosis

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Background: In Brazil a large number of diseases are associated with fever and hemorrhage, some of them widely distributed, many emerging or re-emerging. In this context Brazilian spotted fever (BSF), an acute, febrile rickettsiosis, caused by Rickettsia rickettsii, is one of the most important diseases to be investigated. Because the possibility of emergence of newly identified and previously unknown rickettsiosis, the strategies used in the surveillance of these infectious diseases must be continuously updated. Syndromic surveillance, is increasingly being employed to detect new diseases and identify new agents, including during clusters and outbreaks, could be adapted to investigate both emerging and reemerging, new and old rickettsiosis.

Methods: Retrospective and descriptive study of an hospital-based surveillance of acute febrile hemorrhagic syndrome (acute onset fever and at least one hemorrhagic sign) at the Hospital das Clínicas da UNICAMP (HC/UNICAMP), a regional referral university hospital in Campinas, São Paulo, Brazil, a BSF endemic area.

Results: Situation 1: A cluster 6 cases with a case-fatality of 50% in a nearby rural area. All cases were confirmed as BSF by a four-fold rise in IFA titer (2 cases), immunohistochemistry and Rickettsia isolation (1 case), Rickettsia isolation (1 case) and clinical and epidemiological correlation (2 cases). **Conclusion:** a cluster of BSF. **Situation 2:** An international traveler from Portugal died a few days after arriving in Campinas, with an acute febrile disease with rapid progression to hemorrhagic suffusion and shock. Rickettsia were isolated from skin lesions and blood clot, later identified as characterization of R conorii. **Conclusion:** a rickettsiosis in an international traveler by a never previously detected species in Brazil. **Situation 3:** A sub-acute febrile disease with petechial exanthema in a woman from Campinas and that reported previously contact with ticks and two sick dogs. The Nested-PCR performed in blood clot was positive for Ehrlichia sp infection. **Conclusion:** the first human Ehrlichiosis case reported in Brazil.

Conclusion: In these cases the hospital based syndromic surveillance was an important tool to detecting a cluster of a previously known rickettsiosis in Brazil; an imported Rickettsia infection and an emerging Rickettsiales pathogen in Brazil. Syndromic surveillance is adequate for the identification of emerging pathogens.

7.015 Information Technology Integration for Global Public Health Surveillance

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Background: Coordinating disease surveillance for epidemic intelligence purposes at the national and international levels provides critical information for early identification of potential health threats. Local, national and regional disease surveillance systems have evolved in the absence of international standards for specific data types, resulting in a wide variety of unique data bases containing valuable information. Current information sharing across the various reporting systems (human, veterinary and wildlife) happens via human-intensive, time-consuming activities such as the exchange of e-mails or faxes.

Methods: An open architecture information technology solution is presented that addresses data exchange and aggregation enabling global disease surveillance without the need for expensive harmonization of data across the various systems. The system design leverages current network technology to ensure secure exchange of information in a manner that provides anonymity for individuals and respects national sovereignty.

Results: Aggregating information from all sources via the use of database adaptors, metadata, publish/subscribe web services, a metadata catalog, an applications layer and an "integration backbone" results in a global public health surveillance system. Applications automatically process the metadata in real time to detect significant events using numerical, temporal and geographic criteria for alerting human operators when appropriate. The metadata catalog provides a substantial information resource for human exploration using visualization tools and data mining applications.

Conclusion: Electronic connectivity of disease surveillance information systems for epidemic intelligence is achievable using metadata with current network technology. The resulting aggregation of data in a metadata catalog enables automatic processing to generate alerts and support analyses of disease trends and patterns. The extensible and expandable open architecture system design permits future growth via additional data sources, applications and users.

7.016 Evaluation of a Surveillance System for Single-Use Instruments in Tonsil Surgery. Can a Surveillance System Monitor the Safety of Single-Use Surgical Equipment?

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Background: The Department of Health introduced single-use instruments for tonsil and adenoid surgery in the United Kingdom in January 2001, based on the advice given by the Spongiform Encephalopathy Advisory Committee to minimise the theoretical risk of transmission of variant Creutzfeldt-Jakob Disease (vCJD) via surgical instruments. Concerns were raised on the potential intra-operative hazards due to the quality of the instruments and increased risk of postoperative complications, especially haemorrhage. In Wales, following objective laboratory tests of the available single-use instruments, new highly specified single-use instruments for tonsil and adenoid surgery were introduced in February 2003 together with a surveillance system to monitor both instrument problems and complications. The objective of the study is to evaluate the Single-use Instrument Surveillance Programme (SISP) using a framework for evaluating surveillance systems.

Methods: The Centres for Disease Control and Prevention (CDC) Updated Guidelines for Evaluating Public Health Surveillance Systems methodology was used. A description of the SISP, a summary of its usefulness and an analysis of its attributes as a public health surveillance system was performed.

Results: The SISP appears to be an effective surveillance system that meets its objectives, attains the standards set and is acceptable and useful to the majority of users. The operation of the SISP is simple and sufficiently flexible to adapt to changes. The sensitivity and quality of the SISP data were straightforward to evaluate with comparative data.

Conclusions: The safe use and procurement of single-use instruments for tonsil and adenoid surgery was ensured with this surveillance system. This demonstrates that it is possible to monitor surgical outcomes and advise on the impact of changes in instrumentation within a short time period.

This is the first time that the functionality of a set of single-use surgical instruments and the potential complications associated with their use has been monitored in this way.

This evaluation demonstrates an alternative approach to the safe introduce of new health technology on a large scale, when evidence on its efficacy and safety is inconclusive. This is of potentially profound importance to public health practice.

7.017 Syndromic Surveillance of Infectious Gastroenteritis Using Non-Prescription Drug Sales: Results of 3 Canadian Retrospective Studies

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Background: Support for a public health surveillance resource based on over the counter (OTC) sales of non-prescription products will depend on whether sales patterns reflect underlying community infectious disease. The usefulness of OTC sales-based syndromic surveillance for infectious gastroenteritis (GE) is investigated in three studies using retrospective data under large outbreak and non-outbreak conditions.

Methods: Retrospective OTC sales and epidemiological case data from two large Canadian waterborne outbreaks were investigated. Regional temporal patterns of weekly aggregated OTC sales, Emergency Room visits (ERV) and notifiable cases related to infectious GE were compared. Weekly reported cases were categorised by bacterial, parasitic and viral infections, for comparisons with OTC sales. Then, Generalised Linear Models (GLM), smoothed with natural splines and adjusting for holidays and day-of-week effects, were used to assess relationships between daily OTC sales and ERV.

Results: OTC sales gave early evidence of heightened community GE activity in two large waterborne outbreaks. Under non-outbreak conditions, weekly OTC sales patterns were temporally similar to ERV, likely reflecting community Norovirus activity (high in winter), but not notifiable bacterial and parasitic infections (high from late spring through early fall). GLMs showed a positive relationship between ERV and OTC sales only on the same day. Beyond this, the relationship was negative; heightened sales on a particular day would see decreased ER visits from 1 to 8 days thereafter.

Conclusion: GE-related OTC sales patterns reflect certain aspects of underlying community illness and would be good early indicators of large-scale community GE outbreaks (intentional or unintentional). Results support the concept of monitoring OTC sales trends to forewarn public health officials of burgeoning community GE. Reacting with increased vigilance and interventions could reduce infection rates and consequently, burden of illness. A web-based syndromic surveillance system utilising OTC sales is currently under evaluation in Canada.

7.018 Capacity Building and Diagnostic Support for Avian Influenza in Sub-Saharan Countries

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Background: The active circulation of the H5N1 highly pathogenic avian influenza (HPAI) virus in the African continent represents a serious threat for animal and human health. The Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in Padova, Italy, as an OIE/FAO Reference laboratory and in the framework of the FAO Technical Cooperation Programmes (TCP), has delivered training and provided diagnostic assistance to over 40 countries in Africa.

Methods: Diagnostic capacity building IZSVe staff delivered six training courses focused on Avian influenza diagnosis in different African Central Veterinary Diagnostic Laboratories. Laboratory assessment missions were also performed to evaluate upgrading requirements.

International diagnostic support Since February 2006, IZSVe received

and analysed for AI over 700 samples from 13 African countries.

Strengthening the network Validated diagnostic protocols were provided to all laboratories, with intensive support to the national laboratories of affected countries. Expert advise and reference reagents were provided to attendees.

Results: Diagnostic capacity building a total of 97 laboratorists from 44 countries attended the courses. Selected laboratory personnel received specific training about the use of diagnostic reagents and kits in the diagnosis of HPAI. Up to now, two laboratories (the National Veterinary Research Institute in Nigeria and the Laboratoire National d'Appui au Développement Agricole in Ivory Coast) improved their diagnostic capacities and are now able to diagnose avian influenza autonomously.

International diagnostic support and strengthening the network IZSVe provided confirmatory diagnosis for HPAI in 47 cases and Newcastle disease in 49 cases to countries of that areas. Training efforts in developing countries led to personal contacts between African and European scientists. This resulted in improved networking and in the provision of diagnostic reagents and protocols, all of which have contributed to facilitating the diagnosis of H5N1 in Africa.

Conclusion: The current H5N1 panzootic is unprecedented in its spread and, for the first reported time, vast areas of developing countries are being affected. It is therefore essential that international organizations and reference laboratories fully support the implementation of veterinary services and diagnostic capacities in order to make the control of HPAI affordable for developing areas. The experience reported herein may represent a first step to achieve this goal.

7.019 Prevalence of Antibiotic Resistance in the Community in the Southern Part of the Netherlands

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Background: Worldwide methicillin-resistant Staphylococcus aureus (MRSA) is an increasing problem. Moreover, an increased prevalence of antibiotic resistant S. aureus has been observed in healthy persons and in patients without any history of hospitalization or contact with health care institutions. The present study was performed as only limited information concerning the prevalence of antibiotic resistant S. aureus in the community is available.

Methods: A random sample of 4000 inhabitants living in a large city was taken from the Municipal Administration. Each person received an envelop by mail containing study instructions, an informed consent, a questionnaire, a nasal swab for taking a sample from the anterior nare, and material for returning the swab to the laboratory. The nasal samples were analyzed for the presence of S. aureus using selective media. The susceptibility to commonly used antibiotics was determined including penicillin, methicillin, macrolides, tetracycline, fusidic acid and mupirocin. The presence of MRSA was confirmed using a real-time PCR.

Results: In total, 2323 swabs out of the 4000 were received, (i.e. a spontaneous response rate of 60%) and S. aureus strains were isolated from 487 swabs (21%). Resistance to penicillin, macrolides and tetracycline was found in 71%, 4% and 3% respectively. The prevalence of resistance to mupirocin was 1% and to fusidic acid 5%. Two methicillin resistant strains were found.

Conclusions: The spontaneous response rate of 60% was relatively high. However, the percentage S. aureus isolated was slightly lower than one should expect. The low prevalence of antibiotic resistant S. aureus found in the population analyzed suggest that resistance among the healthy population outside the hospital is not an important risk factor for the prevalence of resistance in hospital isolates.

7.020 HIV/HBV, HIV/HCV and HIV/HTLV-1 Co-Infection Among IDU Admitted Patients in Razi Hospital Ahvaz, Iran (2001-2003)

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Objectives: To assess the prevalence and risk factors for HBV, HCV and HTLV-I co-infection in the Iranian HIV positive Injecting Drug User patients admitted in hospital.

Methods: Analyses were based on 154 male IDU patients recruited to Razi hospital in Ahvaz. city during 3 years (2001–2003). All of them had been tested for HIV infection (Elisa-antibody & Wesernblot), HBV surface antigen, HCV antibody and HTLV-1 antibody.

Results: Among those tested, prevalence of HIV infection was 60.52%. Among those HIV infected, HBV surface antigen, HCV antibody and HTLV-I antibody were 30% and 50%, and 16.33% respectively.

Conclusions: Co-infection with HBV or HCV or HTLV-1 is common among HIV-infected IDU patients admitted in Razi hospital. HIV disease outcomes appear to be adversely affected by HBV/HCV/HTLV-I co-infection.

Keywords: Co-infection, hepatitis B, hepatitis C, HTLV-1, HIV.

7.021 Excess Death Attributable to Influenza in the Czech Republic: Final Results for the Years 1982–2000

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Background: Annual influenza epidemics differ in duration and magnitude. Influenza infection is often underestimated, being easily mistaken for one of other acute respiratory infections (ARI) since it has similar clinical symptoms. The aims of this study are to find correlation between mortality and influenza morbidity and to model mortality in different weeks of the year outside the influenza epidemic.

Methods: Data on daily deaths from all causes and deaths from diseases of the circulatory system in the Czech Republic were available for 1982–2000 (Altogether 2 197 654 and 1 225 088 deaths reported, respectively). Data on the incidence of influenza and other ARI were taken from the surveillance programme. The weeks in which ARI morbidity exceeded the epidemic threshold and at the same time, circulation of influenza virus among the population was reported by the National Reference Laboratory for influenza were considered as influenza epidemic weeks. Analysis was based on the assumption that outside the epidemic periods, deaths are distributed according to the Poisson distribution with a linear trend depending on time and with periodic behaviour during the year. The mortality is only expected to increase in the epidemic compared with non-epidemic period.

Results: When comparing the weekly morbidity from acute respiratory illnesses and weekly mortality for all causes of death, the peaks of these two parameters almost overlap. In the epidemic period (162 weeks) 47.5% of findings were above the unilateral 95 % tolerance limit of the model, compared with the non-epidemic period (777 weeks) with only 4.6% of finding above this limit. The mean estimated excess of annual deaths from all causes was 2661 (25.99 per 100 000 population), min -918; max 6172. The median of deviations of the estimated number of deaths from the actual number of deaths is negligible, that is, 0.8 (95% CI -0.4; 12.2) for the non-epidemic period, being equal to 188.2 (95% CI 178.6; 283.6) for the epidemic period. Similar results were found for deaths from diseases of the circulatory system accounting for 55.7% of all deaths in the study period. The median of deviations of the estimated number of deaths due to diseases of the circulatory system from the actual number of deaths is 2.4 (95% CI -0.4 to 10.6) for the non-epidemic period, being equal to 126.0 (95% CI 120.4 to 196.4) for the epidemic period, $p<0.001$.

Conclusions: The presented results confirm clearly and unambiguously excess in death rates during the influenza epidemic periods, depending on the duration and magnitude of the epidemic. We estimate that 2.17% of all cause mortality throughout the study period was attributable to influenza in the Czech Republic. Vaccination against influenza proved both effective and cost-effective and therefore is to be recommended as the most important preventive measure.

7.022 Evaluation of Influenza Epidemiological Surveillance System in Brazil, 2000 to 2004

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Background: Influenza is a high magnitude respiratory infectious disease with pandemic potential. Since 2000, Brazil has implemented an epidemiologic surveillance system of influenza (ESS_Flu). In this work we evaluate the ESS_Flu to verify fulfillment of the objectives, to characterize the attributes and to recommend improvements.

Methods: Data and information was collected from the information system (SIVEP_Flu), Guidelines to Epidemiologic Surveillance, among other documents. We used Guidelines to Evaluation of Surveillance Systems in Public Health from CDC-Atlanta to proceeds this evaluation. Qualitative and quantitative attributes had been evaluated. Analysis of sensitivity included different case definitions (fever + cough; fever + throat pain; fever + cough + throat pain) of flu like disease (FL) and positive predictive value (PPV) was calculated using indirect immunofluorescence test (IIF) as standard gold. The evaluated period was of 2000 the 2004.

Results: The ESS_Flu uses the sentinel surveillance strategy. During 2000 to 2004 it was registered 4,637 cases of SG; 266 (6%) had been positive for influenza in IFF and seven strains had been identified in 10 Brazilian states. The system is considered simple, flexible, with good acceptability. However, the representation for age group, data quality (fulfillment of case definition of FL), and opportunity in laboratory results was unsatisfactory. PPV was 9.2% and case definition 'fever + cough' presented the greatest sensitivity (70%).

Conclusion: ESS_Flu fulfills its objective to monitor influenza strains in Brazil. It is necessary to improve the opportunity of laboratory results and case definition. We recommend using indicators of opportunity to monitoring laboratory response and changes in case definition of flu like disease including fever + cough in suspect cases.

7.023 Mapping Analysis on Emerging Infectious Diseases in Guangdong Province, China

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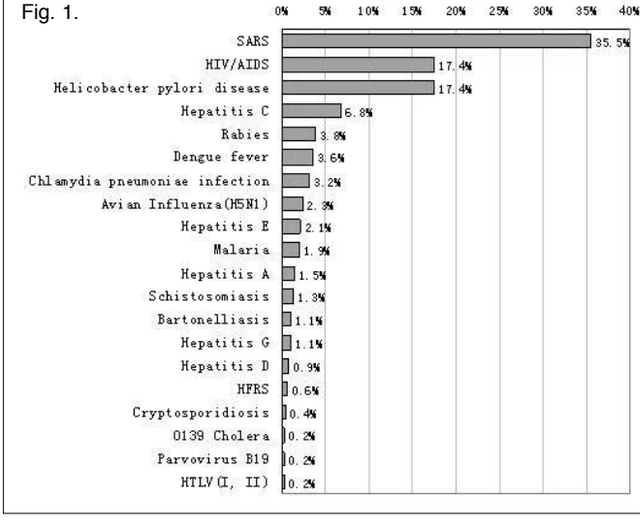
Background: A key step for understanding the research of emerging infectious diseases (EIDs) is to map the current studies on EIDs. But, there were no such studies on mapping the whole studies on EIDs in China. The purpose of the analysis is to understand the research fields in EIDs undertaken in Guangdong Province, China and identify gaps that may exist.

Methods: List of EIDs in China were obtained based on the announcement of the Ministry of Health of China and publications. Numbers of papers on EIDs published in China were collected from the search engine of http://ckrd.cnki.net/grid20/Navigator.aspx?ID=1.

Fig. 1.



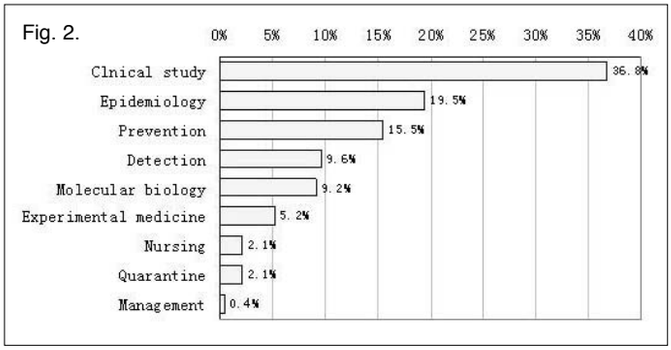
Results: There have been 31 EIDs up to now in China since 1975.

The papers published on EIDs in Guangdong were accounted for 9% of those in China (2,069/ 23,033) from 2002 to September 2006.

A total of 478 papers on EIDs were published in Guangdong in 2005. The top 10 EIDs from the 478 papers were SARS(35.5% of total 478), HIV/AIDS (17.4%), Helicobacter pylori disease (17.4%), Hepatitis C (6.8%), Rabies (3.8%), Dengue fever (3.6%), Chlamydia pneumoniae infection (3.2%), Avian Influenza(H5N1) (2.3%), Hepatitis E (2.1%) and Malaria (1.9%) Fig. 1.

Approximately 1/3 of the papers are concentrated in the clinical study (36.8%), following by prevention (15.1%), detection (9.6%), molecular biology (9.2%), experimental study (5.2%), Nursing (2.1%), Quarantine (2.1%), and management (0.4%) Fig. 2.

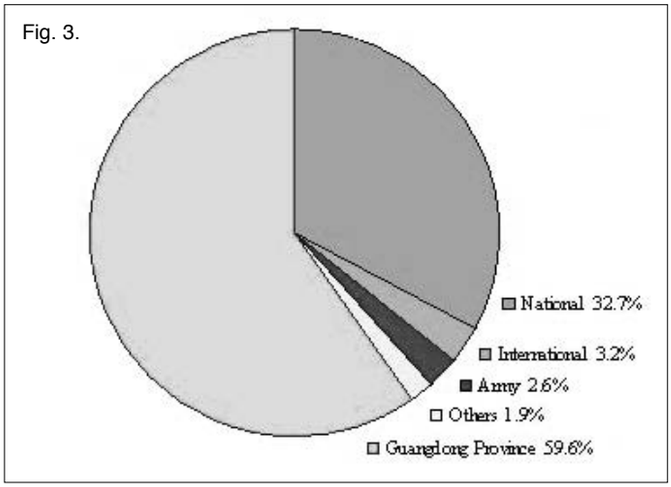
Fig. 2.



The institutions of the authors and co-authors of the papers published in Guangdong includes centers of disease control and prevention at different levels, hospitals, universities, biotechnology institutes, blood centers, entry-exit inspection and quarantine bureaus, gene technology companies, and so on.

A total of 12 EIDs had 156 funds supporting for research in Guangdong in 2005. Regarding the funding resources, 59.6% were from Guangdong Province, 32.7% were national, 3.2% were international, 2.6% from army, and 1.9% from others Fig. 3.

Fig. 3.



Conclusion: There are 31 EIDs up to now in China since 1975. During 2002 to September 2006, the papers published on EIDs in Guangdong were accounted for 9% of that in China. The study fields included clinical study, prevention, detection, molecular biology, experimental study, nursing, quarantine and management. A total of 12 EIDs had 156 funds supporting for research in Guangdong in 2005.

The findings showed the overview of the research of the EIDs in Guangdong, China. It is suggestive that some gaps might be the evaluation of the projects and management research.

7.024 Emergence of Vibrio Infections in Wounds—Change in Surveillance or Climate?

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Background: Infections caused by Vibrio spp. (non-toxinproducing, non-agglutinating) became compulsory to report according to the Swedish Communicable Disease Act from the 1 July 2004. During these 2.5 years after the alteration of the law a total of 8, 25, and 39 cases have respectively been reported.

The summers of 2005 and 2006 were warmer than normal and the temperature in the seawater temperatures around Sweden were consequently also warmer. The growth of the Vibrio bacteria is promoted by a temperature above +20oC and an optimal salt content of 0.4-1.7%, nevertheless it has also been found in fresh waters in Sweden.

Methods: The number of reported cases with a domestic acquired severe wound infection caused by Vibrio spp. was extracted from the Swedish national surveillance database.

Results: An increase in reports of persons with severe wound infections after swimming in seawater has been observed since the number of reported persons in 2004 was one and in 2005 three persons and 2006 eight persons. The majority of persons were elderly with underlying conditions and wounds/scars on the legs prior to bathing/swimming. In all cases, wounds became infected with Vibrio bacteria from water. Different Vibrio spp. were detected, all except one person had an infection with V. cholerae non-O1 non-O139. The other person had an infection with V. vulnificans after an accident with a boat where he was hit by a propeller. The accident happened in the waters west of Sweden which has a higher halt of saline than the Baltic Sea where most of the other cases were infected. One person infected with V. cholerae non-O1 non-O139, took a bath in a wooden hot tub filled with seawater from the Baltic Sea.

Several persons developed septicaemia and during 2006 three persons died.

Other European countries have also in 2006 reported Vibrio infections after swimming in seawater.

Conclusions: Several severe Vibrio infections emanating from wounds have been detected after contact with brackish water, with the outcome in some cases being fatal. The question remains: Is the emergence of severe Vibrio infections due to changes in climate or surveillance?

7.025 Prevalence of Panton-Valentine Leucocidin (PVL)-gene Positive Methicillin-Resistant Staphylococcus aureus in Austria

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Objective: Infections due to Panton- Valentine leucocidin (PVL) gene positive Methicillin resistant Staphylococcus aureus in the community (CA-MRSA) are a recent worldwide phenomenon. Aim of the presented study was to determine the prevalence of PVL-gene positive CA-MRSA in Austria.

Methods: A total of 2433 non duplicated S. aureus strains, isolated in 2005 at the Institute of Hygiene, Medical University Graz, Austria, were investigated. The isolates were characterised by routine laboratory standard methods and determination of the resistance pattern according the CLSI (formerly NCCLS) guidelines, subsequently polymerase chain reaction tests for S. aureus-specific genes, the mecA-gene and the Panton-Valentine leukocidin (PVL) gene were performed.

Results: 83 (3.4%) out of the 2433 S. aureus isolates were MRSA, approved by the resistance to oxacillin, evidence of S. aureus-specific genes and detection of the mecA-gene.

17 (20.5 %) out of the 83 MRSA strains showed a positive reaction in the PCR for the PVL- gene, and were defined as CA-MRSA. 14 patients demonstrated clinical signs of infection, 3 PVL-gene positive MRSA strains were detected on the basis of CA-MRSA screening. All Patients with CA-MRSA had no risk factors for colonization by health-care associated MRSA, such as recent hospitalization and/ or contact with health-care workers. All PVL-gene positive CA-MRSA strains were non-multiresistant, in addition to the resistance to oxacillin these CA-MRSA strains exhibited resistance only to tetracycline, ciprofloxacin and gentamycin with 35.3%, 29.4% and 17.6% respectively. Resistance to fusidic-acid could be detected in 6 CA-MRSA strains (35.3%).

Clinical features of the 14 CA-MRSA patients were superficial to deep soft tissue abscess formation with single through multiple abscesses, after surgical intervention and antibiotic therapy all patients recovered without sequelae. Pneumonia could not be observed in any case.

Conclusions: The results of this study suggest that the occurrence of PVL-positive CA-MRSA in Austria is more frequent than suspected, with 20.5% of the investigated MRSA isolates containing the PVL-gene. The worldwide trend of emergence of CA-MRSA could be confirmed in this study and thus call for closer systematic local surveillance.

7.026 The Impact of New and Emerging Infections on the UK

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The impact of emerging infections identified over the past 20 years in the UK has been significant. This work reviews the estimated economic and indirect impacts of emerging infections in the United Kingdom, focusing on HIV, SARS, vCJD and hepatitis C (HCV) as examples.

The annual cost of treating those currently infected with HIV in the UK is estimated at GBP 400 million, with the cumulative lifetime treatment costs for those known to be infected predicted to exceed GBP 5 billion by 2007.

The burden of disease due to HCV is expected to increase dramatically in the UK over the coming decades, as chronic sequelae develop in those already infected. These sequelae are severe and very costly to treat, and some patients require liver transplants at a cost of up to GBP 52,000 each. This will place a major burden on health services, with the cost of investigation and treatment estimated at GBP 40 million for the estimated 35,000 HCV infected people in Scotland alone. These estimates exclude the substantial compensation costs of up to GBP 25,000 for those infected via blood or blood products, and the many indirect economic impacts of the disease.

The impact of variant CJD in the UK has been enormous, despite relatively few cases to date. The emergence of BSE and subsequent identification of a link to vCJD in humans devastated the UK agricultural sector, with annual costs of hundreds of millions of pounds. Estimation of the cost to the health sector is more complicated and direct estimates are unavailable, however the total cost of treating a vCJD patient has been estimated at GBP 45,000.

The emergence of SARS in 2003 created widespread panic, and the resulting economic impact was huge. No transmission of the SARS virus was detected in the UK, however the burden was substantial in terms of control and prevention activities and impacts on air travel and tourism, and is still ongoing via funding of research.

These examples demonstrate the immense impact that these unpredictable infections can have, and reinforce the importance of thorough planning to minimise the potential burden they impose. This work reviews in more detail the economic burden and indirect impacts of these selected infections on the UK.

7.027 Surveillance of Influenza A/H5N1 in Human: Need for a Sensitive Case Definition

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Background: The Belgian health authorities have defined a standard operating procedure in order to detect any possible cases of influenza A /H5N1 and to take all necessary measures.

Methods: This procedure is detailed in the 'national influenza plan' and includes case definitions as well as the procedure to apply if confronted with a suspected case of influenza A/H5N1. All health professionals were informed.

Results: Between January–June 2006, an alert was given for 25 suspected patients. Among them, 7 (28%) were classified as 'no case', 1 (4%) as 'possible case' and 1 as 'probable case'. For all other cases (64%), the degree of contact with birds was impossible to estimate while patients had nevertheless some clinical symptoms and a travel history in an affected area. In order to include these patients in the surveillance, a new level in the case definition was added; 'case for testing'.

Among the 18 tested patients, 4 (22%) had a positive result for Influenza virus, 3 for Influenza A (2 H1N1, 1 H3N2) and 1 patient positive for Influenza B. The sensitivity of the strict case definition is low with 25% (95% IC 1.3-78) while the specificity is 93% (95% IC 64-99.6). The sensitivity is ameliorated if we remove the needs for contact with birds, going up to 75% (95% IC 22-98.7) while the specificity is decreasing to 71% (95% IC 42-90%).

Conclusion: In a pre-pandemic phase, the main objective of surveillance is to detect all suspected cases. The sensitivity of the case definition needs to be as high as possible. The Belgian experience, working with a case definition where the need for close contact with birds was removed, should be considered in order to increase the sensitivity.

7.028 Clinical and Epidemiologyc Presentation of Acute Chagas Disease Cases in Outbreaks Related to Oral Transmission, Brazil—2005/2006—Preliminary Data

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Background: American Trypanosomiasis or Acute Chagas Diease (ACD), caused by Trypanosoma cruzi infection, is responsible for at least 3,000 million chronic cases in Brazil (2006). Nowadays, ingestion of contaminated drink or food is responsible for occurrence of several outbreaks of ACD.

Methods: We report clinical, laboratorial and entomological characteristics observed in 5 outbreaks in 2005–2006.

Results: Outbreaks occurred in Navegantes municipality/Santa Catarina state (n=24), Redenção/Ceará (n=8), Macaúbas/Bahia (n=7), Santarém/Pará (n=20) and Ibipitanga/Bahia (n=6), with 65 confirmed cases of ACD, including 6 deaths (lethality=9,2%). Diagnosis was confirmed by direct parasitological blood exam in 27 cases (41%). IgM or IgG blood antibodies test (n=25 and n=8 respectively), PCR (n=1) or epidemiologic method (n=4) were confirmatory only in presence of suggestive clinical and epidemiological features. Incubation period was 3-21 days; time interval between symptoms begin and diagnosis was 27-48 days (media=34 d). Clinical manifestations included fever (80%), myalgia (78%), headache (71%), face/lower limbs edema (70%), transaminases elevation (41%), exantema (37%). Digestive manifestations occurred, as epigastralgia (55%), vomiting (52%), diarrhea and hepatomegalia (25%), as well atypical ones as hyperbilirubinemia (18%) and digestive bleeding (26%). Cardiologic presentation: ventricular repolarization alterations (34%), pericardic effusion (29%), cardiomegaly (26%), pleural effusion (23%). Benzonidazole is the drug of choice for treatment in Brazil. The infection was associated to ingestion of sugar cane juice (3 outbreaks) and bacaba (palm tree fruit, in one); there are concerns about the contaminating vehicle in 2 outbreaks. Entomological investigation found Triatoma. tibiamaculata, T. sordida, Panstrongylus lutzi, Rhodnius robustos, R. pictipes and P. lignarius vectors, not necessarily colonized, with T. cruzi infectivity rate higher than 30%. The most important reservoirs seen by triatomine stool analysis were marsupials, rodents and avians.

Conclusions: Oral transmission of T. cruzi is responsible for many outbreaks of ACD. The lack of diagnosis opportunity may be responsible for the high death rate. We saw high taxes of digestive manifestations, with atypical characteristics. Only 41% of cases were confirmed by the recommended diagnostic method (parasitologic blood exam) in these outbreaks. Prevention and control measures for handling foods or drinks are been implemented by the MoH and states Surveillance to avoid contamination by T. cruzi during it preparation.

7.029 Cases of Chikungunya Virus Infections Imported in Italy: Use of a Standardized Panel of Bio-Molecular Tests

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Background: A large epidemic of a mosquito-borne viral disease, Chikungunya, has begun in 2005 in Indian Ocean. In few months several countries have experienced a dramatic increase of cases. Currently the outbreak is still ongoing in many countries in Asia and Africa, mainly in the Indian Subcontinent. As expected, in European countries several cases in returning travelers appeared, for the most part in France. The recent attention raised on imported cases of Chikungunya Virus (CV) infection alerted the infectious disease physicians also in Italy.

Methods: Following to the WHO alert, at National Institute for Infectious Diseases L. Spallanzani, a case definition of suspected CV infection, based on epidemiological and clinical data, was developed. Bio-molecular tests were implemented, and made available to other institutions. Several laboratory methods have been used to diagnose the infection, according with the different phases of the diseases. Virus detection has been obtained by molecular biology tests and viral culture. Ab detection has been preformed by IgG and IgM IF assays and in some cases confirmed by microneutralization tests. The RT-PCR for CV has been added to a pre-existing panel of exams for febrile patients coming from affected areas, that includes PCR for malaria and RT-PCR for Flaviviruses, including Dengue.

Results: Using this approach, CV infection has been confirmed by PCR and seroconversion in five patients returning from Mauritius; in two cases CV has been isolated. Other four cases have been considered as suspected having serological evidence that exposure to CV has occurred (high-titer positive IgG), and supporting epidemiological/clinical data. Among these 9 patients fever and arthralgias were present in 100%, while rash, headache and linphoadenomegaly were present in, respectively, 71%, 43% and 29% of patients.

Conclusion: The CV cases among European tourists illustrate the need to consider CV in the differential diagnosis of fever and joint pain in people returning from the affected areas. The role of biomolecular tests in facilitating a rapid diagnosis should be considered: the application of a standardized diagnostic approach, updated on the basis of ongoing epidemiological data may be very useful for an early recognition of imported diseases. However, the increasing attention to CV fever should not reduce physicians's awareness on the need for a comprehensive approach to the diagnosis of other infections, such as dengue and malaria, in travellers returning from areas where their transmission occurs.

7.030 Comparison of Human Metapneumovirus Strains from Alto Adige with Strains from Surrounding Regions in Italy and Austria

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Background: Human Metapneumovirus (HMPV), in addition to paediatric patients, has been increasingly described in elderly people and has been found to cause more severe infections in immunosuppressed patients.

Methods: In our laboratory in Bolzano serving a regional hospital routine HMPV diagnosis is based on direct immunofluorescence assay (DFA) using monoclonal antibodies. Species diagnosis is confirmed by reverse transcriptase-PCR. During 2005 we performed a preliminary epidemiological survey to compare the local strains to those of surrounding regions by doing also sequencing analysis of the N-Genes as well as the L-genes of the obtained isolates.

Results/Discussion: By alignment with established Dutch reference strains we identified three different genotypes circulating in our region with predominantly type B strains being detected in this season. This is in contrast to published data from nearby Italian regions from the 2003/2004 winter season where predominantly A2 strains have been characterised. Also our study in the Austrian Tyrol region from the same season detected A-type strains only. Whether this is a further indication for similar strains cycling in a wider geographic area with the respective types replacing each other on a seasonal basis remains to be clarified although absolute sequence identity due to the high variability of RNA viruses was rarely detected. Similar HMPV types circulated in both the paediatric and adult patients and, of interest, the two isolates originating from bone marrow transplant recipients (BMT) were of different genotypes. Both findings rather exclude hospital transmission in our BMT patients as has been suspected in lung transplant patients (Larcher et.al., J.Heart & Lung Transplant., 2005).

7.031 Cyclic Occurrence of Epidemics of Shigella sonnei Shigellosis in Israel

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Background: Shigellosis is highly endemic in Israel. S. sonnei has been responsible for approximately 90% of the cases of shigellosis and has been involved in several country-wide epidemics of the disease in the last decade.

Methods: We analyzed data collected by a sentinel laboratory-based surveillance network established in Israel in the mid-nineties. For computation of incidence rates, estimates of the catchment population of the different sentinel laboratories were used. Pulsed- field gel electrophoresis (PFGE) was employed for the genotypic characterization of S. sonnei isolates.

Results: A cyclic occurrence (every 2 to 3 years) of large and propagated epidemics of S. sonnei shigellosis has been observed in the last decade with more than 50% of the cases occurring among children aged 1 to 4. The incidence rates of culture-proven S. sonnei shigellosis in this age group ranged between 2.5 to 12 cases per 1,000 in epidemic and non-epidemic years, respectively. Communities living under conditions of crowding with a high number of children below the age of 5 were the epicenter of these epidemics. The investigation of consecutive outbreaks affecting the same communities revealed that shigellosis due to S. sonnei conferred around 60% protection against the recurrent homologous disease for a period of at least 2 years. Characterization by PFGE of a large number of S. sonnei strains isolated during these outbreaks indicated a high level of clonality with the minor differences within the PFGE profiles probably due to the frequent human passages of the epidemic strain.

Conclusions: We assume that the outbreaks of shigellosis occurring in children of the age group 1–4 years are followed by an increase in the level of natural immunity to the homologous Shigella organism (S. sonnei) which will also provides the level of herd immunity sufficient to prevent the onset of a new epidemic. After one or two years, the decay in the level of LPS antibodies together with the intake of a new cohort of naïve newborns leads to the decrease in the level of herd immunity below a critical level. This allows, under high and continuous risk of exposure, the renewal of the epidemic transmission of S. sonnei.

7.032 Concurrent Winter Excess Mortality During Christmas Holiday Periods in Four Nordic Countries

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Background: We described the temporal correlation of periods of excess mortality in Demark, Finland, Norway and Sweden to explore if presence or absence of concurrent excess mortality peaks facilitates detection of underlying causes and if using common methodology has added value for surveillance of mortality in Nordic countries.

Methods: We obtained weekly numbers of all-cause deaths between 1994 and 2004 and fitted a Poisson regression model, including terms for trend and seasonality, to total and age stratified series. To exclude epidemic periods the model was re-run after excluding 25% of the highest residuals. Weeks with excess mortality were weeks where the observed number of deaths was >1.64 standard deviations of residuals. We compared excess mortality estimates of the four countries.

Results: We observed concurrent excess mortality around the Christmas holiday period (week 52, week 1) in 9 of 10 winter seasons. In 3 of these 9 seasons, peak influenza activity occurred simultaneously and mortality peaks were large. In the remaining 6 seasons, peak influenza activity occurred after the Christmas period. Here, mortality curves were typically M-shaped, with a first peak around Christmas and a second peak at the time of peak influenza activity. Excess mortality in the Christmas period was confined to the age groups 45–64 years and 65+ years.

Conclusion: By comparing excess mortality across four Nordic countries using common methodology, we identified excess mortality peaks around the turn of the year. The peaks occurred regularly and independently of influenza activity. This has not been described previously. The cause for these peaks has yet to be determined but our finding that the peaks occur annually at the same time and simultaneously in all four countries speaks against infectious or environmental causes. The results demonstrate the added value of using common methodology for surveillance of mortality in Nordic countries.

7.033 21st Century Zoomancy? An Integrated Approach to Detecting Future Threats

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Background: Most emerging infections which affect humans are zoonotic in origin. Those organisms which are able to infect both domestic and wildlife animal species are more likely to emerge as zoonoses. Emerging diseases have the potential for impact that may extend beyond local or national boundaries, exerting profound effects on human and animal health, trade, travel, and the economy. An effective capability for recognising the emergence of a zoonosis or a new disease requires new partnerships and ways of working to ensure detection, diagnosis, and surveillance. Such new systems must involve systematic, transparent and timely processes of identification and assessment of potential new threats.

Method: The joint Human Animal Infections and Risk Surveillance (HAIRS) group is a multi-agency and cross-disciplinary group comprising a number of agencies with responsibility for human and veterinary public health, epidemiology, and food safety in the United Kingdom. Its remit includes hazard identification, and risk assessment, management and communication, and the group acts as a forum to identify and discuss infections with potential for interspecies transfer (particularly zoonotic infections). Horizon scanning activities are based on the daily logging and review of reports relating to national or international infectious dis-

ease events. Incidents discussed by HAIRS are detected through such horizon-scanning, or via surveillance activities, or are directly referred to the group. Since its formation in 2004 HAIRS has considered a range of infections, and has been able to advise policy makers and others working in human and animal health.

Conclusion: A new group was established to identify, monitor and assess the risk to the population from emerging infections. Because the HAIRS group meets regularly each month, issues of potential concern that are identified by either the human or animal health side are assessed rapidly, allowing appropriate action to be taken in a timely manner. The multi-agency format has proved to be robust in its integrated horizon scanning and risk assessment approach.

Presented on behalf of the HAIRS group.

7.034 WITHDRAWN Implementation of Brazilian Centre of Strategic Information in Health Surveillance (CIEVS) Improving National Relevance Events Detection Capacity

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Background: The expansion of circulation of H5N1 virus, treat of pandemic SARS and Anthrax use in terrorism are examples of the need for improving surveillance at national and international levels. Implemented in March 2006, CIEVS has been a model for strategic response to emergencies, defined in the International Health Regulations, to be implanted in all countries, mainly developing ones, such as Brazil. The purpose of this study was to analyze CIEVS performance since its creation.

Methods: We described CIEVS structure, models and emergency responses during March-November 2006.

Results: CIEVS follows international models, like Strategic Health Operations Centres from Canada and WHO-Geneva. It was created to improve reporting system, monitor epidemics, support field investigation teams and promptly respond to epidemiologic emergencies. It is accessible 24 hours a day, all over the country, by e-mail or free call service. Brazil is developing a wide net of collaborators including states surveillance services, Department of National Defense, laboratories, assistance/epidemiology expertise, to be ready to emergency events. To present date CIEVS monitored 152 events, such as Human Rabies, Acute Chagas Disease, Yellow Fever, epizooties and/or death of animals that could precede the occurrence of diseases in humans, Hantavirus, Human Influenza, among others, and investigated 25 events in loco. Median of interval between beginning of event and reporting was 9.5 (0-80/n=62) days and between reporting and end of investigation was 11 (0-214/n=89) days. A weekly summary report is distributed to states including information of person, time and place, type of event, hypothesis, and other.

Conclusions: CIEVS was considered an important strategy to strengthen Brazilian Surveillance System, improving reporting, information systems and timeliness of actions. Number of reporting increased since CIEVS implantation. It was adopted field technology to help analysis and data interpretation. Finally, we suggested expand this service to states that composed Brazil territory standardizing surveillance.

7.035 Assessing the Risk from Emerging Infections

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Introduction: Since the 1970s over 30 previously unknown infectious diseases have emerged for which there are no fully effective treatments. Most of these are zoonoses. A range of activities have been developed to try and identify new threats as they emerge in order to facilitate a timely response including control. The purpose of this work is to develop a rapid, systematic, objective, transparent method for assessing the risk to the UK population from new and emerging infections arising throughout the world.

Methods: Previous works on risk assessment relevant to public health were reviewed to define best practice and consistency of approach. Two major projects were identified: HPZone—a Department of Health (England) funded project to develop a risk management model and another by Defra as part of the prioritisation goal of their Veterinary Surveillance Strategy. The risk assessment tool was then developed, which utilises algorithms comprising a series of decision questions.

Results: Three parameters were used: **Probability of infection:** the likelihood of an infectious threat causing infection in the UK human population; **Impact on human health:** the scale of harm caused by the infectious threat in terms of morbidity and mortality, and; **Context:** difficult to assess, but may significantly impact upon perception of the level of risk and the requirement for a response.

Separate risk assessment algorithms were devised for each because combination into one parameter would have resulted in loss of crucial information. Each algorithm uses all readily available scientific information, and rapidly identifies current gaps in knowledge. For a new agent, the gaps may be significant and are clearly documented in the assessment. This risk assessment tool is qualitative rather than quantitative and potential infectious threats are assigned a level of risk (minimal, low, moderate, or high) for probability, impact and context. Having devised the risk assessment tool, it has been applied to a range of emerging infections by a multidisciplinary, animal and human infection group and proved invaluable in documenting and communicating risk.

Conclusions: The rapid risk assessment tool has been developed for potential emerging infectious threats. It provides a rapid, standardised way of estimating risk and identifies not only current gaps in knowledge but the need for an expert formal risk assessment. It can be used to promote risk-informed decision-making, and ensure that all decisions, including complex ones, become easier to explain and justify at each stage of the decision process.

7.036 Building on Routine Surveillance Infrastructure for Pandemic and Emerging Respiratory Infections Response

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Background: Lessons learned from SARS and avian influenza (H5N1) involving humans have underscored the importance of strengthening surveillance infrastructure for early detection and ongoing monitoring of emerging respiratory infections. Developed following WHO guidelines during the post-SARS outbreak period, Canada's emerging/re-emerging respiratory infections surveillance strategy has evolved in order to reflect the current situation, namely developing capacity for the changing demands and information needs of other emerging severe respiratory illness (SRI) outbreaks, such as pandemic influenza in particular.

Methods: National working group consultations over a 3-year period considered the following in the development of a severe respiratory illness and pandemic influenza surveillance plan: formal evaluation of the national routine influenza surveillance system (FluWatch), survey of provincial SRI systems, expert reviews of surveillance infrastructure and gap analyses, minimum national standards for reporting, development and testing of electronic reporting platforms, and scenario-based testing of surveillance plans.

Results: A 3-pronged strategy was developed linking together routine influenza surveillance, enhanced recommendations for front line vigilance targeted to current pandemic alert levels, and hospital-based surveillance for severe respiratory illnesses not yet diagnosed (SRI-NYD). Surveillance guidelines and data management tools were developed to support the strategy including: pandemic influenza surveillance plan; respiratory illness outbreak investigation protocol for data sharing; questionnaire/database for investigation of severe emerging respiratory infections.

Conclusion: Early detection of an emerging respiratory disease will depend on alert and informed primary care providers working closely with public health and laboratory professionals. The strategy, which is adaptable to the evolving risk circumstances of the pandemic alert period, aims to promote an environment of awareness for increased vig-

ilance and appropriate early response. The development of the strategy has provided an opportunity to build on current infrastructure, establish and reinforce communication links between primary care providers and public health and laboratory professionals at local/provincial/territorial and federal levels. Evaluation of the system will be needed to ensure plans are implemented, accepted and tested and performance barriers are identified.

7.037 Tularemia in Germany: The Tip of the Iceberg?

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Background: Tularemia is a very rare, notifiable zoonosis in Germany. In the last four decades only 0 to 5 human cases were notified annually. Since November 2004, several lines of evidence including outbreaks in human or animals and confirmed infections in indigenous hare and rodent populations have indicated a re-emergence of tularemia in different German states. Unfortunately, reliable basic information on the seroprevalence in different geographical regions permitting the identification of risk factors does not exist.

Methods: Combining a sensitive screening assay (ELISA) with a highly specific confirmative immunoblot test, we performed a serological investigation on 2436 sera from a population-based, cross-sectional health survey of the city population of Leutkirch, Baden-Wuerttemberg. Epidemiological data were obtained by a standardized questionnaire.

Results: Surprisingly, a total of 56 sera out of 2436 samples gave positive results indicating a seroprevalence of 2.27%. These data were significantly different from the 0.226% positive samples (15 out of 6632 sera) reported in a nation-wide study performed in 1997. Stratification of the data indicated putative risk factors like hunting or working as a farmer. Gender, outdoor activities, exposure to ticks or pets seemed not to be associated with a higher risk for a tularemia infection.

Conclusion: F. tularensis can cause a wide variety of clinical syndromes ranging from asymptomatic infection to severe, sometimes fatal disease. Missing epidemiological data on its spatial and temporal distribution in an endemic country complicate an appropriate risk assessment necessary for public health authorities to be prepared for an adequate outbreak management. This is of special concern regarding the extraordinary potential of F. tularensis as an agent of biological warfare or bioterrorism. Our investigation performed in a presumed low-risk area demonstrated that tularemia might be seriously underestimated in Germany and probably in other central European countries.

7.038 Enhancing Epidemic Surveillance and Response Systems in Developing Countries: Evaluation Framework and Pilot Application

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Background: Electronic surveillance systems (ESS) could improve early disease identification and containment for pandemic influenza and other emerging infections. Current guidelines for ESS evaluation were designed for US-based systems, but pandemic diseases could first emerge in less developed countries, where limited resources hamper surveillance. This project examines the use and evaluation of ESS in resource-poor areas, focusing initially on pandemic influenza and the Early Warning Outbreak Recognition System (EWORS), a hospital-based ESS in 4 Southeast Asian countries and Peru.

Methods: First, in order to better address surveillance challenges unique to resource-poor areas and identify technologically appropriate and sustainable system improvements, existing ESS evaluation frameworks were modified. On-site evaluations in countries with EWORS are used to examine system utility in identifying disease outbreaks, refine the evaluation framework, identify parameters critical for epidemic containment, and identify enhancements. Information is gathered through questionnaires provided to system operators; on-site system observation; surveillance data analysis; and interviews with system personnel, and country and on-site international public health officials. A generic model of pandemic influenza emergence, detection, and response in a resource-poor setting also is being developed for quantitative ESS evaluation.

Results: A site visit to Lao PDR, the first EWORS network evaluated, was completed in September 2006. EWORS was initiated in 2003 and operates at 7 sites throughout the country, with the hub in the Ministry of Health. It has captured >230,000 suspected infectious presentations, identified outbreaks, and guided decision-making; and is integrated into the national pandemic influenza plan. Reporting timeliness improved markedly since implementation. Methods to improve communication and data collection, transmission, and analysis are needed and under evaluation. Certain important cultural and operational factors affecting EWORS utility might not have been captured by existing ESS frameworks.

Conclusions: This pilot evaluation identified needed improvements to EWORS and modifications to ESS evaluation frameworks. The longer-term goal of this project is to iteratively apply and improve an ESS evaluation framework to varied resource-poor settings, and to define the potential role of ESS in epidemic detection and response in those environments.

7.039 The Influenza Surveillance in Brazil, 2000–June 2006

R. Malaguti, **L. Daufenbach**, L. Campos, F. Barros. Ministry of Health, Brasilia, Brazil

Background: The Ministry of Health began in 2000 the influenza surveillance system nationwide which aims to monitor virus strains circulation concomitant with morbimortality indirect data analyzes, assist annual vaccine composition update, and to detect outbreaks early in order to initiate infection control measures promptly. This system involves 59 sentinel medical units in 26 of 27 states of Brazil and 27 public laboratories.

Methods: One of the adopted data source strategies for the surveillance system was the sentinel medical units which collects weekly aggregate epidemiological data and near five clinical samples of suspected flu cases from its care demand, which were tested by immunofluorescence staining, and further by polymerase chain reaction (PCR) for antigenic characterization.

Results: From 2000 to June 2006, the influenza like illness (ILI) proportion reported by sentinel units varied from 5% to 25%; 9,239 samples were collected and 1,865 (20.1%) were respiratory virus positive, which 39.5% (738) for Flu (598 Flu A; 140 Flu B), 29.5% Respiratory Sincicial Virus, 15.0% Adenovirus and 16.5% Parainfluenza. In the period of January to June 2006, the main strains detected by the sentinel units were A/NC/20/99 H1N1; A/Wyoming/03/2003H3N2; B/Shangai/361/02 and B/Jiangsu/10/2003. Were detected, notified and investigated in the period of 2002-June 2006, 22 influenza like illness (ILI) outbreaks in different institutional settings with distinct attack rates ranging from 3.5% to 25%. Strains identified in outbreaks and/or in the sentinels surveillance routine may integrate the annual vaccine composition employed in the south hemisphere.

Conclusion: The viral antigenic characterization has been showed matching with the annual vaccine´s composition strains. The surveillance system has been useful to identify the viral circulation strains and how it reflects on the disease epidemiology, besides timely outbreaks detection and investigation; these activities are substantially collaborating for controls and prevention measures establishment in Brazil.

7.040 The Study of Dengue Outbreak Investigation in Sumsung District, Khon Kaen Province, Thailand 2005

A. Pensiri[1], P. Chaiyaseth[1], W. Lertpongpipat[2], P. Pripul[3], P. Bunyong[1], R. Dumrongmongkolkul[1], **K. Suwannarong[1]**, Khon Kaen Dengue Outbreak Investigation Team[1]. [1]Khon Kaen Provincial Health Office, Amphur Muang, Thailand; [2]Sumsung Hospital, Amphur Sumsung, Thailand; [3]Sumsung District Health Office, Amphur Sumsung, Thailand

This study describes the dengue outbreak investigation at Moo 3, Tambon Kammad in Sumsung district, Khon Kaen Province, Thailand in 2005. Four patients were reported with DSS (dengue shock syndrome) to Epidemiology section of Khon Kaen Provincial Health Office during May-June 2005. The Khon Kaen Surveillance Rapid Response Team (KK-SRRT) investigated the cases and hypothesized this area as dengue infected area since April 2005. Patients' information with flu like symptoms that went to Kammad Village Health Office, Sungsung Hospital and other health offices for treatment during 1 April 2005 to 29 June 2005 were extracted. Individual blood samples were also drawn and transferred to the Khon Kaen Associate Medical Sciences Center for ELISA tests for dengue. Forty two of the 70 cases (60.0%) with histories of flu-like symptoms have signed informed consent form and willingly participated in this study. We found that 18 cases (42.8%) showed positive laboratory result on recent dengue infection which included 8 males and 10 females. Most of them (12 cases, 66.7%) went to Kammad Village Health Office for treatment. About 38.9 % (7 cases) were diagnosed as common cold, 22.2% (4 cases) as tonsillitis and 27.8% (5 cases) others with other symptoms. Only 2 cases (11.1%) were identified as dengue infection. After considering of the household data, we determined that the disease onset among 2 suspected index cases started on 21 April 2005. These cases were also found to reside in geographically distant areas. From these results, we can conclude that this area had dengue infection since April 2005 but had not been reported. Such time lag may well have caused the recent outbreak to occur. These show significant evidence-based results for our local and national health administrators to promote knowledge and skill for local health officers for the diagnosis of dengue and other notifiable diseases through training and improvement of standard guidelines on prevention and control as well as focus on frequent task monitoring.

7.041 Rapid Identification and Characterization of Multiple Drug Resistance and Virulence Factors in Staphylococcus aureus Using a Novel Pathogen Detection System

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Background: Main strains of Staphylococcus aureus (S. aureus), a major cause of nosocomial and community infections, contain toxins and virulence elements that are likely responsible for the severity of illness. Early recognition and treatment is essential. We have developed a rapid, high throughput screen for the simultaneous detection of S. aureus and important virulence and resistance factors. The IBIS T5000 Biosensor System is a novel pathogen detection system, which uses broad-range PCR followed by time-of-flight (TOF) mass spectrometry. Accurate molecular mass is determined from all PCR products and base composition data derived using propriety signal processing software. The purpose of this study was to evaluate the ability of the IBIS T5000 Biosensor System to determine resistance and virulence markers among a set of well characterized S. aureus recovered from clinical samples.

Methods: We developed a high-throughput assay that can characterize all S. aureus isolates simultaneously, using multiple broad-range PCR primers, that target conserved sites in housekeeping genes and the S. aureus-specific resistance and virulence genes. The assay employs a tufB gene marker fro broad detection and differentiation of all Staphylococci, and an additional specific marker on the nuc gene. Resistance to methicillin, erythromycin and mupirocin are determined by using markers on the mecA, mecl-R, ermA, ermC and mupR genes. Virulence factors determined by the PVL gene elements are also evaluated. Using this system we examined over 50 clinical isolates of S. aureus as well as a number of other Staphylococcal sp. The total collected data set was compared to an identical set of data gathered by traditional assays.

Results: For the characterized strains tested, there was a high correlation between conventional testing and the results obtained by the IBIS T5000 Biosensor System.

Conclusion: The automated system described here is ideally suited for rapid monitoring and surveillance for highly virulent S. aureus outbreaks. Results from this prototypic evaluation will be used to characterize clinical samples.

SESSION 8 (Poster Session)
Emerging Zoonoses

Saturday, February 24, 2007
Room: Klimt Ballroom 1
11:15–12:30

8.001 Epidemiology of Hantavirus Cases Among US Personnel Training Near the DMZ, Republic of Korea

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Background: Korean Hemorrhagic Fever (KHF) is a rodent-borne hantavirus throughout northeastern Asia. The primary reservoir for KHF is the stripped field mouse, Apodemus agrarius. During October-November 2005, there were four cases of KHF reported among US Soldiers.

Methods: During a five-year rodent surveillance program, hantaviruses were identified and a portion of the genome sequenced to determine focal variability. During the fall, 2005, four cases of hantaviruses in US Soldiers were reported. Blood from each of the patients was assayed for virus and the same genome sequenced. Epidemiology investigations identified the locations where the Soldiers had previously trained.

Results: Previous nucleotide sequencing demonstrated distinct differences in the nucleotide sequence of the hantaviruses at different training sites. Comparisons of the nucleotide sequence of the patients were matched with rodent hantavirus groups at areas where the Soldiers had previously trained >20 days prior to the onset of symptoms. Habitat characteristics identified tall grassy margins along the perimeter and within the center of the training sites, allowing for migration of rodents from one grassy area, as primary risk factors associated with dust (firing weapons and vehicular traffic).

Conclusion: The identification of the location of transmission of hantaviruses is important to mitigate disease transmission, e.g., habitat modification, dust reduction policies, and modifications of Soldier activities. Furthermore, this identifies the length of the incubation period.

8.002 Identification of Brucella spp and Brucella melitensis Vaccine Strain Rev.1 in Cattle in Iran by Analysis of the PstI Site Polymorphism of Its omp2Gene

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Introduction: Brucellosis is a worldwide zoonosis causing reproductive failures in livestock and a severe multi-organ disease in humans. The long-term serological studies have indicated that 5% of sheep and 0.8% of cattle was infected with brucellosis. Brucella melitensis biotype1 in sheep, goats' cattle and man are the predominant infective biotype. It was shown that although the vaccine prevented abortion, it did not provide protection against infection. There are some evidence, the real possibility that attenuated Brucella vaccine Rev.1 strains used in animal's vaccination can represent a source of human and animals' brucellosis.

Methods: 50 cattle aborted fetus samples from different farms and locations were collected after Rb51 and S19 vaccination in these farms. All samples were examined. Brucella strains were isolated from 50 (100%) of the samples and all of the strains were identified as Brucella melitensis by biochemical characteristics, agglutination with nonspecific A and M sera…Genomic DNA Rev1, Rb51 and S19 vaccines and DNA samples were extracted. All DNA isolates were amplified using 2 pairs of specific primers Omp2a R, F and Omp2b R, F using a PCR machine. These PCR products were then restricted using Pst1 enzyme.

Results: According to the working hypothesis, DNA fragments obtained from Brucella vaccine Rev.1 strainsand a brucella fetus sample should produce three fragments,(pattern 3) an intact 200-bp fragment from the amplified Omp2a gene and two largest fragments 238 and 282 bp,. In contrast, B. abortus biotype 1, 2, 4 and (Rb51.S19) vaccines should produce two. fragments 550 and 200 bp and some brucella fetus samples DNA should produce only the two fragments a 200-bp fragment and a 282-bp fragment, (pattern 2) belongs to Brucella melitensis biotype 1, 2, 3or Brucella abortus biotype 5, 7, 9.

Conclusion: Therefore the cattle in our areas were infected with Brucella melitensis biotype 1 could have originated from Brucella melitensis Vaccine Strain Rev.1 (as determined by PCR-RFLP of the omp2a gene digested with Pst1 pattern 3), This study have highlighted some of the potential hazards associated with use of the Rev.1 vaccine in national control program.

8.003 The Effect of Lifestyle on Brucellosis Among Nomads in Khuzestan Province, Iran, 2004

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Objective: To investigate the effect of lifestyle and related risk factors on brucellosis among nomads in Khuzestan, Iran.

Study: Descriptive, cross-sectional study.

Place and Duration of Study: North area of Khuzestan in the south of Iran from March 2004 to June 2004

Patients and Methods: 3,594 person took part in this study by randomized cluster sampling. The diagnosis of brucellosis was made by measuring Brucella antibodies (Wright and 2ME). Wright and 2ME with titers equal 1/80 or more were concerned positive. Risk factors, such as exposure to animals, ingestion of high risk foods (unpasteurized dairy products) were derived from questionnaires which were filled for every person. The results were analyzed by descriptive-analytic statistical methods with SPSS soft ware.

Results: 228 of 3,594 were positive for Wright and 2ME (prevalence=6.3%). Consumption of raw milk in 109 (%), fresh cheese in 228 (100%), uncooked meat in 190 (%), closed skin contact with animal in 82 (%), contact with gestational products of animals in 26 (%) and living with animal in one place in 46 (%)

Conclusion: This study showed that the frequency of brucellosis among nomads in Iran because their life style (close contact with goats, consumption of unpasteurized dairy products) are high.

8.004 Seroprevalence of Brucellosis Among Nomads in Khuzestan Province, Iran, 2004

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Background: Brucellosis is a disease of domestic and wild animals that is transmittable to human (zoonosis). The disease exists worldwide, especially in the Mediterranean basin, and countries such as Iran. The disease is endemic in Khuzestan.

Virtually all infection derives directly or indirectly from animal exposure. Nomads who are living in the north of Khuzestan because their life style and close contact to domestic animals such goats are highly infected. This study conducted to determine the prevalence of infection among nomads of Khuzestan a province in the north of Iran.

Methods and Materials: This is a descriptive, cross-sectional study, which was done in Khoozistan, Iran, during spring 2004. 3,594 persons, 49.9% male and 50.1% female took part in this study by randomized cluster sampling. The range of age was 2–65 years with mean age of 35 years. The diagnosis of infection was made with serologic tests (to measure Brucella antibodies); RoseBengal, the serum agglutination test (SAT) named Wright and 2ME (to differentiate immunoglobulin classes). Those with positive Wright tests were retested with 2ME. Wright and 2ME with titers equal 1/80 or more were concerned positive. Epidemiological data, such as occupation, exposure to animals, travel and ingestion of high risk foods (unpasteurized dairy products) were derived from questionnaires which were filled for every person. The results were analyzed by descriptive-analytic statistical methods with SPSS soft ware.

Results: 287 of 3,594 were positive for Rose Bengal test (prevalence =7.98%). 283 of 3,594 were positive for Wright test (prevalence =7.87%). 228 of 3,594 were positive for 2MEtest (prevalence=6.3%). 283 of 287(98.2%) those with positive results for Rose Bengal were positive for Wright test. 228 of 283 (80.5%) those with positive Wright test were also positive for 2MEtest.

Conclusion: Seroprevalence of Brucella in Iran is about 3% (according previous studies). This study showed that the prevalence of Brucella infection among nomads in Iran is 7.9%, so this population are highly infected, because their life style (close contact with goats, consumption of unpasteurized dairy products).

We recommend that all nomads with low back pain, sweating with or without fever must be treated for brucellosis and preventive measures in domestic animals such as use of Rev-1 vaccine and serologic testing and elimination of reactor animals.

8.005 BIOCHIP Mosaics with 6 Hantavirus Serotypes for a Reliable Serological Detection of Hantavirus Infections

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Background: Hantaviruses are the causative agents of hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS), two severe, and often fatal human diseases. Mortality caused by HFRS varies between 1–10% depending on the Hantavirus serotype. The mortality rate of HPS is approximately 40%. Chronically infected rodents excrete viruses with their droppings. Humans can become infected by inhaling contaminated rodent excrement particles. Distribution of Hantaviruses is nearly world wide. A high number of Hantavirus infections are not clinically identified as such and often misinterpreted as flu-like infections, renal failures or acute respiratory distress

syndrome of unknown genesis. Because viremia lasts only for a few days and specific antibodies often appear 2 days after onset of the first symptoms, the detection of anti-Hantavirus antibodies is of high importance even at an acute stage.

Methods: We developed a BIOCHIP mosaic for the indirect immunofluorescence with 6 BIOCHIPs on each reaction field: Hantaan virus (HTNV), Puumala virus (PUUV), Seoul virus (SEOV), Saaremaa virus (SAAV), Dobrava virus (DOBV) and Sin Nombre virus (SNV) for simultaneous detection of specific antibodies against these 6 Hantavirus serotypes. For the evaluation of the new BIOCHIP mosaic we examined 5 sera panels (n=165) using Titerplane® technique to detect antibodies of class IgG and IgM. The samples originate from: (I) 52 patients with PUUV infections from Finland, (II) 5 Asian SEOV patients, (III) 19 SNV patients from Canada, (IV) 25 healthy Canadian blood donors and (V) 64 healthy blood donors from northern Germany.

Results: Antibodies against one or more Hantavirus type were detected in 97% (IgG, panels I-III), in 90% (IgM). Specific IgG was found in 2% of the samples from blood donors (panels IV and V), IgM in 4%.

Antibody prevalences (IgG / IgM) of the serotypes HTNV, PUUV, SEOV, SAAV, DOBV and SNV in sera from patients with (I) PUUV-, (II) SEOV- and (III) SNV infections

IgG / IgM	Antibody prevalence [%]					
	HTNV	PUUV	SEOV	SAAV	DOBV	SNV
(I) PUUV, n=52 / 54	79 / 46	100 / 96	53 / 25	57 / 6	57 / 11	86 / 76
(II) SEOV, n=5 / 5	100 / 100	100 / 60	100 / 80	100 / 80	100 / 60	100 / 40
(III) SNV, n=19 / 17	5 / 6	55 / 47	0 / 0	0 / 0	0 / 0	80 / 53

Conclusion: The new Hantavirus Mosaic showed a high sensitivity and specificity for differentiation between Hantavirus infections and healthy blood donors. In many cases the identification of the serotype was possible. The mosaic is a valuable tool for antibody seroprevalence establishment.

8.006 The SARS-Coronavirus Nucleocapsid Protein: A Protein with Multifarious Activities

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The SARS-CoV nucleocapsid (N) protein is a major structural component of the virus capsid and is postulated to play important roles in viral pathogenesis, replication, and RNA packaging. Besides these, the N protein has also been predicted to be involved in a variety of other important functions in the viral life cycle. Here we present and discuss recent data obtained from our laboratory showing the diversity of host pathways that this protein may be involved in. We have tested the capability of N protein to self-associate using its ~140 amino acid interaction domain implying that N may be involved in various regulatory activities in the infected cell. Mammalian cell expression studies further proved our in-silico predictions that the 46kD N protein is a phosphoprotein. Immunoflouresence, in-vitro phosphorylation and c-DNA substraction techniques subsequently were used to prove that the serine - phosphorylated N was stable and localized in the cytoplasm and co-precipitated with the membrane fraction. N was a substrate of cyclin dependent kinase (CDK), glycogen synthase kinase (GSK3) and casein kinase II (CKII). Phosphorylated N translocated to the cytoplasm by binding 14-3-3 (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein) thus revealing a phosphorylation dependent nucleoplasmic shuttling mechanism. The N protein directly inhibited the activity of the cyclin-CDK complex resulting in hypophosphorylation of retinoblastoma protein with a concomitant downregulation in E2F1 mediated transactivation. Our data clearly points towards N having a major and multifarious role to play in SARS-CoV life cycle and pathogenesis.

8.007 Puumala Hantavirus, Myodes Glareolus and Their Habitat Preferences

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Background: In Europe, Puumala hantavirus (PUUV) is the etiological agents Nephropathia epidemica. The bank vole (Myodes glareolus) is the carrier rodent and fluctuations in its population density correlate with epidemics in the human population. The aim of the study was to determine habitat preferences of M. glareolus.

Methods: Rodents were captured during the years 2001 to 2003 in Belgium. The test site was an, approximately, 170-hectare terrain that showed a variety of significantly different habitats. It was known to harbour various PUUV-positive rodent species. Habitat properties, trapping indexes, rodent characteristics and seroprevalences were obtained. Rodent sera were screened for PUUV IgG and HTNV IgG positivity, the species and density of trees, understore vegetation, scrub wood, grasses and mosses were determined and soil humidity was determined.

Results: Habitat 1: 39.6% of the M. glareolus and 39.3% of the A. sylvaticus were IgG positive. Traps in a biotope with a vegetation index of over 6 were significantly more successful. **Habitat 2:** 10.9% of the M. glareolus and 60% of the A. sylvaticus IgG positive. Traps in a biotope with a vegetation index of over 6 were, as in habitat 1, still successful. **Habitat 3:** 11.7% of the M. glareolu and 0.0% of the A. sylvaticus IgG positive. Trapping success was no longer linked to the vegetation index and appears random.

Conclusion: A decreasing diversity in food-producing plants and trees (represented by the vegetation index), significantly decreased the absolute numbers of M. glareolus. A determining factor for M. glareolus abundance was the presence of mosses for nesting. In a habitat where abundance (food, shelter) is the rule, M. glareolus and A. sylvaticus closely interact as evidenced by the similar PUUV antibody seroprevalences in both rodent species, this dramatically changes in favour of M. glareolus when habitat conditions change.

8.008 First Fatal Hantavirus Cases in India, by an as Yet Unknown New Hantavirus Serotype

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Background: No clinical hantavirus cases have been reported so far from India. Puumalavirus (PUUV) is the most important hantavirus in Europe and Russia, inducing generally mild acute renal failure (ARF). PUUV is spread by bank voles, but both this virus and rodent reservoir are absent from India. However, wild rat-transmitted Seoul virus (SEOV) is cosmopolitan, causing thrombocytopenia, ARF and liver involvement, thus perfectly mimicking leptospirosis. Thottapalayamvirus (TPMV) is the only indigenous hantavirus in India, isolated from a shrew (Suncus murinus), captured near Vellore. No human TPMV pathogenicity has been demonstrated so far. However, Suncus murinus is an insectivore, not a rodent.

Methods: In the Chennai and Cochin area in South-India, leptospirosis-suspected but MAT & ELISA-IgM seronegative ARF cases were prospectively investigated. Hantavirus screening was performed by IgG & IgM strip immunoblot assay (SIA) with recombinant SEOV and PUUV, together with IgG & IgM ELISA for PUUV. RT-PCR was used as confirmation test.

Results: We found 7/60 (12 %) positives for SEOV, i.e. showing a higher band intensity for SEOV than for PUUV, and 3/60 (5 %) PUUV-positives. All IgG SIA-positive cases, except 1 SEOV, were also IgM SIA-positive. Two cases were fatal: one Chennai patient (male, 72 y) developed fever, confusion, ARF, jaundice, and thrombocytopenia (100.000/mm*³*), and died after 1 day of peritoneal dialysis. The positive PUUV-bands in SIA were confirmed by positive ELISA IgG. The second fatal case was a Cochin female patient (64 y), who presented with fever, myalgiae, dyspnoea and abdominal pain. She developed jaundice, pronounced thrombocytopenia (7000/mm*³*), disseminated intravascular coagulation, severe ARF requiring dialysis, and ARDS with extreme hypoxia, prompting mechanical ventilation. She died in refractory shock on day 7. SIA was clearly positive for PUUV-bands, and confirmed by positive PUUV IgG & IgM in ELISA. However, RT-PCR on all seropositive Indian samples appeared negative for SEOV and PUUV, as well as for TPMV. Neutralisation tests are in progress.

Conclusions: The fatal Cochin case is reminiscent for 'hantavirus pulmonary syndrome' caused by Sin Nombre virus (SNV), described so far only in the New World. Indian seropositive PUUV results should be interpreted as cross-reactions with TPMV or another yet unknown, but PUUV-like hantavirus strain. Due to their genetical relationship, SNV infections with fatal ARDS can also cross-react with PUUV. Recently, several other ARDS cases in India have been found ELISA-positive for PUUV.

8.009 Identification and Molecular Characterization of Hantaviruses (Dobrava, Puumala and Tula) Detected in Rodents in the Transdanubian Region of Hungary

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Background: Dobrava, Puumala and Tula hantaviruses belong to the genus Hantavirus, family Bunyaviridae and carried by different rodents. Dobrava virus transmitted by yellow necked (Apodemus flavicollis) and striped field (Apodemus agrarius) mice, while the main host of Puumala and Tula virus is bank vole (Clethrionomys glareolus) and European common vole (Microtus arvalis), respectively. Dobrava hantavirus causes severe hemorrhagic fever with renal syndrome (HFRS) whereas Puumala virus rather causes nephropathia epidemica (NE) in Europe. The goal of this study was to determine the prevalence of Dobrava, Puumala and Tula hantaviruses in Apodemus, Clethrionomys and Microtus rodents in the Transdanubian region of Hungary.

Methods: The rodents were trapped in three different location of the Transdanubian region of Hungary during the summer and autumn of 2005. The rodents were dissected and lung tissues were used for hantavirus detection. The viral RNA was extracted from lung suspensions with TRIzol reagent according to the manufacturer's recommendation. Dobrava and Puumala hantaviruses were detected by SYBR Green-based real-time RT-PCR, using newly designed virus specific primers, while in case of Tula virus standard RT-PCR was used. Positive samples were selected for sequence and phylogenetic analysis.

Results: During the study period 22 Apodemus sp. (11 A. agrarius, 10 A. flavicollis and 1 A. sylvaticus), 12 Clethrionomys glareolus and 46 Microtus arvalis were tested for the presence of hantaviruses. Out of the 22 Apodemus rodents 3 A. agrarius were infected with Dobrava virus, while—first time in Hungary—17 Microtus arvalis found to be positive for Tula virus. None of the 12 C. glareolus tested were positive for Puumala virus. Phylogenetic analyses showed, that Hungarian hantavirus strains—Dobrava as well as Tula—were most closely related to those of isolated in Slovakia and Slovenia.

Conclusion: In this study we reported the occurrence of Dobrava, Puumala and Tula hantaviruses in the Transdanubian region of Hungary. Based on the clinical experience and our new data from the region we concluded that extended reservoir studies as well as serological investigations might be important in the future.

8.010 Major Increase in Human Hantavirus Infections, Germany, 2005—Geographic Distribution and Risk Factors

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Background: Over the period 2001–2004, between 150 and 250 cases of symptomatic hantavirus infections (HVI) were reported annually in Germany. In 2005, reports increased strongly to 450 cases. Many cases occurred in regions where HVI were previously unknown to be endemic, including urban areas. We carried out a nation-wide case-control study to identify risk factors, and to determine the clinical course of HVI.

Methods: Descriptive epidemiologic data (age, sex, location, etc.) were available for all 450 cases with laboratory-confirmed HVI. For the case-control study, cases reported between May and August 2005 were recruited via the local public health offices in the national surveillance system. Controls matched by sex and place of residence were selected by sequential digital dialling. Telephone interviews were conducted with standardised questionnaires which covered sociodemographic and clinical characteristics, and various potential risk behaviours. Independent risk factors for HVI were identified by conditional logistic regression.

Results: In the study, 154 case-control pairs were analysed. Of the cases (median age 42 years, 32% females), 73% were hospitalised and 7% required hemodialysis. In the state of North Rhine-Westphalia, 60% of cases lived in metropolitan areas. The majority of cases in other federal states lived in smaller towns or rural areas. In multivariable analysis, independent risk factors for HVI were working as construction worker (OR 4.8, 95% CI 1.4-17), noticing mice close to the place of residence (OR 3.0, 95% CI 1.4-17), and living less than 100 meters away from forested areas (OR 2.5, 95% CI 1.3-4.7).

Conclusions: In 2005, human HVI occurred not only in previously known endemic areas but spread to many additional urban and rural areas. An increase of infected rodent populations could have contributed to this trend. Risk factors identified in this study demonstrate the need for preventive measures (eg, targeted information campaigns for persons with occupational exposures, or those living near forested and mice infested areas). Close monitoring of rodent density and infection status could contribute to a better prediction and control of human HVI.

8.011 Epidemiology of Rift Valley Fever

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Rift Valley fever (RVF)poses a significant threat to domestic ruminants and human health. The recent RVF outbreaks in the Arabian Peninsula, the first outbreaks outside Africa, have the implication that it is likely that RVF virus will now spread further into non-endemic RVF regions since it is capable of utilizing a wide range of mosquito vectors.

Our aim was to determine whether an epidemic of RVF results from spread of a single virus strain or it is triggered by an increase activity of multiple strains. A total of 113 isolates of RVF virus recovered over a period of 60 years in 15 African countries, Madagascar and Saudi Arabia were characterised by partial genomic sequencing of 535-nucleotide segment of the G2 glycoprotein. Phylogenetic analysis indicate that circulating strains are compartmentalized and belong to one of 15 genotypes.

A RVF outbreak can result either from rapid spread of a single strain over vast distances or from an increased activity of multiple virus strains circulating at an endemic level within a country. It appears that multiple strains of RVF virus can co-exist in endemic areas during prolonged dry periods. When favourable enviroclimatic conditions occur, individual strains of the virus may become disseminated rapidly over vast distances and adapt to diffrent ecological conditions.

8.012 EDEN in Sweden: Rodent-borne Zoonoses in Changing Environments

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Anthropogenic climate change is held to induce occurrence and spread of zoonotic diseases. The pan-European project Emerging Diseases in a changing European Environment (EDEN http://eden.cirad.fr/) was

launched in 2004 to study European ecosystems and environmental conditions in relation to global climatic change and the influence thereof on the spatiotemporal distribution and dynamics of zoonoses, their reservoir hosts and causative disease agents.

The Swedish part of EDEN involves the monitoring of rodent populations and their zoonotic agents, e.g. Bank voles (Clethrionomys glareolus) and the concomitant Puumala virus (PUUV, Bunyaviridae) that causes Nephropathia epidemica (NE) to 100s of humans each year in northern Sweden. Though Bank voles are widely distributed throughout most of Sweden, NE appears to be absent south of Lat. N 59°.

In 2005 we collected >1.400 small mammals on available nation wide sites for sampling of environmental data (National Inventory of Landscapes in Sweden, NILS http://www.nils.slu.se). Collected specimens are screened to identify zoonotic agents, and the results are used to; describe biodiversity/phylogeography among rodents and disease agents: map and correlate findings to local habitats: identify areas of high risk: follow-up and couple to changes through series of field/satellite data and risk predictions under different scenarios of climate change.

Initial analyses suggest that PUUV, or a PUUV-related virus, circulates among south-Swedish Bank voles respectively north-Swedish Northern Red-backed voles (Clethrionomys rutilus). The previous region is one without notified cases of NE and the latter is out of range of Bank voles geographical distribution. Whether this reveals new and/or old viruses in new and old hosts in until now unstudied places render further investigations.

The Scandinavian Peninsula represents a delimited ecosystem with short postglacial history, where landscapes and rodent communities are well documented. Thus it constitutes a highly suitable study area since regional climatic conditions vary distinctly along a vast South-North gradient and the structure of the presented study system makes it feasible as a general model. The EDEN project will develop a set of methods, tools and skills which can be used by decision makers for risk assessment and decision support both at the EU and at the national or regional level.

8.013 Characterization of Specific IgE Antibody Related to Antigen 5 of Echinococcus Granulosus

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Cystic Echinococcosis (CE), caused by infection with larval Echinococcus granulosus (E.granulosus) has public health importance not only in areas of endemicity but also in countries or regions where migration of infected people and exchanges of livestock occurs. In human cystic hydatid cases, which are sero-positive, IgG and IgE antibodies against cyst fluid antigens predominate. Differences in the antibody class switching response have been noted between asymptomatic patients and more advanced cases that had undergone surgery. Asymptomatics preferentially induce IgG and surgical cases IgE, which suggest a class switching as the disease progresses.In helminth infections, IgE production may be responsible for the protective immune response to the parasite or immune-mediated pathology or both. Anaphylactic reactions, such as urticaria, edema, respiratory symptoms, and anaphylactic shock often complicate the course of Cystic Echinococcosis (CE). To investigate the role of the IgE immunoreactive Antigen 5 (Ag 5) in the sero-positive patients with CE, we determined N-terminal of 57 kDa subunit of Ag5 responsible for IgE and C-terminal of this active antigen related to induction of IgG specifically. Immunoblotting analysis showed that specific IgE to 57-kDa subunit related to inter-chain disulphide band of two 22 kDa and 38-kDa component of Ag5 and conformational epitope on this subunits. In addition, since the 57 kDa component arise from the removal of the C-terminal portion of 22 kDa subunit of Ag5, thus IgE specifically recognized N-terminal of 22 kDa subunit which remain bounds to the other component, whereas IgG reacted with C-terminal of 38 kDa component of Ag5. Recognation of the specific binding site on the 57 kDa subunit of Ag5 could leads to understanding the mechanism regulating IgE/IgG production in some immune circumstances that IgE tends to some dominate, whereas in other IgG predominates.

8.014 Recent Isolations of Mokola Viruses from Domestic Mammals in South Africa

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Background: The aetiologic agent of rabies was initially thought to be antigenically unique until the isolation of rabies-related viruses in the late 1950s from Nigeria. Since then, these lyssaviruses have been recovered from domestic and wildlife mammals from several African countries including South Africa. It is presently believed that the rabies-related viruses are widely endemic in this country. Mokola virus (MOKV) belongs to genotype 3 of the Lyssavirus genus within the Rhabdoviridae family.

Methods: Brain samples of a canine (Canis familiaris) [404/05] and a feline (Feline domesticus) [173/06] were submitted to the Onderstepoort Veterinary Institute (OVI) for routine rabies diagnosis. Lyssavirus antigen was detected by the fluorescent antibody test (FAT) and confirmed by virus isolation in neuroblastoma cells. Antigenic characterisation of the rabies samples was performed with a panel of 16 anti-nucleocapsid monoclonal antibodies (N-Mabs) (Centre of Expertise for Rabies, Canada). Total viral RNA was extracted from the original brain material using Trizol and reverse transcribed using standard procedures. Complementary DNA was subsequently amplified with the N1(+)/N2(-) primer set, purified using spin columns and sequenced with the BigDye Termination Sequencing Ready Reaction Kit. DNA sequencing information was compared with the nucleoprotein sequence data for other Mokola virus isolates from southern Africa.

Results: FAT performed on the brain material showed a positive reaction for lyssavirus antigen. Antigenic and genetic characterisation further confirmed that the 2 rabies virus isolates were indeed Mokola (genotype 3). Whereas MokV173/06 clustered with viruses from South Africa, MokV404/05 was found to be more closely related to those from Zimbabwe.

Conclusions: Mokola virus infections are considerably more common than is presently reported and an increased surveillance for these lyssaviruses is warranted. Surveillance and knowledge of the distribution of the antigenic and genetic virus variants are essential for an essential and economical rabies control programme.

8.015 Molecular Epidemiology of Canine Rabies in the Free State Province of South Africa

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Background: The Free State province has been historically associated with endemic rabies in the Cynictis penicillata (yellow mongoose). Recently rabies viruses of the mongoose biotype have been isolated from the domestic dogs. Rabies in the domestic dogs in the Free State province has been lately reported with some transmission into other domestic animals.

Methods: A molecular epidemiological study was performed on a cohort of 85 rabies viruses collected from domestic dogs between 1995 and 2006. The cytoplasmic domain of the glycoprotein and the G-L intergenic region of the 85 rabies virus isolates was amplified and sequenced. Phylogenetic trees were reconstructed from an alignment of a 592-bp region of the genome under investigation. The neighbouring provinces and countries were also compared.

Results: The phylogenetic analyses on the rabies viruses in this study sample revealed two main clusters. The first cluster was composed of the compact canid rabies biotype circulating in the domestic dog population. The second cluster was composed of virus isolates resulting from spillover events from cynictis penicillata and was further differentiated into 3 sub-clusters. Rabies viruses of the mongoose rabies biotype clustered according to their geographic origin.

Conclusion: The genetic analysis performed in this study indicates the spillover of infection into domestic dogs by mongoose rabies biotype.

The canid rabies viruses from the Free State province are more closely related to those obtained from Lesotho with 99% sequence homology. This study has great importance in terms of disease control measures in the province and the neighbouring provinces/countries.

8.016 Disease Burden of Puumala Virus Infections in Finland

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Background: Puumala virus (Hanta virus genus) infection causes a mild form of hemorrhagic fever with renal syndrome. Reservoir is the bank vole with presumed mode of transmission by inhalation of excreta. Neither specific treatment nor vaccine exists against Puumala infection. In Finland, it has been a notifiable disease since 1995: laboratories report electronically to the National Infectious Disease Register (NIDR). Large epidemics occur every few years. Occupational and recreational activities may give a wide variety of exposure. To evaluate the disease burden of Puumala virus infections in Finland, we analyzed surveillance data from NIDR, complementing it by other relevant national registers.

Methods: Cases were serologically confirmed (IgM) Puumala virus infections reported to the NIDR during 1995–2005. The vital status at 7 and 28 days from the date of diagnostic specimen was available for cases reported during 2004–2005 from the National Population Information System by linkage with of the national identity codes. Data on hospitalizations related to Puumala virus infections during 1996-2005 were retrieved from the National Hospital Disease Registry by use of the International Classification of Diseases (ICD) code 10. Notifications on occupational diseases during 1995–2002 were obtained from the Finnish Occupational Diseases Registry.

Results: A total of 16,047 cases were reported to NIDR (range by year, 757–2577); average annual incidence of 28 cases per 100,000 population (range by year, 15–49). Most of the cases (85%) were aged 20–65 years and 63% were males. Case fatality proportion at 7 and 28 days was 0.1% and 0.18%, respectively. Overall, 7,990 hospitalizations related to Puumala virus infections (range by year, 461–1279) were identified; the mean length of stay was 5.4 days. During 1995–2002, 399 occupational Puumala virus infections were notified (range by year, 24-91); most (80%) were among males and related to farming and forestry work (96%).

Conclusions: The incidence of Puumala virus infections is highest in Finland across Europe. Deaths are extremely rare but more than half of the cases diagnosed are hospitalized. The disease affects mostly males in working age. Although data on occupational infections is scarce, farming and forestry work are prominent. Further analyses based on the methodology of ongoing European pilot study on communicable disease burden might provide comparable results.

8.017 Different Epidemiological Pattern of Infections of Rickettsiae of the Spotted Fever Group and of the Typhus Group in Humans in Northern Afghanistan

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Background: Rickettsiae have worldwide occurrence and human rickettsial diseases are widely recognized as emerging infections in several parts of the world. In Afghanistan rickettsiae were recently detected in cat fleas, however their epidemiology and medical importance are unknown.

Methods: 140 sera from residents of the Northern Afghan area of Kunduz were tested for antibodies against Rickettsia (R.) conorii (spotted fever group), R. typhi (typhus group) and Orienta (O.) tsutsugamushi. Antibodies were detected by commercial immunofluorescence (IFA; R. conorii, R. typhi) and by commercial ELISA (R. conorii, O. tsutsugamushi). For 133 sera information on gender, age group and urban or rural residence of donor were available and results were matched for these parameters.

Results: No antibodies against O. tsutsugamushi were detected at all. 26/133 sera (19.5%) tested positive with titers of 1:64 or higher in IFA against R. conorii. Antibody prevalence increased from 4% (age group 50 years). Sexes did not differ in antibody prevalence. In urban residents, the seroprevalence was higher (22.8%) when compared to rural residents (4.3%). For R. typhi, 49.6% were antibody positive (> 1:32 in IIFA) with the highest prevalence rate in the age group < 20 years (66.7%) and decreasing rates with higher age groups (36.4% in age group > 50 years). No differences neither in sex nor residency were found.

Conclusions: The data of the study suggest that epidemiologic characteristics of endemic rickettsial diseases differ significantly between the spotted fever group and the typhus group. Risk of infection for R. conorii shows a typical pattern reflecting increasing risk of exposure to vector tick species with increasing life span and higher risk in urban areas. In contrast, risk of infection with flea transmitted R. typhi (typhus group) seems to be highest during the early life span and decreasing during life. This different epidemiological pattern can reflect different vector behavior and/or human social behavior in different age groups. It further provides first evidence for short life length of antibodies against typhus group rickettsiae present in Northern Afghanistan. No evidence for circulation of O. tsutsugamushi in Northern Afghanistan was detected.

8.018 Emerging Nephropathia Epidemica Hotspots in Germany in 2004 and 2005: Molecular Characterization of Four Different Puumala Virus Subtypes

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Background: Due to a large increase in the number of human cases in 2004 and 2005, hantavirus infections are emerging infections in Central Europe. In Germany the number of reported cases increased dramatically from about 140–240 annual cases (2001–2003) up to 448 human cases in 2005. Neither the particular hantavirus strain(s) involved nor the reasons for the outbreaks are known.

Methods: Rodents were trapped and screened for hantaviruses in the four regions (Lower Bavaria, Cologne/North-Rhine Westphalia, Osnabrück/Lower Saxony, Sennickerode/Lower Saxony) with high increases of human cases. Screening of rodents for hantaviruses was performed in lungs, livers and kidneys by real time-RT-PCR and by detection of antibodies in rodent sera. Rodent organs positive in the real-time RT-PCR were further tested by conventional PCR and the S and partial M segments were sequenced. Nucleotide (nt) sequences were compared to known sequences of hantaviruses.

Results: In all four regions mentioned Puumala virus (PUUV) was identified as the hantavirus circulating in bank vole populations and thus being the probable cause of human infections. S and partial M segment nt sequences showed a low level of divergence (3–5 %) within PUUV strains from the same geographical area. In contrast, a nt sequence divergence of up to 16% was observed in both M and S segments between the PUUV from Northern (Cologne, Osnabrück, Sennickerode) and Southern Germany (Lower Bavaria). Comparison of the PUUV in the four regions confirmed that at least four different PUUV subtypes are presently circulating in bank vole populations in Germany. Two of the four subtypes form singe clades in the respective phylogenetic analysis of available PUUV strains that have not been recognized so far.

Conclusions: The emerging activity of PUUV in 2004/2005 in Germany could be traced to more than one subtype of PUUV. General changes in natural endemic cycles affecting the whole central European area seem to be the driving force for the emergence of various regional PUUV subtypes in different areas of Germany. Although the PUUV subtypes identified displayed a considerable level of genetic diversity, they all seem to be fairly capable of causing human disease resulting in the increase of human cases observed in 2004 and 2005.

8.019 A Primate Animal Model for Orthopoxvirus Infections

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An atypical epizootic infection was observed in a colony of 50 New World monkeys composed of different species including a group of marmosets (Callithrix jacchus) and cotton top tamarins (Saguinus oedipus). During an outbreak in autumn 2002, a total of 15 animals died. In the course of the disease all animals exhibited erosive-ulcerative lesions of the oral mucous membranes. Later stages were characterized by skin lesions presenting as hemorrhagic lesions distributed randomly over the body, but primarily on face, scrotal regions, soles and palms. Some lesions developed into erythematous papules, only single papular lesions became incrusted. All animals died within a week after onset of clinical symptoms.

From 5 animals samples from different organs were collected at necropsy. Electron microscopy revealed virus particles with orthopox-like morphology within intracytoplasmatic inclusions. With orthopox-specific real-time PCR assays virus genomes could be detected in the organs. Sequence data showed that all 5 animals were infected with the same virus and phylogenetic analyses revealed that this orthopoxvirus, tentatively named calpoxvirus, is related to but distinct from known cowpox virus isolates. Virus was isolated on Vero cells. Intravenous as well as intranasal application to marmosets reproducibly induced the disease and resulted in rapid death within 2 to 3 days after the first symptoms appeared. We therefore suggest that the calpoxvirus / marmoset system might be a useful non-human primate model for the study of orthopoxvirus pathogenesis and the development of vaccines and antiviral therapies.

8.020 Crimean Congo Hemorrhagic Fever Suspected Cases in Albania: What at Last?

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Background: Balkan Peninsula is endemic region for Crimean Congo Hemorrhagic Fever (CCHF) and Hemorrhagic Fever with Renaldrome (HFRS), and every year sporadic cases, or even outbreaks of these diseases are observed. Common symptoms are high fever and hemorrhagic manifestations. CCHF cases occur almost every year in Albania, where the most endemic region is the Northeastern part of the country. During spring, summer and autumn months of the years 2003-2006, serum samples from 31 CCHF suspected cases in Albania were tested. For differential diagnosis, samples were tested also for a probable hantavirus or leptospira infection.

Methods: Viral RNA was extracted from serum samples from all suspected cases and RT-PCR was performed using primers specific for CCHF virus. In addition, serum samples were tested by IFA for the presence of antibodies specific to CCHF virus. All samples which resulted negative for CCHF were tested for hantaviruses and leptospirosis, by both serology and PCR, as these pathogens are included in the differential diagnosis of the disease.

Results: In total, 10/31 (32.2%) cases were laboratory confirmed as CCHF, while 4/31 (12.9%) were HFRS cases and 10/31 (32.2%) were leptospirosis cases. Concerning CCHF, 8 cases were positive by both serology and PCR, while two cases were positive only by serology. Laboratory diagnosis of HFRS and leptospirosis was set by detection of specific IgM antibodies. CCHF cases were observed from mid-May to July, HFRS from July to September, while leptospirosis from April to October. All CCHF cases were observed in Northeastern part of Albania, where leptospirosis cases were also detected.

Conclusions: Both CCHF virus and hantaviruses are endemic in Albania and all suspected cases have to be tested for both viral pathogens. In addition, cases have to be tested also for leptospirosis, as the disease is endemic in the region accounting for a similar percentage as CCHF, and has similar clinical signs and symptoms, complicating the differential diagnosis.

8.021 The Blind Leading the Blind: How the Nigerian Press Reported the First Cases of Avian Influenza in Africa

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Background: On the 8th of February 2006, an outbreak of the (A)H5N1 virus was confirmed for the first time in Africa, in a farm in Kaduna State, northern Nigeria. While health officials had prepared for the possibility of migratory birds carrying the virus to Africa, Nigeria was not expected to be primarily at risk, nor was it prepared to respond. It took 1 month from the date of onset of the outbreak until it was confirmed and declared. Effective communication is key to the management of infectious disease outbreaks and the press is an important means of disseminating information. This is particularly important for avian influenza due to all the uncertainties. In Nigeria, the most populous country in Africa with a vibrant press, and literacy rate of about 50% the potential for a communication disaster was huge.

Methods: We examined the 3 most widely circulating newspapers in Nigeria prospectively every day for the 2 months after the outbreak was reported. Every story related to avian influenza was extracted. This was categorized and analsysed for being 1. factually correct, 2. educative, or 3. inflammatory. A further in-depth qualitative analysis was done to examine specific themes.

Results: In this 2 month period, 72% of the articles studied were news articles, 17%feature articles, while 11% were editorials. Of all the articles on avian influenza, 40% got their facts wrong. The most common errors related to the risk of disease in humans. Just 60% could be described as having any educative value and 10% can be deemed inflammatory.

Discussion: Most of the reports only reinforced the uncertainties surrounding avian influenza. There is an urgent need to educate the Nigerian press (as in other countries) on their role and power in the dissemination of information during infectious disease outbreaks and the effects this could have on peoples' decisions. To do this, journalists themselves need to be proactively educated on the difficult field of emerging infectious disease.

8.022 Virologic Characterization of a Poxvirus Zoonosis in Northern Italy

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Background: Poxviruses are a family of large, dsDNA viruses that have received increased attention in recent years due to fears of bioterrorist attacks and zoonotic transmission. Recent outbreaks of monkeypox, tanapox, and Passatempo infections of humans have underlined the importance of the careful handling of exotic and farm animals. Few data are available on European orthopoxvirus strains involved in zoonotic events, and so far no there are no reports on orthopoxvirus zoonosis from Italy. We report a case of human orthopoxvirus zoonosis in Northern Italy.

The Clinical Case: In January 2006, an Italian male who had been scratched by a diseased cat presented at the infectious disease clinic with a suspected orthopoxvirus (OPV) lesion on the right hand, accompanied by systemic symptoms such as moderate fever and malaise.

Methods: Laboratory investigation involved all currently available technologies for the differential diagnosis of orthopoxvirus infections (electron microscopy, PCR, phylogenetic analysis, viral isolation, immune response detection). Viral DNA was detected by both SYBR Green I based Real-Time PCR specific for CrmB and classic PCR targeting HA. Further characterization was carried out by RFLP analysis and sequencing of CrmB and of full length HA gene. Humoral immune response was analyzed by IF and microneutralization assays, and cellular immune response was established by intracellular detection of IFN-gamma after in vitro PBMC stimulation with both patient's virus isolate and reference vaccinia virus.

Results: Orthopoxvirus infection was identified by electron microscopy, PCR and virus isolation. The diagnosis was confirmed by detection of both cellular and humoral specific immune response. RFLP and phylogenetic analysis of both CrmB and HA were unable to conclusively identify the poxvirus species. In fact, both viral genome regions had strong similarity to cowpoxvirus, though some similarity was also noted with camelpox virus.

Conclusion: Further investigations is necessary to fully characterize the Italian isolate and to definitively assign a position in the Orthopoxvirus family. Enhanced diagnostic capability for orthopoxvirus infections, resulting from global awareness of bioterrorism menace, will be helpful to identify new orthopoxvirus infections in animals and humans, and to improve the knowledge on ortopoxviruses circulation in Italy.

8.023 Interaction of SARS Coronavirus with PBMC-derived Dendritic Cells

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The severe acute respiratory syndrome (SARS) of humans is caused by a novel coronavirus of zoonotic origin termed SARS-CoV. The virus induces severe injury of lung tissue as well as lymphopenia and a destruction of the architecture of lymphatic tissue by yet unknown mechanisms. In our study we analysed the interaction of SARS-CoV with dendritic cells (DCs), the key regulators of immune responses. Monocyte-derived DCs were infected with SARS-CoV and analysed for viability, surface marker expression and interferon-α (IFN-α) induction. SARS-CoV infection was monitored by quantitative RT-PCR, immunofluorescence analysis and recovery experiments. SARS-CoV infected both immature and mature DCs although replication efficiency was low. Immature DCs were activated by SARS-CoV infection and by UV-inactivated SARS-CoV. Infected DCs were still viable on day 6 post-infection, but, MHC class I upregulation was missing, indicating that DC function was impaired. Additionally, SARS-CoV infection induced a delayed activation of IFN-α expression. Therefore, we conclude that SARS-CoV has the ability to circumvent both the innate and the adaptive immune system.

8.024 New Data on Seroprevalence, Incidence and Risk Factors for Q Fever in Germany

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Background: Q fever is a notifiable and endemic zoonotic disease in Germany. The causal agent Coxiella burnetii, has an extensive reservoir, including farm animals. Between 2000 and 2005 the annual number of cases in Germany varied between 117 and 416 cases. Two large outbreaks with several hundreds of affected persons occurred in 2003 and 2005. The average annual incidence in south west Germany is 0.5-1 per 100.000 population. However, population based seroprevalence studies of C. burnetii antibodies in the German population have not been performed in recent years.

Methods: We performed a serological investigation on 2447 sera with a commercial ELISA (Virion/Serion, Würzburg, Germany). Uncertain results were confirmed with a indirect immunofluorescence assay (Focus, USA/Genzyme Virotech, Germany). The material derived from a population-based, cross-sectional health survey in a county where no Q fever cases have been reported in the last 5 years through the national notification system. Data on possible risk factors for recent infection was obtained from all participants by a standardized questionnaire.

Results: A total of 181 sera out of 2447 samples gave positive results for IgG Phase 2 antibodies indicating a seroprevalence of 7.4%. Of 20 positive results for Phase 2 IgM antibodies by ELISA, four could be confirmed by the immunofluorescence assay.

Statistical analysis showed a significantly positive association between phase II IgG antibodies and profession as farmer (OR 2.0, CI 1.1-3.5, $p<0.01$). No other statistically significant correlation was found according to the remaining variables studied.

Conclusion: Due to the mild clinical signs of acute illness (up to 50% of the cases), Q fever is for sure a underestimated disease. The presented data confirms this hypothesis. Interpretation of immunofluorescence IgG phase II antibodies as a possible indicator for recent infection would reflect an incidence of the disease of about 160 per 100.000 population. In addition to known risk areas (e.g. intensive sheep keeping), intensive farming regions seem generally to be endemic. This supports the requirement of keeping the awareness high to be able to identify local outbreaks and take adequate measures.

8.025 Outbreak of Leptospirosis Among Triathlon Participants, Germany, 2006

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Background: Leptospirosis is a notifiable infection in Germany. In 2005, 58 cases were reported to the national surveillance system, resulting in an incidence of 0.07 per 100.000 inhabitants. Most of the cases occurred sporadically. Travelling abroad is the single most reported risk factor, but direct contact with contaminated freshwater through recreational activities (i.e. swimming, fishing, canoeing) seems to play an increasing role in recent years. In August 2006 a case of leptospirosis was detected by routine surveillance. Explorative assessment of possible risk factors by the LGA revealed swimming in the Neckar River during the triathlon as the most likely source of infection. In order to determine the possible extent of the outbreak an outbreak investigation was performed.

Methods: An email survey was conducted to collect clinical information and data on possible risk factors from the participants. All triathletes were asked to fill out a questionnaire and give a serum sample for the investigation of leptospiral antibodies by IgM and IgG ELISA and by MAT.

Results: 507 participants swam the 1,7 km distance in the Neckar on August 6th. 177 (35%) responded to the email, 71 (40%) of them reported being ill (mostly gastroenteritis) after the triathlon; three patients had been hospitalized with severe clinical signs of leptospirosis, and were laboratory confirmed. However, one of these patients had a negative MAT while the ELISA tests have been positive. Two additional participants with mild symptoms of disease (fever, myalgia, headache) revealed only positive IgM ELISA results. Another 16 triathletes (3%) had positive IgG ELISA titers

One participant had a positive MAT and IgG ELISA antibodies but reported no clinical symptoms. Further investigations revealed a leptospirosis typical illness with hospital admission after the triathlon last year. Hence at that time no laboratory tests were performed.

Conclusion: This outbreak demonstrates that participants of triathlons with freshwater exposure are at increased risk of leptospirosis in Germany. Laboratory tests have to be further evaluated for their clinical use. Heavy rains that preceded the triathlon were likely to have increased leptospiral contamination of the river. Therefore the risk of waterborne infections under such conditions should be regarded by health care providers and occupational and recreational users. In febrile patients with history of freshwater exposure leptospirosis has to be considered.

8.026 Emergence of New Feline Coronavirus Variants Following Interspecies Transmission in an Animal Shelter

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The group 1 coronaviruses FCoV (feline coronavirus) and CCoV (canine coronavirus) are closely related and interspecies infections are possible. New virus types have emerged from recombinations between FCoV and CCoV. In cats a recombination in the S-gene between species specific FCoV type I and CCoV resulting in FCoV type II has emerged. In dogs the equivalent recombinant type is designated as CCoV type I, for its close genetic relationship to FCoV type I in the S- and M-genes. In contrast to CCoV type I in the dog with genetic drift in both S- and M-genes, nothing was known about the M-gene evolution in FCoV.

We investigated 87 fecal samples of cats collected in an Austrian animal shelter with close contact between dogs and cats for coronavirus RNA of the group 1. Positive samples were submitted to further S- and M-gene typing. The S-gene PCR was designed to discern FCoV type I from FCoV type II and CCoV type II. Two different M-gene PCRs were used. The first detects FCoV and CCoV independently of their genetic type, the second was originally designed for the detection of recombinant CCoV type I. 12 randomly chosen M-gene amplification products of feline samples were sequenced and investigated by phylogenetic analysis. Further RT-PCRs were designed to sequence other parts of the 3'-end of the viral genome.

Of 87 randomly chosen cats 46 were coronavirus shedders. In the S-gene only species specific type I was detectable. Surprisingly, in the M-gene, 56.5% of the samples tested positive in the PCR originally used for the detection of CCoV type I. Four of 12 M-gene sequences showed the highest identities of 86-88% to CCoV type I, bearing traits of both feline and canine coronaviruses. Never the less the sequences showed point mutations different from any group 1 coronavirus presently known. Preliminary results of the sequencing of further parts of the viral genome sustain the assumption that the shelter served as a “melting pot” for different FCoV and CCoV strains and that new possibly more virulent virus types may emerge any time under comparable circumstances. Our investigations show the high potential of coronaviruses for recombinations following interspecies transmissions, which could eventually have some relevance also in other species including humans.

8.027 Comparative Analysis of Brazilian Vaccinia Virus Strains

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Background: Although the WHO declared global smallpox eradicated in 1980, recent events around the globe have raised concern over emergent poxvirus infections. Vaccinia virus (VV) strains largely infecting cattle and their handlers have been isolated repeatedly in Brazil. Genetically distinct populations are currently circulating in the country and even within the same outbreak. To further investigate the origin and evolution of these viruses we present a comparison of Brazilian VV strains isolated from multiple hosts and geographic areas.

Methods: The diversity of 4 essential and non-essential genes involved in DNA replication, virion structure and viral pathogenicity was examined among VV strains. Phylogenetic analyses were performed on available data using parsimony and Bayesian methods with the programs PAUP* and MrBayes.

Results: Results indicate high nucleotide similarity (96%-100%) among these genes when compared to reference strains. The Brazilian strains form two phylogenetic groups that are not distinguished by host, clinical manifestations or geographic origin. An 18-nucleotide deletion in the A56R gene (hemagglutinin), previously proposed to identify all Brazilian VV, is characteristic of only one of these groups. All Brazilian VV examined thus far possess the B19R (/ IFN receptor) gene; however, the vaccine strain Lister, a proposed progenitor of Brazilian VV, does not possess this gene at all.

Conclusions: Despite genetic similarities, variation among strains consistently suggests that multifactoral events best explain the genetic diversity present in Brazilian VV. The history of VV in Brazil suggests vaccinia may have been introduced prior to the vaccination campaigns of the 20th century. The absence of the B19R gene in the Lister genome makes it unlikely that this vaccine strain lead to the establishment of VV in Brazil. High similarity of Brazilian VV to the laboratory strain VACV-WR suggests that some isolates may be derived from an escaped vaccine strain, NYCBH (New York City Board of Health), not Lister or IOC (Oswaldo Cruz Institute) as previously hypothesized. Furthermore, circulation of multiple genetic types in overlapping regions is supportive of hypotheses where recombination may play a significant role in the evolution of these viruses.

8.028 The 3a Accessory Protein of SARS Coronavirus Specifically Interacts with the 5'UTR of its Genomic RNA, Using a Unique 75 Amino-acid Interaction Domain

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More than four years have passed since the outbreak of the severe acute respiratory syndrome (SARS) epidemic and still very little is known about the molecular biology and pathogenesis of this deadly virus. Among the accessory proteins of the SARS coronavirus (SARS-CoV), the 3a protein has been shown to interact with the spike, envelope and membrane glycoprotein and has recently been established to be a structural component of capsid. Recent studies suggest that the 3a protein may functions as an ion channel and may promote virus release. In order to further characterize the functional properties of this protein, we initiated studies to check its RNA binding activity. Using yeast three-hybrid system, electrophoretic mobility shift assay (EMSA) and Ultraviolet (UV) cross-linking technique, we have shown that the 3a protein is capable of binding specifically to the 5' untranslated region (5' UTR) of the SARS virus genomic RNA. Further we have mapped the interaction domain of 3a protein responsible for this RNA-protein interaction using a series of deletion mutants and define it to the central 75 amino acids. This RNA binding motif of 3a does not share homology with any other known RNA binding protein and may have an important role in viral capsid assembly and pathogenesis.

8.029 Emergence of Methicillin Resistant Staphylococcus aureus (MRSA) Infections in Horses in a Veterinary Hospital: Are There Relations to MRSA from Human?

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Background: MRSA became a major problem of nosocomial infections and infection control in human medicine worldwide, moreover invasive MRSA have emerged and spread among the community (community MRSA). Therefore the most recent emergence of MRSA in companion animals and livestock needs particular attention with respect to the question of mutual transmis-sion between humans and animals, and to MRSA infections as an emerging zoonosis.

Methods: Samples where taken from equine patients with wound infections (nose, wounds). Detection of MRSA by means of selective agar medium (Chromagar MRSA); susceptibility testing (microbroth

MIC); PCR demonstration of antibiotic resistance genes, typing by multilocus sequence typing and spa-sequence typing.

Results: From 2003 until 2006 31 cases of sporadic nosocomial infections in horses in a veterinary university hospital have been recorded. Isolates from 29 animals exhibited an uniform pat-tern of characteristics with regard to molecular typing, antibiotic resistance phenotypes and resistance genes: nearly identical Sma-I-macrorestriction pattern, MLST ST254, spa-sequence type t036, SCCmec element of type IVd, resistance to beta-lactam antibiotics (mecA), oxytetracy-cline (tetM) and gentamicin (aph2'-aac6').

MRSA from humans originating from nosocomial infections in Central Europe and also exhibiting MLST ST254 differed from horse MRSA by spa sequence type 6009 and by containing SCCmec elements of types Iva and IVc respectively. MLST ST254 was so far not found among community MRSA in Europe.

Two isolates were strikingly different: they belonged to clonal lineage ST398 and contained an SCCmec IVa element.

In 2006 MRSA ST398 had also been recorded in Germany from dog, foal, pig, as well as from veterinary staff. Furthermore 7 cases of ventilator associated pneumonia have been observed in 3 German hospitals caused by MRSA ST398.

Conclusion: MRSA ST254 (t036) from horses are obviously not related to nosocomial and to community MRSA from humans, although a common ancestor can not be excluded.

MRSA exhibiting ST398 are obviously able to colonize and cause infections in humans and in different animal species such as dog, horse and pig. Of particular concern was the subsequent detection of MRSA ST398 not only in outpatients but also in association with ventilator assocated pneumonia in hospitals.

8.030 Q Fever in Germany—Epidemiology and Clinical Manifestation

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Table 1 Frequency of symptoms in 50 patients with acute Q fever

symptom (No of cases)	percent
general	
exhaustion	98
fever	88
weight loss	70
chillings	64
arthralgia	64
myalgia	52
respiratory	
cough	38
pleuritic chest pain	24
shortness of breath	10
cns	
severe headache	68
disorientation	4
gastrointestinal	
nausea	42
vomiting	20
diarrhea	4

Background: Q fever is a notifiable disease in Germany with an incidence of about 0.05-0.1 per 100.000 population. In south Germany, where the pilot-study was performed, the incidence is 5 to 10 times higher. Primary infection is often symptomless, signs of acute illness have no typical manifestation. An influenza-like illness, often with pneumonia or hepatitis is the most frequent manifestation. We report about a case series of Q fever patients.

Methods: Patients with laboratory confirmed Q fever notified through the national surveillance system where contacted by the responsible local health departments. Personal or telephone interview using a standardized questionnaires was performed on a voluntary basis, including demographic data, symptoms and duration of disease, treatment and possible risk factors.

Results: During the 7 months pilot phase in 2006, from 57 contacted patients 50 (87%) responded. 21 cases belonged to two outbreaks (13 and 8 patients). Half of the patients (54%) reported to have been diagnosed with pneumonia and 8% with hepatitis. Specific symptoms are listed in table 1. 42% of the patients where hospitalized. Illness lasted for about 2 weeks (5-65 days, mean 14, median 17). The male:female ratio was only 1.45:1 and much lower as reported elsewhere. All age categories were represented (5-81 y, mean 46 y), including 3 children. Both outbreaks and 3 of the sporadic cases where related to birth-giving animals (sheep or goat). 45% of sporadic cases had direct contact to sheep or goat, and a further 14% had been in areas (i.e. field, pasture, hiking trail), which are generally used by sheep or where sheep droppings were present. However, 31% of cases did not report about any of the possible risk factors asked for.

Conclusions: The study highlights, that surveillance of Q fever is essential to establish the distribution and magnitude of the disease. The hospitalization rate in our case series is 2 to 10 times higher as reported in most other case series. Q fever in children seems to be not so rare as often assumed. Further studies are needed to investigate this issues.

8.031 Extracellular Gelatinases of Aeromonas sp—The Influence of Physicochemical Factors on Proteases Expression/Activity

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Background: Aeromonas spp. are ubiquitous inhabitants of aquatic ecosystems such as freshwater, coastal water and sewage. They are increasingly being reported as important pathogens from humans to lower vertebrates. These bacteria have a broad host range, being often isolated from fish with hemorrhagic septicaemia, as well as from humans with diarrhoea. Nevertheless, the pathogenesis and virulence mechanisms responsible for selected Aeromonas infection in different species are not clearly understood. Several microorganisms, many of which putative human pathogens, have been reported as producing enzymes with collagenolytic and elastinolytic activities. Since collagen is a major component of many tissues, this enzymatic activity is relevant for the invasiveness capacities of the bacteria.

Methods: For this work we used A. caviae (CECT 838), A. hydrophila (CECT 839), A. salmonicida (CECT 894), A. sobria (CECT 4245), A. veronii bv veronii (CECT 4257), A. veronii bv sobria (CECT 4246) as reference strains. Culture medium was applied on gelatine zymography for enzymatic activity evaluation. The effect of environmental variations was carried out at 10-30ºC, 5.0-50 ups (salinity) and pH 5-9, to determine the differences on the expression of gelatinases.

Results: The zymographic analysis confirmed that all the reference strains used in the study express different metallo- or serine- gelatinases, as revealed by the use of specific protease inhibitors (1,10-phenanthroline and PMSF, respectively). Concerning environmental variations, a major effect of temperature and salinity on the growth of Aeromonas sp was verify, whereas the effect of pH was not so clearly revealed. These results are correlated with gelatinases expression.

Conclusions: The report describes the preliminary characterization of extracellular proteases of Aeromonas sp. establishing relationship with environmental conditions for their expression, namely concerning the effect of temperature, salinity and pH variations. These proteases can be important contributors to the virulence characteristics of Aeromonas. By providing new highlights on the behaviour of microorganisms in different environmental conditions we intend to contribute both for public and animal health, for instance on better and safety food production and on the development of fish vaccines.

SESSION 9 (Poster Session)
Establishing and Coordinating Outbreak Surveillance and Response

Saturday, February 24, 2007
Room: Klimt Ballroom 1
11:15–12:30

9.001 Role of Applied Epidemiology Training Program in Response to Outbreaks of Emerging Infectious Diseases in Central Asia

S. Ajeilat, **D. Nabirova**, M. Favorov. Central Asia Regional Office, Division of Epidemiology and Surveillance Capacity Development, Coordinating Office of Global Health, U.S. Centers for Disease Control and Prevention, Almaty, Kazakhstan

Background: Central Asia Region (CAR) is hyperendemic for emerging infectious diseases (e.g., HIV, tuberculosis, hemorrhagic fevers, and plague). In February 2003, CDC began a two-year Applied Epidemiology Training Program (AETP) in CAR with goals similar to CDC's Epidemic Intelligence Service: develop epidemiological capacity to detect and respond to public health threats, which include outbreaks of emerging infectious diseases.

Methods: AETP combines didactic and in-service training in evidence-based methods of field epidemiology, surveillance, communication, laboratory and computer skills, program evaluation, and applied research.

Results: To date, 64 outbreak investigations, 22 surveillance evaluations, and 7 research studies have been conducted; 5 studies are in process. 26 investigations focused on potential biothreat agents. When an avian influenza (AI) outbreak occurred among poultry in Kazakhstan, AETP fellows investigated a possible human case and assisted the Ministry of Health to develop an AI epidemic national preparedness plan. Similarly, brucellosis studies (233 cases in total) led to improved information sharing between human and veterinary surveillance systems, and initiation of health promotion activities. Three hemorrhagic fever investigations (57 cases) led to implementation of standard case definitions and laboratory tests to improve surveillance. Investigation of two leptospirosis outbreaks (257 cases) supported the need for better sanitation and water system repair. Three botulism outbreak investigations (61 cases) resulted in health education on proper food preservation. AETP investigation of HIV outbreak among children (76 cases) in Kazakhstan contributed to reorganization of blood collection system and strict infection prevention control in hospitals in the country.

Conclusions: As a functional part of the MoH, AETP promotes development of evidence-based public health in Central Asia. The program plays a major role in response to emerging infectious diseases through epidemiological investigations. Further AETP development will include strengthening of laboratory support for outbreak investigations.

9.002 High Isolation Hospital Beds for Patients with Highly Infectious Diseases: an Inventory of Resources in Europe

F.M. Fusco[1], V. Puro[1], P. Skinhoj[2], K. Ott[3], H. Siikamaki[4], H.R. Brodt[5], G. Sheehan[6], R. Hemmer[7], K. Mansinho[8], A.I.M. Hoepelman[9], P. Follin[10], M. Campins Marti[11], P. Brouqui[12], B. Bannister[13], G. Ippolito[1]. [1]National Institute for Infectious Diseases L. Spallanzani, Rome, Italy; [2]Rigshospitalet, Copenhagen, Denmark; [3]West Tallinn Central Hospital, Tallinn, Estonia; [4]Central Hospital Helsinki University, Helsinki, Finland; [5]Klinikum der Johann Wolfgang Goethe Universitaet, Frankfurt, Germany; [6]University College of Dublin Mater Misericordiae Hospital, Dublin, Ireland; [7]Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg; [8]Hospital de Egas Moniz, SA, Lisbona, Portugal; [9]University Medical Center, Utrecht, Netherlands; [10]Swedish Institute for Infectious Disease Control, Stockholm, Sweden; [11]Universitat Autonoma de Barcelona, Barcelona, Spain; [12]Faculte de Medecine, Universite de la Mediterranee, Marseille, France; [13]Royal Free Hospital, London, United Kingdom

Background: in order to prevent the spreading of Highly Infectious Diseases (HID) in the hospital setting, isolation measures are essential. According to the European Legislation, collective measures, i.e. structural and engineering characteristics, should have the priority over individual protective measures. In particular, the availability of hospital rooms equipped with negative pressure and anteroom is recommended. EUNID (European Network for Infectious Disease), an EC-funded network of experts in the management of HID, performed an inventory of hospitals provided with High Isolation Hospital Beds in participating countries, their placement and technical features.

Methods: a questionnaire was sent to partners. Answers were discussed and reviewed during the meetings of EUNID project. We define as High Isolation Room (HIR) a single or double room provided with at least negative pressure and anteroom.

Results: Data were obtained from Austria, Denmark, Estonia, Finland, France, Germany, Greece, Ireland Italy, Luxembourg, Portugal, Spain, Sweden, The Netherlands, United Kingdom. The hospitals with HIR with 6 air changes per hour (acph) are 92, for a total of 558 beds, while 88 hospitals have HIR with <6 acph, with 317 beds. In addition, 6 hospitals provided with HIR with -6 acph and direct connection with BSL 3–4 laboratory have a total of 14 beds, and 5 hospitals with HIR with < 6 acph and direct connection with BSL 3–4 laboratory, 11 beds. Hospital beds in HIR equipped with Intensive Care capabilities are 291. The HIR are mostly located in the same building as other hospital facilities, and are mostly sealed. The exhausting of the air is directly to outside with or without HEPA filtration. Detailed data are available for each country.

Conclusions: Overall, the number of hospital beds in HIR in the EUNID European countries appear adequate to manage the sporadic introduction of HID cases and small outbreaks. However, differences exist among countries, thus, to manage HID emergencies a transnational collaboration is required. Of note, larger outbreaks or epidemics could be difficult to handle with current HIR capacity, and should require the use of healthcare facilities other than HIR, preferably airborne isolation rooms without anteroom.

9.003 A Core Curriculum for Health Care Workers on Training in Management of Highly Infectious Diseases

A. Baka[1], F.M. Fusco[2], V. Puro[2], N. Vetter[3], P. Skinhoj[4], K. Ott[5], H. Siikamaki[6], H.R. Brodt[7], P. Follin[8], B. Bannister[9], G. De Carli[2], C. Nisii[2], J. Heptonstall[10], G. Ippolito[2]. [1]Hellenic Center for Infectious Disease Control, Athens, Greece; [2]National Institute for Infectious Diseases L. Spallanzani, Rome, Italy; [3]Otto-Wagner-Spital, Wien, Austria; [4] Rigshospitalet, Copenhagen, Denmark; [5]West Tallinn Central Hospital, Tallinn, Estonia; [6]Central Hospital Helsinki University, Helsinki, Finland; [7]Klinikum der Johann Wolfgang Goethe Universitaet, Frankfurt, Germany; [8]Swedish Institute for Infectious Disease Control, Stockholm, Sweden; [9]Royal Free Hospital, London, United Kingdom; [10]Scarborough and North East Yorkshire NHS Trust, Scarborough, United Kingdom

Background: Infectious Disease (ID) medicine is formally recognised as an independent specialty in most countries in Europe, and there is continuing debate about the ways in which the specialty should evolve. The European Union of Medical Specialists (EUMS), has agreed a core curriculum for training in clinical ID, updated in 2002, to facilitate the development of common standards of training within Europe. Similarly a core curriculum was developed by the Infectious Diseases Society of America. However, neither models mention training in preparedness for highly infectious disease (HID) emergencies, or the management of patients who have, or might have HID.

Methods: EUNID (European Network for Infectious Disease) is an EC-funded network of experts in the management of HID patients. Between April 2005 and April 2006, EUNID partners from Austria, Denmark, Estonia, Finland, Germany, Greece, Italy, Sweden and United Kingdom developed a consensus core curriculum and a prototype training course centred on this curriculum, through questionnaires, suggestions, comments, and amendments of draft documents shared between partners using the project web site, and a consensus meeting.

Results: The proposed course consists of two modules. Module 1 (knowledge), based on didactic teaching, aims to introduce the trainees to the clinical aspects of HID and their impact on public health, and to the principles of infection control (including selection and use of personal protective equipment, disinfection and waste management), through didactic teaching and class-based discussion. Module 2 (practical skills) consists of three skill stations (i.e. use of respiratory equipment and other personal protective equipment); a lecture incorporated into an on-site tour of a functioning high security infectious disease units, and four clinical scenario exercises, during which trainees work as a small groups to manage a patient or an outbreak. The assessment of trainee performance consists of a pre-course test, in-course assessment of performance in the skill stations and clinical scenarios, and an end-course test.

Conclusions: The EUNID curriculum and course have been developed to contribute in the creation of a common framework for the training of healthcare professionals in the EU in the management of patients with HID. Integrated with existing training curricula in Europe, it will also be relevant to doctors in other medical specialties and settings and to other groups of hospital-based healthcare workers who may provide care to a patient with HID.

9.004 Piper-the Pandemic Influenza Primary Care Reporting System for Scotland

L.E. Wilson, J. McMenamin. Health Protection Scotland, Glasgow, Scotland, UK

Background: Scotland has a number of influenza surveillance schemes, including flu-spotter practices, and enhanced surveillance general practices that submit clinical samples for virological testing. This information feeds annually into the European Influenza Surveillance Scheme. Information from the systems is seasonal, and limited geographically. The utilisation by Scottish community physicians (general practitioners) of the same administration system in over 80% of settings—the General Practice Administration System for Scotland—offers an alternative approach to influenza surveillance with some additional benefits. We aimed to develop an enhanced primary care surveillance system of influenza-like illness in Scotland, that would record influenza vaccine uptake and estimate vaccine effectiveness in season in real time.

Methods: A software programme was designed by Campbell Software to extract vaccine uptake data on influenza and pneumococcal vaccine. In the winter of 2005/6 this was used to report on vaccine risk groups and facilitate billing claims. PIPER, the **P**andemic **I**nfluenza **P**rimary car**E** **R**eporting system was designed as an extension of this system.

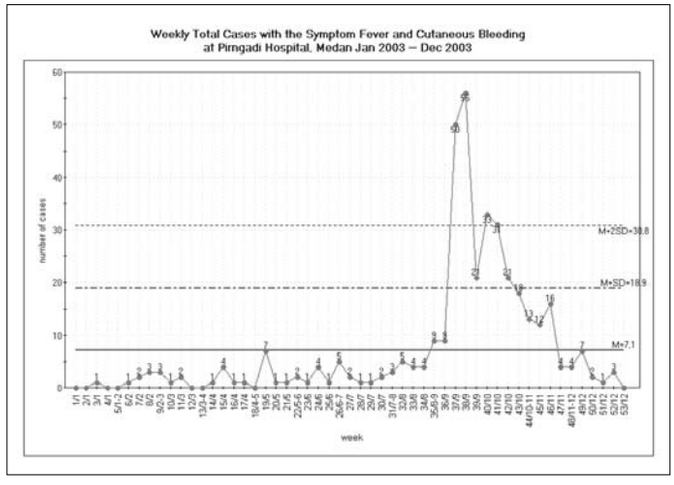
Results: READ codes (similar to SNOMED II), used in general practice to identify clinical concepts, have been selected to identify clinical risk groups, and symptoms. Reports on vaccine uptake by risk group are currently produced on a weekly basis. The PIPER database has been designed to allow integration of demographic variables, consultations for influenza-like illness and acute respiratory illness, antibiotic and antiviral medications and vaccine status. Data from general practice will be encrypted and transferred by e-links. The potential for daily automated extraction of data has been included. Piloting of the PIPER system is scheduled for January 2007.

Conclusion: PIPER has the capacity to support pandemic influenza planning by relating vaccine, antiviral and antibiotic effectiveness in the prevention of influenza-like illnesses and acute respiratory illnesses.

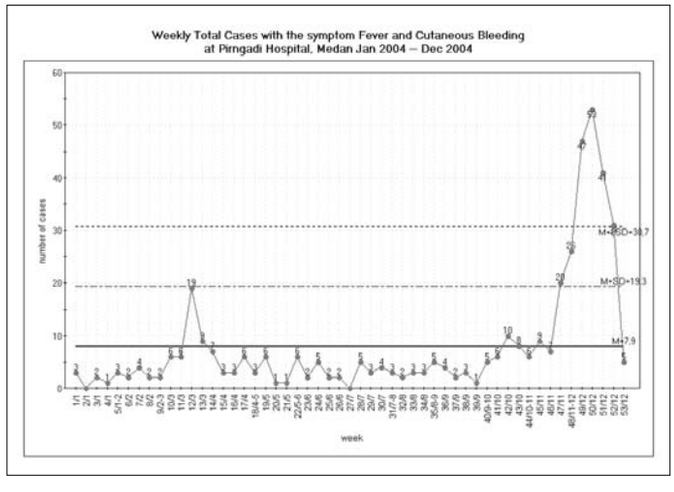
9.005 EWORS Utilization as a Complementary Surveillance Tool for Detecting Dengue Outbreaks

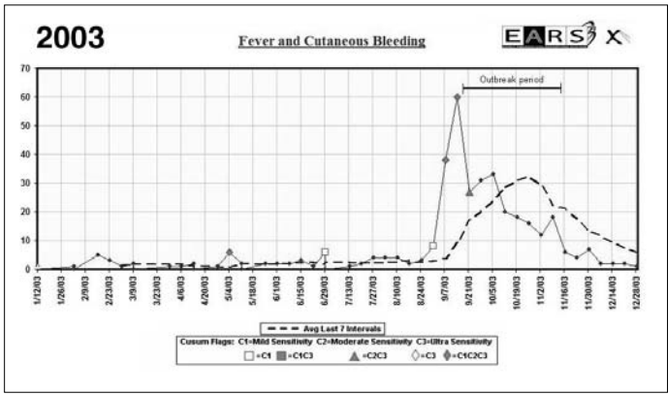
H. Siswoyo[1], **K. Laras**[2], A. Suwandono[1], E. Tresnaningsih[1], T. Adimidjaja[1], C. Simanjuntak[2], R. Larasati[2], J. Glass[2]. [1]National Institutes of Health Research and Development, Ministry of Health, Jakarta, Indonesia; [2]United States Naval Medical Research Unit No. 2, Jakarta, Indonesia

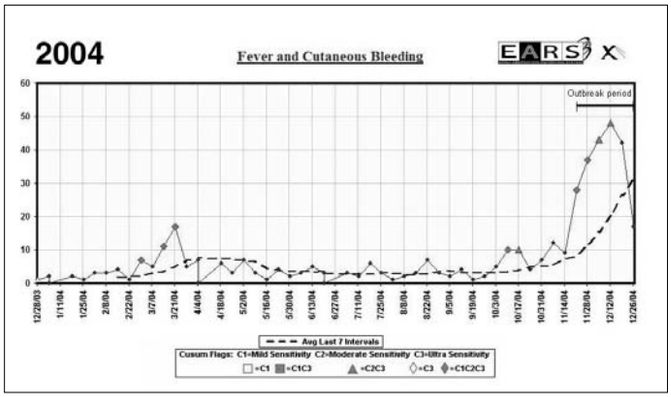
Background: Electronic syndromic surveillance for early outbreak detection may be a simple, effective tool to rapidly bring reliable and actionable outbreak data to the attention of public health authorities in the developing world. The Early Warning Outbreak Recognition System (EWORS) is a syndromic surveillance system that collects demographic and sign/symptom data on individual patients presenting at a hospital clinic or emergency room with suspected infectious disease. Data is emailed at least once a week to a central data management and analysis center. Data may be analyzed at the hospital or the hub for case count trends over time as well demographic trends in abnormal signals. The purpose of this project is to assess EWORS' efficacy to detect dengue hemorrhagic fever outbreaks.



Methods: EWORS data from RS Pringadi hospital in Medan, North Sumatra (2003–2004) was analyzed for hemorrhagic fever syndrome (fever and cutaneous bleeding) and compared to city-wide dengue data from the Medan health office (2003–2004). Daily counts of patients recorded in the EWORS database was analyzed using the US CDC's EARS algorithms—C1 (mild sensitivity), C2 (moderate sensitivity), and C3 (ultra sensitivity)—and historical mean ± 2 standard deviation (SD) threshold. The abnormal signals were compared with the outbreak alerts from Medan health office.







Results: From 2003 to 2004 the Medan health office declared dengue outbreaks on 17 September 2003 and 23 November 2004. Analysis using EARS detected significantly abnormal signals by C1, C2, and C3 as early as 7 September 2003 and 21 November 2004. From 2003 to 2004 there were 15 abnormal signals detected by EARS, 8 of which occurred outside the outbreak periods. Six of these signals were characterized by brief, unsustained increases in fever/cutaneous bleeding cases. In comparison, the historical mean ± 2 SD threshold detected abnormal signals starting 10 September 2003 and 2 December 2004. No abnormal signals were detected outside the outbreak periods.

Conclusion: This suggests that EWORS data analyzed for fever and cutaneous bleeding using the US CDC's EARS algorithms may be an effective tool at early detection of epidemic dengue in a community. More detailed analysis of data quality and comparison of the algorithms, in particular, evaluating other syndromes is further required to demonstrate its effectiveness as an early warning outbreak surveillance tool.

9.006 Epidemic Intelligence Activities in Emergency Operations Centres (EOC) in Europe-Risk Assessment and Communication Centre at ECDC

M. Ciotti, **P. Vasconcelos**, T. Van Cangh. ECDC, Stockholm, Sweden

Background: Within its mandate, ECDC is setting up a Risk Assessment and Communication Centre (RACC), to cooperate with the Members States (MS) and international partners regarding public health events. This facility will serve as the hub to coordinate and support epidemic intelligence (EI) activities as well as the investigation and response to outbreaks at European level. The goal is to establish a regular coordination of risk assessment and its integration in emergency management activities, filling a gap in the European public health framework; see figure below.

Methods: Activities were developed to ensure the deployment of the RACC and its contribution to the integration of risk assessment and risk management at European level, including a survey conducted among MS, regarding existing EOC capacities at National Surveillance Institutes. Simulation exercises involving MS and international partners are organized to test the RACC Public Health Event Operations Plan.

Results: Among the activities, answers to the survey provided by 21 countries, evaluated, among other aspects, the following:

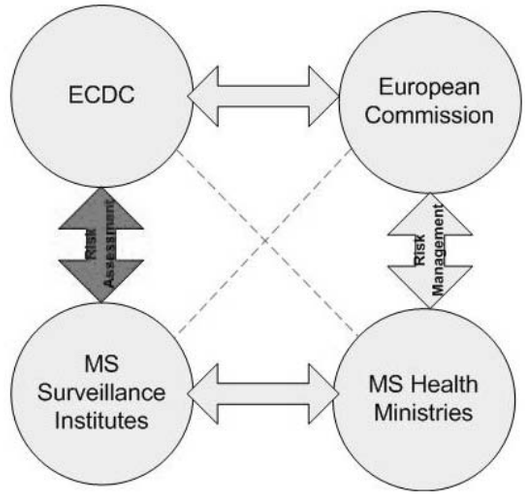
Facilities: Almost half of NSI (48%) have (19%) or will soon have (29%) their own EOC. One third (33%) of all NSI has access to another EOC in their country.

Tools and Equipment: Only 29% of all NSI have videoconference equipment. All of them have access to Internet and fixed telephone. Only 1 country (5%) has satellite communications.

Procedures: Only 10% have SOPs regarding communications with ECDC, 14% with WHO and 29% with the European Commission.

Security: 29% of the NSI reported that 1 to 5% of the information handled requires confidentiality.

Conclusion: To establish a RACC that will support a complete integration of risk assessment and risk management at European level, further improvement of procedures and equipment at both ECDC and MS is required. The result of this effort will certainly be shown with the development of simulation exercises.



9.007 Influenza Surveillance in the US Military in Europe: 2001 to Present

W. Dick[1], W. Aldous[2], A. Snyder[1], G. Kallstrom[2], J. Maza[1]. [1]U.S. Army Center for Health Promotion and PReventive Medicine Europe (USACHPPMEUR), Landstuhl, Germany; [2]Landstuhl Regional Medical Center Virology Laboratory, Landstuhl, Germany

Background: Laboratory surveillance of influenza is conducted in the United States European Command (USEUCOM) as part of the U.S. Department of Defenses (DoD) global influenza surveillance program. The USEUCOM area includes the largest number of Americans living and deployed outside of the U.S. (over 90 countries and territories, most are in Germany), and many service members and beneficiaries live off post in surrounding communities. The USEUCOM population has varying rates of influenza vaccine coverage (very high in service members, low in family members). Enhanced surveillance during 2006-2007 will yield a wealth of data to allow comparison of subtypes and circulating strains to those in Europe, the U.S., and the DoD overall. Clinical data on outpatient visits for Influenza-Like Illness at Military Treatment Facilities in the USEUCOM area complements laboratory surveillance, utilizing the Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE).

Methods: Descriptive epidemiological study design utilizing active laboratory surveillance and syndromic surveillance.

Results: The number of laboratory-confirmed cases detected in the USEUCOM has increased as the number of surveillance sites has grown, and the ratio of influenza type A:B has resembled that in the U.S. (2002–2003 season) as well as Europe (2005–2006). All 15 laboratory-confirmed cases in USEUCOM service members in 2005–2006 had

been previously vaccinated and were positive for type B; unlike the U.S., the circulating strain of influenza B in the European community was a different lineage than that in the 2005–2006 vaccine. Preliminary 2006–2007 data shows confirmed cases of types A and B in the USEUCOM, the DoD, the U.S., and in Europe.

Conclusion: Influenza surveillance in US EUCOM can identify outbreaks and determine circulating (sub)types and emerging strains, and contributes importantly to DoDs global program. USEUCOM surveillance activities also benefit host nations through data sharing and collaboration, which are critical for pandemic planning.

This research was supported in part by an appointment to the Postgraduate Research Participation Program at the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and USACHPPM.

9.008 Risk of Gastroenteritis After Participation in Triathlons in a River, Germany 2006

C.H. Winter[1], I. Piechotowski[2], A. Wiedenmann[3], U. Muller[4], **S.O. Brockmann[2]**. [1]Robert Koch Institute, seconded to Baden-Wurttemberg State Health Office, FETP Germany, Berlin, Germany; [2]Baden-Wurttemberg State Health Office, District Government Stuttgart, Stuttgart, Germany; [3]Administrative District Lörrach, Public Health Department, Lorrach, Germany; [4]Administrative District Heidelberg, Public Health Department, Heidelberg, Germany

Background: Sports activities like swimming in fresh waters can be associated with a significant risk of gastroenteritis. This was reported for microbiologically uncontrolled freshwaters as well as for bathing sites complying with current microbiological EU standards. We investigated a leptospirosis outbreak among participants of two triathlons with a 1.8 km swimming course in the river Neckar. In this context, the incidence of gastroenteritis and possible risk factors were examined. There is no officially registered and controlled bathing beach on the Neckar, and usually the water does not meet microbiological EU bathing water quality standards. Monitoring results in the week between the triathlons revealed values between guide and imperative levels of the EU standard 76/160/EEC.

Methods: A questionnaire was sent to all participants, which included questions about gastroenteritis symptoms following the triathlons and possible risk factors. Our case definition was diarrhoea, vomiting or nausea within 14 days after triathlon. The relative risk of gastroenteritis due to ingestion of river water was calculated. Fisher's exact test was applied to test for statistical significance.

Results: A total of 922 persons participated in the two triathlons. Of the 235 triathletes (25.4%), who responded to the questionnaire, 49 persons (20.9%) have suffered from gastrointestinal symptoms. Under the assumption of a maximum recall bias this would correspond with an attack rate of at least 5.3% in the entire cohort. 48 (92.3%) of the cases remembered to have swallowed water during the triathlon. Swallowing river water was associated with a five times higher risk of developing gastroenteritis (RR 5.3, Confidence Interval 1.1-25.1, p=0.03).

Discussion and Conclusion: Swimming in surface waters may be associated with an increased risk of gastroenteritis depending on the degree of fecal water pollution, even when current microbiological water quality standards are met. The presented data highlight that swimming in sewage contaminated surface waters correlates with a significant risk for gastroenteritis, which is concordant with other recent epidemiological studies. Furthermore this may coincide with severe and life-threatening illness like leptospirosis. Our findings demonstrate the necessity of keeping fecal pollution of recreational waters to a minimum, and show that appropriate risk communication to the public and to politicians deciding about standard regulations is essential.

SESSION 10 (Poster Session)
Agents of Bioterrorism

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

10.001 Biological Weapons Nonproliferation Status in the United States

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Background: Nearly 35 years after signing of the Biological Weapons Convention (BWC), biological weapons still pose significant threats to human health and political stability. Global biological security, tested by natural diseases such as AIDS, SARS and influenza, thus also is threatened by man-made influences and factors.

Methods: A selective review of pertinent "open-source" materials from the U.S. government, government auditors, and published news over the past few years was carried out. Items were reviewed for information viewed as being supportive of biological weapons non-proliferation (BWNP) efforts, or tending to enhance proliferation risks. The effects of science, national defense, and global politics on BWNP were evaluated. Specific items and their likely impact were discussed and an overall assessment was made of the status of BWNP in the U.S. The reviewers were 2 microbiologists and 1 physician.

Results: The synthesis of a polio virus in 2002 in the U.S. and the genetic modification of the mousepox virus that negated prior vaccination in 2001 in Australia illustrate the sort of scientific developments that are cause for concern to security analysts. In 2004, President Bush outlined four essential pillars of the US national biodefense program: threat awareness; prevention and protection; surveillance and detection; and, response and recovery. The section on threat awareness included two items that might be cause for concern, namely "Red Teaming" and "threat anticipation." In addition, there is a burgeoning increase in the construction of high-security biodefense laboratories. Global political calculations include lingering questions about BWC inspections and the apparent misuse of intelligence in the global "War on Terrorism."

Conclusion: The ISID should work energetically with other organizations to improve BWNP in the U.S. To quote from the invitational message to this meeting: "…members of ISID can make a difference with clinical recognition, global reporting, laboratory discovery, worldwide education, policy development, prevention and control."

10.002 Development of Diagnostic Microarray for Detecting Priority Biothreat Bacterial Pathogens

L. Papazisi, C. Sung, G. Bock, S. Ratnayake, C. Ong, B. Remortel, H. Howell, T. Minogue, M. Barsic, R. Fleischmann, **S. Peterson**. TIGR, Rockville, MD, USA

Sensitive diagnostics is a primary key in the line of defense against infectious agents. Accurate detection methods enable the healthcare network professionals to identify potential causes and sources of the outbreaks in order to take measures for containment, eradication or prevention. Here we describe the development of a diagnostic microarray technology aimed at identifying the unique genomic regions from select pathogens of NIAID Categories A, B, and C, as well as others of interest to the biodefense community. Genome sequences of biothreat agents were obtained from various publicly available databases. An in-house modified version of the Pick70 software was used to design specific 70mers. Oligomer sequences were subjected to BlastN searches against several nucleotide databases for filtering out non-specific 70mers. Our microarray contains

ca. 8000 genomic markers representing over 50 species. In addition, we have designed 14,000 markers for representing specifically genes known to be associated with virulence or conferring antimicrobial resistance. Oligomers were printed onto both aminosilane and epoxide slides. All DNA samples were labeled with Cy3 or Cy5 dyes for hybridizations. During ongoing extensive validation experiments, genomic markers have shown a sensitivity ranging 50-100% and a specificity as low as 93%. These results highlight the power of predictive bioinformatics approaches for genomic markers discovery, and, at the same time, they underscore the need for experimental validation.

10.003 Investigating of Bacillus cereus sensu Lato Diversity by Novel Gene Discovery (NGD) Approach Through Comparative Genomic Hybridization (CGH)

L. Papazisi[1], D. Rasko[2], S. Shallom[3], S. Ratnayake[1], G. Bock[1], T. Minogue[1], B. Jarrahi[1], J. Rilstone[1], E. Snesrud[1], J. Gilbert[1], J. Hasseman[1], Q. Sun[1], O. Økstad[4], A. Kosto[4], R. Fleischmann[1], **S. Peterson**[1]. [1]TIGR, Rockville, MD, USA; [2]University of Texas Southwestern Medical Center, Dallas, TX, USA; [3]Virginia Tech, Blacksburg, VA, USA; [4]U. Oslo, Oslo, Norway

Bacillus cereus (Bc) sensu lato members display a variety of phenotypes and pathological effects in their respective hosts. There is a growing body of evidence to suggest that genomic diversity within this group is relatively large and that exchange of genetic elements among members of this group are commonplace. In evolutionary terms, B. anthracis (Ba) has recently emerged from within this group as a clonal complex displaying remarkable homogeneity. Given the flow of genetic information within this group, we were motivated to define novel genes for the purposes of creating a species DNA microarray to better understand the ancestral relationships that exist among its members. We conducted novel gene discovery approach on short insert random genomic libraries from nine diverse Bc strains. The amount of DNA sequence characterized among strains ranged from 104 kbp to 3.4 Mb. Approximately, 2/3 of these genomic fragments had a no database species hit. Among the strains investigated, the number of "unique ORFs" relative to Ba strain Ames varied between 19 to as many as 1054. Based on this large amount of sequence information we have designed the first Bc/Ba species microarray containing 30,000 genomic markers. We will present an analysis of these data along with a preliminary demonstration of the use of an expanded species DNA microarray in a large-scale comparative genome hybridization study.

10.004 Investigating the Genomic Diversity of Yersinia pestis and Y. pseudotuberculosis Clade Members through Novel Gene Discovery (NGD) Approach Utilizing the Microarray Technology

T. Minogue[1], **L. Papazisi**[1], S. Ratnayake[1], M. Barsic[1], G. Bock[1], M. Nikolich[2], R. Zsigray[3], R. Brubaker[4], L. Lindler[2], R. Fleischmann[1], **S. Peterson**[1]. [1]TIGR, Rockville, MD, USA; [2]DHS, Washington, DC, USA; [3]U. New Hampshiere, Durham, NH, USA; [4]Michigan State University, East Lansing, MI, USA

Novel gene discovery (NGD) was applied to investigate the extent of Y. pestis (YPE) and Y. pseudotuberculosis (YPTB) species diversity. Six YPE, Pestoides A, B, C, D, E and J, and three YPTB, serotypes IB, IV and III were chosen to represent the most diverse clades within the species. Short insert random genomic libraries were spotted onto the microarrays and screened by hybridization. Upon the normalization of the hybridization signal ratios, a probabilistic approach was utilized to identify the vectors containing inserts with novel sequences. This method proved to be highly sensitive. Among the strains investigated, the number of relatively unique ORFs ranged between 15 -1032. A significant number of novel read sequences from all nine genomes had no database species hit. This was also reflected in the finding that the hypothetical genes constituted the majority of the novel genomic content discovered. The greater part of the rest of the sequences showed significant homology to those originating from the Enterobacteriaceae family members. Mobile extrachromosomal elements, transporters, genes products involved in the DNA metabolism, protein fate and synthesis constituted the majority of ORFs with predicted functions. The data obtained from the NDG are allowing us to understand the relationship between YPE and YPTB, and elucidate aspects of their genome evolution.

10.005 World Health Organization: Global Laboratory Directory and Network: Anthrax and Tulaeremia

R. Leuenberger, O. Cosivi, M. Chu. World Health Organization, Geneva, Switzerland

The 2005 International Health Regulations require countries to establish capacity to manage 'Public Health Emergencies of International Concern, irrespective of their origin or source', and provides the legal framework for international cooperation on outbreak alert and response. WHO's newly established Bio-Risk Reduction programme promotes a strategy to manage the consequences of the emergence of infectious agents across the entire spectrum from natural occurrence, accidental release, to possible deliberate use. Key activities are (i) development of the Global Laboratory Network Directory (GLaDNet) which aims at enhancing the international cooperation between laboratories to be better prepared to respond to outbreaks; (ii) development of standards and guidelines on laboratory biosafety, laboratory biosecurity, and the management of disease specific risks (anthrax, tularaemia, brucellosis); (iii) raising awareness among the public health and scientific community about the potential misuse of life science research; and (iv) strengthen the collaboration between health and security sectors at national and international levels to ensure efficient public health response whenever needed. One component of the GLaDNet is working with laboratories that handle high risk pathogens. Its purpose is to provide (i) tools for generating fast, accurate and internationally comparable laboratory results; (ii) a mechanism to share information and specimens during outbreaks; (iii) a repository for the collection of epidemiological data on the occurrence of specified pathogens worldwide and regular reports thereof; and (iv) support for an effective and representative international decision-making process for setting research priorities. A first step in achieving these goals was the survey of WHO member state laboratory capabilities in relation to the diagnosis and detection of B. anthracis carried out in 2003. A second survey round is being designed with a view to update the information provided by the 121 participating laboratories, to include additional laboratories, and to map existing networks. By its efforts to increase global collaboration between laboratories, GLaDNet works to increase global equity in the access to best practice disease-specific information, efficient laboratory diagnostics, and the decision-making process for setting research priorities.

10.006 Developing and Maintaining Evidence-Based Guidelines for Bioterrorism Agents

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Background: In response to the heightened awareness and perceived threat of bioterrorism agents, the Health Protection Agency (HPA) first published evidence-based guidelines to the CDC Category A agents on its website in 2001. The guidelines are intended for healthcare, laboratory and public health professionals to guide clinical and public health action in the event of a deliberate release. The challenge is to maintain good quality practical guidelines for use in the event of a UK-based incident.

Methods: Each guideline is written by a small team of clinical, laboratory and public health practitioners with specific expertise. The guidelines are maintained and developed through continual monitoring of the scientific literature (e.g. PubMed, Science Direct) and other information sources, including websites (CIDRAP, ProMed). Guidelines may also change and evolve because of decisions made at relevant national committees, through Exercises (planning and lessons identified), through evidence

gathered by related projects, or through comments from healthcare, laboratory and public health professionals. The original named experts are involved in any major review of the guidelines and the guidelines undergo a planned yearly review. The work is overseen by the Deliberate and Accidental Releases (DAR) website Steering Group.

Results: The guidelines which are published on the HPA DAR website have continued to develop and improve. There are guidelines covering anthrax, smallpox, botulism, plague, tularemia, viral haemorrhagic fevers, brucella, Q-fever, glanders and melioidosis. The guidelines can be triggered in the event of an incident to give clear advice on public health management, including advice on treatment and prophylaxis for these agents. The website has also developed to include general documents for the investigation of 'unusual incidents' and much of the information available is also relevant when considering naturally occurring outbreaks or accidental releases.

Conclusion: The practical evidence-based guidelines on the DAR website raise awareness of bioterrorism threat agents and provide practical advice on clinical, laboratory and public health action for healthcare professionals in the event of an incident involving these organisms.

10.007 The Survival and Transmission potential of CDC Category A Bioterrorism Agents

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Background: A comprehensive understanding of the survival and transmission potential of Category A bioterrorism agents is essential for health protection planning and guidance. The information can be found in publications many of which utilise and extrapolate from the same historical studies but vary in their interpretation. This study reviewed the available data in the scientific literature to collate and analyse the evidence for survival, transmission routes and transmission risks of these organisms.

Methods: The organisms responsible for anthrax, tularemia, plague, smallpox and viral haemorrhagic fevers (VHF—specifically Lassa, Ebola and Marburg) were included. Literature searches were on organism names (current/ previous) and key words relevant to survival (survival characteristics in soil/pasture, water/liquids, carcasses/tissues, body fluids, fomites, physical inactivation and disinfection of organisms) and transmission (infectious dose, transmission routes, and transmission risks).

Results: In carcasses, tissues and body fluids, anthrax spores and smallpox virus can survive for years, while tularemia, plague and VHF organisms remain viable for weeks to months. On fomites anthrax spores and smallpox virus persist (years), VHF viruses survive for weeks, and tularemia and plague bacteria survive poorly (hours-days). In the environment, only anthrax spores persist long-term and although there is evidence that tularemia bacteria can also survive (weeks) in the environment these bacteria are susceptible to heat and sunlight. Plague bacteria and the viruses (smallpox and VHF) are rapidly inactivated in the environment being susceptible to heat, sunlight or drying. All the organisms are susceptible to chlorine disinfectants. Anthrax, tularemia and smallpox have infectious doses that may be as low as 10 spores/ organisms. VHF viruses are also likely to have a low infectious dose but actual dose is unknown. All of the agents have the potential to be transmitted by inhalation (rare in smallpox), inoculation, or ingestion (of VHF only Lassa). However, only plague, smallpox and VHF show person-to-person transmission. There is documented evidence for autopsy transmission for all these agents except anthrax for which there is evidence from animal necropsy.

Conclusion: Documenting the survival and transmission characteristics of Category A bioterrorism agents is invaluable to assessing risk, developing guidelines for management and health protection planning.

10.008 Impact of Residual Immunity Due to Vaccination in the Past on the Dynamics of Smallpox Epidemics

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Background: In France, smallpox vaccination was compulsory until 1978. Thus, a fraction of the population, vaccinated in the past, could still be fully or partially protected against smallpox. In case of a deliberate reintroduction of the virus, the residual immunity (RI) would impact the susceptibility of vaccinated individuals but also the severity of illness. Partially protected cases could probably develop milder symptoms and could be less infectious than unvaccinated cases. However, these milder cases may be recognised later which would contribute more to disease spread. We studied the impact of RI on dynamics of smallpox epidemics and on efficacy of control interventions.

Methods: We propose a stochastic compartmental model which takes into account different levels of RI of individuals (immune, partially immune, entirely susceptible). We considered that infected cases would develop mild or severe illness with different probabilities according to their level of immunity. This model allows the simulation of contact tracing, isolation of cases, ring and mass vaccination. We studied the impact of varying the isolation rate of severe or mild cases on the simulated epidemic size. All the epidemics were simulated starting with 20 index cases and included a ring vaccination intervention.

Results: Considering that 50% of people vaccinated are only partially immune and the other 50 % fully protected (based on available immunological data), we show that taking into account RI leads to smaller epidemics. If the isolation rate of mild cases was low (20% per day), isolating at least 40% of severe cases per day could allow to control an epidemic. Assuming that mild cases would not be isolated and that they would be as infectious as severe cases, we simulated uncontrolled epidemics. However, when we increased this isolation rate to 10%, the simulated epidemic size dropped significantly.

Conclusion: Overall, the impact of RI appears beneficial on the dynamics of a smallpox epidemic. However our results are very sensitive to the assumption on the relative infectiousness of mild cases compared to severe cases, the fraction of vaccinated individuals partially protected and the relative risk of developing severe disease for partially immune cases. Results of ongoing immunological studies in France will help to refine parametrization of our model and improve the validity of our conclusions.

10.009 Anthrax in South Tyrol, Italy

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Background: In Italy human cases of Bacillus anthracis (BA) are rarely notified. Here we report a case of a farmer who developed cutaneous anthrax after butchering diseased animals without protection.

Methods: Between December 21–29, 2005 a total of 7 animals died on a remote farm in South Tyrol Italy within few hours after onset of symptoms. Three days after contact the 57a old farmer developed an inceasingly severe lesion on the hand for which he sought medical advice one week after the beginning of symptoms. Laboratory diagnosis was made from a smear from the leasion and organs (spleen) of the diseased animals using APICH50 biochemical identification system (Merieux) as well various molecular methods including PCR's for toxin and capsule genes, 16S-rDNA sequencing and RFLP using Qualicon Riboprinter.

Results: Initially typed as B.cereus by the Columbia Agar and API biochemical system, PCR amplification of the protective antigen and capsule genes rapidly revealed BA as species diagnosis which was confirmed by DNA sequencing. Environmental investigation revealed 2

possible sources: Contamination of the local area may exist, as there have been non laboratory confirmed outbreaks on the same farm in the late fifties. The import of hay from a region in northern Italy where animal cases or BA occured may be another possibility, although tested hay samles remained negative for BA spores.

Discussion: The identification and antibiotic prophylaxis of all exposed individuals including the veterinarians performing necropsies as well as the wife of the farmer who presented with unrelated flu like symptoms shortly afterwards was another challenging issue in the present case.

10.010 Biochemical Phenotyping of Burkholderia (B.) mallei, B. pseudomallei and 34 other B.-Species and Construction of a new Identification System

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The genus Burkholderia includes two highly pathogenic agents. In most countries they are classified as biolevel 3 agents and are potential bioterrorism agents. B. mallei causes glanders in solipeds and is a life-threatening zoonosis. B. pseudomallei is responsible for the potentially fatal disease melioidosis. Due to the importance of these bacteria and their potential world-wide distribution, the reliable identification and correct differentiation of these two very closely related pathogens is essential. To date no test for the routine laboratory exists.

The present study was designed to create the basics for an optimized 96 well microtiter plate for the identification of B.-species. 570 biochemical reactions (including 191 amino acid substrates, 191 carbohydrate substrates and 188 enzyme substances) in 160 strains belonging to 36 Burkholderia-species were investigated by means of a miniaturised method (experimental Taxa Profile Plate-System, Merlin, Bornheim-Hersel, Germany).

In total, 44 biochemical reaction groups (taxons) could be discriminated. In five species, including B. mallei and B. pseudomallei, internal homogenous taxons were detected. Every taxon can be clearly assigned to a species and correctly diagnosed. From the 570 biochemical reactions, 88 enable the differentiation of 44 distinct taxons. For 14 taxons a single key reaction could be described. Based on these reactions a 96-well-microtiter-plate was constructed. This optimized, cheap and fast (24h) identification system allows the correct identification of unknown Burkholderia-isolates and can be used in routine laboratory applications.

10.011 Replication of Vaccinia Virus on PBMC is Restricted by Innate Immunity Mediators

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Background: No detailed studies have been conducted on interactions of poxviruses with the immune system, relevant to clarify pathogenic mechanisms of poxviruses infection, and to improve poxvirus vaccine vectors and immunomodulatory strategies. Particularly, the cellular targets for infection and the consequences of infection for cells involved in the generation of antiviral immune responses are not clearly understood.

Methods: The ability of Vaccinia Virus (VV) to productively infect normal PBMC and different cell subpopulations (CD14+, CD4+/CD8+ and CD4-/CD8-) was determined by back titration of infectivity and quantitative detection of viral genome in kinetic studies. In the same experiments the extent of induction of IFN alpha and/or gamma, was measured at both mRNA and protein level.

Neutralizing antisera against IFN alpha and gamma were used to establish if the low replication rate of VV in PBMC is due to the activation of the IFN system.

Results and Conclusions: VV replication efficiency in PBMC is generally low, and is restricted to few PBMC subpopulation (namely CD14+ and CD4-/CD8-), while resting T cells did not support VV replication. Neutralizing antibodies to IFN alpha and gamma strongly increased the replication ability of VV on CD14+ and CD4-/CD8- cells, indicating that these cytokines are induced by VV, and are at least partially responsible for restricted replication in PBCM. Our findings indicate that mediators of innate immunity, such as IFN system, are important factors in the control of poxvirus replication in white blood cells.

SESSION 11 (Poster Session)
Animal Reservoirs for Emerging Pathogens

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

11.001 Experimental Infection of Jungle Crows (Corvus macrorhynchos) with West Nile Virus

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West Nile virus (WNV) is distributed in Africa, the Middle East, parts of Europe, South and Central Asia, Australia, and North America. Although the virus is not distributed in East and Southeast Asia where Japanese encephalitis virus is endemic, the introduction of WNV into these areas would be one of major threats for public and animal health. Wild birds are amplifying hosts of WNV, and the family Corvidae includes many species that act as important reservoirs in North America (e.g. American crows, blue jays). Therefore, Corvidae birds in Asia could also be susceptible to WNV. To investigate the possibility that jungle crows (Corvus macrorhynchos), which inhabit widely in East and Southeast Asia, would be reservoirs of WNV, experimental infection to these birds was performed. Six jungle crows were subcutaneously inoculated with WNV NY99 strain (10^3 plaque-forming units (PFU) / head). Blood, oral swabs, and cloacal swabs were collected at 0 to 7, 10, 14 days post inoculation (dpi). Viremia and viral shedding were titrated by plaque assay on Vero cells, and viral distribution in major organs was examined by TaqMan reverse transcriptase-polymerase chain reaction. All six crows showed clinical signs such as depression and anorexia. Totally, five crows died within 7 days after inoculation. Only one crow did not die and recovered, though this bird showed severe clinical signs. Viremia was observed in six all, and the viral titer in these samples reached from $10^{6.5}$ to $10^{10.9}$ PFU/ml. The virus was also detected in oral and cloacal swabs, and viral RNA was detected in major organs. These data revealed that jungle crows would play an important role as reservoirs of WNV both by mosquito-based transmission and non-vector transmission (e.g. aerosol, cannibalism) in East and Southeast Asia, in the case of introduction of WNV into these areas.

11.002 Impact of Stray Dogs and Rodents in the Endemicity of Bacterial and Parasitic Infections among Communities in Antigua

O.O. Dipeolu. American University of Antigua, St. John's, Antigua & Barbuda

Previous investigations showed endemicity of infectious diseases among populations in the Antigua, a Caribbean island. It was also observed that households usually treat their dogs as stray dogs and many homes are infested with houserats, lizards and houseflies. This investigation was undertaken to elucidate the impact of these observations on the prevalence of infectious diseases in the island.

The first phase of investigation involved laboratory processing of specimens collected monthly from 184 households for 18 months. These included stools from household members and their dogs; nose, throat and ear swabs of household members and materials from dissected rats, lizards and houseflies caught inside the homes. The second phase

involved laboratory processing of soil samples collected weekly for 18 months from popular beaches and community public parks to which stray dogs usually have access. The third phase involved laboratory processing of stools deposited on various days by 311 stray dogs at previously identified scavenging spots.

Commonly found in the stools of household members, stray dogs, cultured materials from dissected rats, lizards and houseflies were cysts of Entamoeba histolytica and bacteria species, including Escherichia coli, Klebsiella pneumonia and Proteus mirabilis. The different bacteria strains demonstrated similar biochemical, physiological and serological characteristics. Larvae of Strongyloides stercoralis were commonly encountered in the stools of household members and stray dogs. Numerous eggs of Toxocara canis and Ancylostoma braziliensis were found in the stools of most of the stray dogs and in the soil samples.

It was concluded that stray dogs, rats, lizards and houseflies are sources of acquisition of some bacteria and parasites by humans in Antigua and are threats to community health Public health education is essential to create sanitation awareness among the community populations and to persuade households to care for their dogs instead of allowing them to roam and scavenge as stray dogs.

11.003 Genetic Characterization of West Nile Virus Strains Circulating in Central Europe

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Background: West Nile virus (WNV) is the most widespread member of the Japanese encephalitis virus (JEV) complex of flaviviruses. The first strain was isolated from a human patient, but later the virus was also detected in mosquitoes, horses, and other hosts in the eastern hemisphere. In 1999, WNV emerged in the USA as well. WNV strains form at least two main phylogenetic lineages: lineage 1 is composed of strains from all around the world, while lineage 2 strains were previously isolated exclusively from sub-Saharan Africa and from Madagascar. The objective of this work was to identify WNV strains, which recently emerged in central Europe, and to reveal the origin and genetic relatedness of these strains.

Methods: The complete genome sequences of three virus strains were determined using RT-PCR-based amplification of overlapping genome fragments and direct sequencing of the amplicons. The nucleotide (nt) and putative amino acid (aa) sequences were submitted for phylogenetic analyses. Strain 97-103 [Rabensburg virus (RabV)] was isolated from Culex pipiens mosquitoes in 1997 in the Czech Republic; strain Hungary-2003 was detected in encephalitic geese in 2003 in Hungary; Hungary-2004 was isolated from a goshawk (Accipiter gentilis) which died due to CNS symptoms in 2004. Further central European isolates were also partially characterized during the investigations.

Results: The goose-derived, Hungary-2003 strain exhibited closest genetic relationship (~98% nt, ~ 99% aa identity) to WNV strains isolated in 1998 in Israel and to the strain that emerged in 1999 in New York. The Hungary-2004 strain showed the highest identity (~96% nt, ~99% aa) to lineage 2 WNV strains isolated in central Africa. The same strain re-emerged in 2005 at the same location in goshawks, in a sparrowhawk (Accipiter nisus) and in a sheep. The 97-103 isolate (RabV) shared 75%-77% nt and 89%-90% aa identities with WNV lineage 1 and 2 strains. Phylogenetic analyses demonstrated that RabV has to be considered either a new lineage of WNV or a novel flavivirus of the JEV group.

Conclusion: The investigations revealed that WNV strains belonging to different genetic lineages are circulating in central Europe. Improved diagnostic methods should be applied by the local public health and veterinary laboratories to detect both lineage 1 and 2 WNV strains in routine diagnostic submissions. Investigations on the occurrence, ecology, and epidemiology of WNV strains circulating in central Europe must be of high priority.

11.004 Risks of Infection Associated with Dogs Visiting Hospitalized People

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Background: The visitation of hospitalized people by pets, a form of animal-assisted activity (AAA), is common in North America. While the benefits of this practice are well documented, the risks of infection (both to and from pets) have not been adequately explored. We set out to characterize the risks of dogs acquiring select hospital-associated pathogens through visitation programs in Ontario and Alberta, Canada.

Methods: Both a prospective cohort study and a nested case-control study were conducted. In the cohort study, 2 groups of dogs were enrolled: 100 dogs that visit people in healthcare facilities (the "exposed") and 100 dogs that participate in other types of AAA but do not visit healthcare facilities (the "unexposed"). Between May 2005 and November 2006, fecal specimens and nasal swabs were collected from each dog every 2 months for 1 year, and submitted to researchers along with a brief log of places visited when in the role of AAA dog, antimicrobial use within the home, dog health status and diet during the period under surveillance. Specimens were cultured for 4 bacterial species frequently linked to human hospital-associated infections: methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), multidrug-resistant E. coli and Clostridium difficile. On completion of the cohort study, a pre-tested questionnaire was mailed to owners of all test-positive (case) and test-negative (control) exposed dogs, inquiring about patient and dog practices during routine visits.

Results: Approximately 90% of the animals completed the cohort study, with attrition rates similar between the exposed and unexposed groups. Analysis of the cohort data showed a trend for exposed dogs to be at greater risk, relative to unexposed dogs, for acquiring certain pathogens. No association between antimicrobial exposure and the acquisition of drug-resistant bacteria was identified. All owners of exposed dogs who were mailed a questionnaire at the end of the study returned it fully completed. Their answers revealed that all of the exposed dogs that had tested positive for MRSA or VRE routinely licked patients as part of their therapeutic visits.

Conclusion: The finding that dogs can acquire hospital-associated pathogens during their interactions with hospitalized people stresses the need for explicit infection control practices to address the potential spread of pathogens through canine visitation programs.

11.005 Developing Animal Challenge Models for Therapeutic Studies Involving Agents of Bioterrorism and Emerging Diseases: Monkeypox, and H5N1 Influenza

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Background: Concerns over the potential use of Variola as a bioterrorist weapon, and newly emerging viruses such as H5N1 influenza becoming serious human and animal health concern have led to developing new countermeasures to protect against these agents. The ability to truly test vaccine efficacy hinges on the appropriate animal model as well as the ability to develop model systems that utilize various inoculation routes. Several critical milestones have lead to enhancements of the smallpox and H5N1 influenza animal models.

Results: Recent data illustrating clinical profiles of monkeypox (Zaire 79) infected monkeys implanted with telemetric devices to monitor full cardiac and respiratory physiology, body temperature, and activity have contributed invaluable information, extending our understanding of disease progression. Telemetric monitoring has significantly enhanced the

breadth and quality of current models and parameters for measureing smallpox vaccine efficacy, and clinical parameters of monkeypox virus infection in cynomolgus monkeys. Data presented demonstrates the severity of illness developed in both respiratory (intranasal) and non-respiratory (intravenous) challenge routes and contributes to the information on disease progression and pathogenesis of this diseases and animal model. When evaluating lethality and mechanism(s) of pathogenesis in mice and ferrets using the intranasal infection route for highly pathogenic H5N1 (A/Vietnam/1203/04 strain), we demonstrated our capability to support medical countermeasure and detection/ diagnostic testing for highly pathogenic avian influenza studies. Following IN challenge, virus recovery from nasal washes and weight loss showed (trend) correlation with challenge dose and dose dependent changes were demonstrated in multiple hematology parameters and clinical (serum) chemistry parameters, especially in liver enzymes. Additionally, we have successfully executed vaccine efficacy trials in ferrets and mice challenged with H5N1.

Conclusions: Our studies have defined both basic pathogenesis and immune responses in these animal models necessary to conduct preclinical and pivotal efficacy studies under the FDA Animal Rule.

11.006 **Tularaemia (**Francisella tularensis holarctica**) in two Squirrel Monkeys (**Saimiri sciureus**) from a Swiss Zoo and Zoonotic Infection of the Veterinary Pathologist**

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Septicemic tularaemia was diagnosed at necropsy in two squirrel monkeys from a Swiss zoo. The monkeys were kept in the same enclosure and died suddenly within one week without prior clinical symptoms. In both animals pathological investigations revealed multifocal acute necrosis associated with bacterial colonies in the lung, liver, spleen, heart and kidney, and focal cerebral gliosis in the brain.

Francisella tularensis holarctica was isolated on bacteriological culture in the liver, spleen and kidney from one animal, whereas E. coli overgrowth was shown in the second monkey. Both animals proved PCR-positive for Francisella tularensis in several organs.

Two days after the necropsy, the pathologist (who did not wear a mask during the examination of the monkeys) developed acute fever which resolved spontaneously within 24 hours. In the following week, the pathologist suffered from progressive thoracic pressure and slightly reduced general condition. A clinical examination eight days after having performed the last necropsy revealed slightly enlarged cervical lymph nodes without further pathologic findings. A CT scan of the chest revealed enlarged hilar and mediastinal lymph nodes and a small nodular infiltrate in the left lower lobe of the lung. The blood exams were in the normal range with the exception of a moderately (87mg/L) elevated level of the C-reactive protein (CRP). The serology for Francisella tularensis was initially negative, however, the repeated serologic examinations showed a seroconversion 3 and 5 weeks after the last necropsy (titers 1:80 and 1:160 by a microagglutination assay, respectively).

Ciprofloxacin was prescribed for 10 days, and 3 weeks later the CRP-level and the CT scan were normal. During the therapy with ciprofloxacin, the pathologist developed a phototoxic reaction which resolved completely after cessation of the drug.

Tularaemia is very rarely observed in wildlife or zoo animals in Switzerland. The last described case was observed in a captive golden-headed lion tamarin (Leontopithecus chrysomelas) in 2004.

In the present case, the veterinary pathologist, most likely, got infected by aerosol inhalation at necropsy of the monkey infected with Francisella tularensis holarctica. The relatively mild clinical course in the present case is typically observed during infections with Francisella tularensis holarctica, as opposed to the typically more severe clinical course of infections with Francisella tularensis tularensis.

11.007 **Illegal Bushmeat Importation into the United States, 2005–2006**

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Background: Bushmeat derived from wild animals is a common source of protein and sustenance in Africa. However, the hunting, preparation, consumption, and global distribution of some types of bushmeat pose a risk for transmission of potentially fatal zoonoses such as monkeypox and Ebola. In the United States, the Centers for Disease Control and Prevention (CDC) restricts the importation of bushmeat made from African rodents, nonhuman primates, and bats on the basis of human health concerns. Understanding the patterns of smuggling of these products may help direct control and educational efforts.

Methods: CDC Quarantine Stations at 18 major points of entry, along with other federal partners, help enforce U.S. regulations restricting the importation of bushmeat. As part of recording daily activities, CDC Quarantine Stations may enter information about seizures of prohibited items into a secure web-based system, QARS (Quarantine Activity Report System). We queried QARS and reviewed reports pertaining to CDC-restricted bushmeat for the period October 1, 2005, through September 30, 2006.

Results: During this period, 50 reports of bushmeat seizures were recorded. The mean weight of seized products per report was 4.1 kg (range 1-13.7 kg). The most common animal identified was grasscutter/cane rat (19 reports, 38%), followed by nonhuman primates (6 reports, 12%), porcupine (2 reports, 4%), and bats (2 reports, 4%). Three African countries accounted for the majority of reports: Ghana (16 reports, 32%), Nigeria (13 reports, 26%), and Cameroon (8%). The ports of entry with the highest number of reports were Newark (24 reports, 48%), Detroit (12 reports, 24%), and Atlanta (8 reports, 16%). Most reports involved passengers from Africa entering the United States via a connecting flight from the Netherlands (22 reports, 44%).

Conclusions: Because QARS reflects only reports received and selected for data entry by CDC Quarantine Stations, the actual amount of bushmeat entering and being seized at U.S. ports is likely much higher. Most bushmeat is associated with travelers coming from West Africa, especially those transiting through the Netherlands. Passenger and cargo inspections targeting these flights can increase the detection of smuggled products. Educational campaigns targeting travelers in their home countries may help heighten awareness of the global health implications of the bushmeat trade and deter smuggling attempts.

11.008 **West Nile Virus Transmission in Resident Birds, Italy**

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Background: Given the speculation that West Nile Virus (WNV) may be disseminated by migrating birds, we hypothesized that the virus would be re-introduced to the largest inland marsh in Italy. Accordingly, and in the framework of activities of the EDEN European project(Emerging Diseases in a changing European eNvironment), we sampled apparently healthy birds there for evidence of locally acquired WNV infection.

Methods: The serosurvey was conducted from April through October 2006 on a total of 599 blood samples from 586 birds belonging to 31 species of passerines and 7 other bird species. Trapping took place using mist nets at the 'Le Morette' study site (N43.80753; E010.81476)(Padule di Fucecchio, Tuscany) which is part of the largest inland marsh in Italy. Morphological characteristics were used to determine species, sex and age of the birds. The migratory or resident status of each bird was determined on the basis of standard references. For species that had both migratory and resident populations, we based status assessment on breeding conditions.

Blood was collected only from birds weighing more than 9 g to have a sufficient amount of serum to be tested. Serum samples were stored at +4°C until taken to the laboratory where they were screened for WNV-neutralizing antibodies by virus neutralization test (VNT) according to standard methods. A 90% neutralization was used as the criterion for a positive test result.

Results: Of the 599 blood samples, 13 were from birds bled twice and 17 were of an insufficient volume to be analysed. Only one bird, an Italian sparrow, adult, resulted seropositive (0,17%, 95% confidence interval 0,04% to 0,9%) with a VNT-titre of 1:10. A total of 6 Italian sparrows were sampled so that the prevalence among this species of birds was 16,7%, 95% confidence interval 3,7% to 57,9%.

Conclusion: Although the seropositivity is not evidence of recent infection, we presume that the seropositive bird was infected locally because the species involved is not migratory.

The evidence of local WNV transmission in Central Italy indicates risk for West Nile fever and meningoencephalitis in the human, equine, and avian populations of Italy, as already hypothesized in 1998 after the WNV outbreak caused by a Senegalese strain probably introduced by migratory birds.

We therefore suggest that WNV be considered in the differential diagnosis of humans and other vertebrates with neurological signs of disease in Italy, also to avoid underestimation of the real prevalence of the disease.

11.009 Pet Amphibian Keeping in Central Europe: Underestimated Contagious Hobby

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Background: Amphibians are increasingly popular pet animals in European households despite their mostly unknown capacity to transmit diseases associated with animal keeping. This study was dedicated to clarify the current epidemiological condition of pet amphibian keeping in Austria.

Methods: Since 1995 pet amphibians from Austrian keepers, mostly frogs and newts, were dissected when deceased; tissue samples, more than 300 fecal samples, and biofilms from the terrarium facilities were tested for identification and characterization of parasites, bacteria and fungi potentially co-infecting man and amphibians.

Results: Such pathogens identified belonged to the taxa Cryptosporidia, free-living, potentially pathogenic ameba (Acanthameba), Salmonella enterica, Weeksella virosa, and Mycobacterium fortuitum.

Conclusion: The microbial fauna of the interface man – pet amphibium – feeder animal – biofilm is characterized by the above-average frequent appearance of a small number of opportunistic pathogens. This obvious bias of the microorganism spectrum may be caused by the special conditions of pet keeping, particularly with regard to unnatural old age, pathogen selection due to host spectrum narrowing, and immunodeficiency due to permanent stress. From the hygienic point of view pet amphibians must be considered as wild animals but not as domestic ones; a common, adapted microbial fauna has not arisen up to now.

11.010 Influenza A Virus Subtype H5N1 Infection in a Cluster of Domestic Cats in an Animal Shelter in Austria

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Background: Domestic and wild cats are reported to develop severe clinical signs and may die due to natural or experimental infection by highly pathogenic H5N1-Influenzavirus. In Styria (Austria) 194 cats were housed in an animal shelter, close to a poultry area where a swan had died from H5N1-Influenzavirus (IvA-H5N1) infection. Influenzavirus A specific nucleic acids were detected in pharyngeal swabs of 3/40 randomly sampled cats 8 days thereafter. All cats were quarantined and monitored for clinical symptoms, virus shedding and antibody production for 6 weeks.

Methods: Pharyngeal and rectal swabs sampled during the quarantine period were examined for the matrix-gene of IvA by Real Time RT-PCR as well as for feline Herpesvirus and Calicivirus specific nucleic acids. Plasma samples were tested for Feline Leukemiavirus (FeLV) antigen (ELISA) and for antibodies against IvA-H5N1, Feline Immundeficiencyvirus (FIV, immunomigration test) and Feline Coronavirus (FCoV, indirect immunofluorescence assay)

Results: IvA specific nucleic acids could not be detected in any sample taken at the quarantine station. Antibodies against IvA-H5N1 were detected in two cats reaching titers of 1:256. None of the cats that had been originally tested positive for IvA or had seroconverted against IvA developed clinical symptoms.

Virological examinations revealed infection with FeLV in 15 cats, FIV in 12 cats, and an antibody titer against FCoV in all but one cat. 57 swabs gave positive results for FCV specific nucleic acids, 17 for FHV-1.

Conclusion: The introduction of IvA-H5N1 infection in a large group of cats, originally demonstrated by detection of viral nucleic acids in pharyngeal swabs, could be confirmed by seroconversion. However, there was no evidence for Influenza associated disease. The absence of morbidity is remarkable as it has to be assumed that several cats of the population had developed an immunocompromised status due to other infectious diseases. Since seroconversion was confirmed in only two cats, horizontal transmission within the cat population is very unlikely.

11.011 West Nile Virus Infection in Horses in Croatia

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The aim of surveing the horse population for the presence of WNV antibodies was attempt to provide information to veterinary epidemiologists. Animals were randomly selected from 8 Croatian regions and total of 980 serum samples were tested. Serum samples were firstly screened by using an home made IgG ELISA and the positive results confirmed using serum-neutralization and complement fixation tests. Of 980 serum samples 4 were found positive for WNV by IgG an home made ELISA. All four were confirmed by SN and only three of them by CFT. To date, there is no evidence that WN virus occured in equine population in Croatia. All positive animals were from a stud-farm located in Dakovo suggesting that the virus activity was concentrated only in that and possibly to restricted geographic area. It's known that migratory birds appear to play an essential role in the introduction of WNV into new regions. From Scandinavian countries to Africa, migrating birds fly in a broad flying zone across Croatia and there are several swampy regions in Croatia where many birds species nest. One of such region is in vicinity of Dakovo, where positive horses were detected. Our findings indicate an active focus of WN virus in horses in Croatia and might suggest a possible role for these animals as sentinels for human risk due to WN virus infection.

11.012 Recreational Drug Abuse and Possible Interference with Anti-orthopoxvirus Immune Response: Implications for Smallpox Vaccination

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Background: Infection with highly immunogenic Orthopoxviruses usually leads to cross protection among species, which has been a prerequisite for successful eradication of smallpox.

Methods: Here we report the rare case of a 17 year old male, who survived a generalised Cowpox virus infection of unusual severity but did not show a proper seroconversion.

Results: Only a very weak immune reaction was observed, which initially appeared to be Cowpox virus specific in immunofluorescence. In Western blotting unusual low antibody titres were observed in early and late serum, restricted to the orthopoxvirus H3L protein only.

No obvious immune deficiencies were detected but high levels of cannabinoids were found repeatedly in the urine and also benzodiacepines upon one occasion and he had been hospitalised for alcohol and cannabis intoxication 2 months prior to infection. As these substances are known to interfere with antibody production, drug induced immune suppression can be suspected as the most likely cause.

Conclusion: This could indicate that otherwise healthy youngsters due to temporary immune suppression by multi drug abuse may serve as a reservoir for rare zoonotic germs lurking for a chance to adapt to human hosts. Additionally long term effects of recreational 'soft' drugs on the host immune response might have the potential to subvert public vaccination efforts against smallpox and rendering certain vaccinees more vulnerable to vaccine side effects.

11.013 African Bats as Natural Reservoir for SARS-Coronavirus

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Background: Asian bats have recently been identified as a potential natural reservoir for the human pathogenic severe acute respiratory syndrome coronavirus (SARS-CoV) that posed a major threat to international public health in 2003. SARS-like coronaviruses (SL-CoV), having nucleotide identities of up to 92 percent to the human pathogenic SARS-CoV, have been detected in bats throughout China. We investigated the seroprevalence of African bats for SARS-CoV.

Methods: With the help of a modified commercial enzyme linked immunosorbent assay (ELISA) we screened 706 bat sera, collected between 1986 and 1999, for their reactivity to SARS-CoV. The sera collection included 27 different species (15 genera) from four locations within the Democratic Republic of Congo and the Republic of South Africa. To confirm ELISA results we used two different Western Blots and a modified commercial Immunofluorescence test.

Results: Overall 6.7 percent (47/706) of all sera showed reactivity in the applied ELISA whereas the emphasis lay within the fruit bat species Rousettus aegyptiacus and the insectivorous bat species Mops condylurus with serological reactivity of 16 and 13 percent, respectively. In nearly all cases (97.3%) the ELISA results could be confirmed by Western Blot analysis. Moreover those results were supported by Immunofluorescence test (IFT) for one third of the tested samples (32.4%). None of the sera had neutralising antibodies or coronavirus nucleic acids.

Conclusion: Our data indicate that different bat species in Africa may also serve as a natural reservoir for SARS-CoV. In fact SL-CoVs are neither newly emerged pathogens nor limited to the Asian continent. More surveillance and viral infectious studies with bats have to be performed to analyse transmission routes from bats to other animals or humans. This is also a necessity to evaluate the risk of a re-emergence of SARS-CoV and other human pathogenic viruses.

11.014 Characterisation of Persistent Viral Infections in Bats

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Background: The great diversity and geographical distribution of bats means it of no surprise that they are a reservoir and transfer vector for many viruses, which once they have crossed the species barrier can be lethal (Filovirus, Flavivirus, Henipavirus, Lyssavirus). We are interested in looking for persistent or latent infection of bats such as herpesviruses and retroviruses. While these are not emerging infections, they have the potential to reactivate and cause zoonoses.

Methods: Polymerase chain reactions were performed using DNA samples extracted from bat brain and spleen tissues. Samples were collected from 5 different species of bats that were sent to the UK VLA from a number of UK zoos. Degenerate primer sets were designed to amplify specific groups of persistent and endogenous viruses.

Results: Preliminary analyses of sequences we have amplified indicates that such proviruses do exist within the bat genome. The work reported here is the characterisation and comparison of these sequences.

Conclusions: Bats are no exception to the diversity of retroviruses among mammals.

11.015 Prevalence of Hepatitis E Virus Antibodies in Occupational Groups with Different Exposure to Swine

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Background: Human and porcine hepatitis E virus (HEV) isolates exhibit partly a close genetic relationship, consequently the possibility of a zoonotic transmission from swine to humans is being discussed. The aim of this study was to determine the seroprevalence of HEV antibodies in occupational groups with different intensity of exposure to swine. We investigated sera of approx. 150 subjects of each of the following professions: farmers, slaughterhouse workers, veterinarians, and hunters; as control group served townspeople. The study was carried out in Austria, a country considered non-endemic for HEV infection.

Methods: For the serological investigations the recomBlot HEV IgG/IgM® (Mikrogen, Neuried, Germany), one of the reportedly most reliable HEV serological tests, was employed. In all sera both HEV IgM and IgG antibodies were determined. The sera were coded, and the investigations were carried out in a blinded manner. In addition, in HEV IgM positive sera the attempt was made to identify HEV nucleic acid by RT-PCR assays.

Results: The results are still preliminay since at the time of submission of this abstract only farmers and some slaughterhouse workers (and a few sera from other professions) have been tested: 50/154 (32%) farmers showed HEV IgM antibodies [11 of which (7%) scored only weak positive], and 67/154 (44%) farmers exhibited HEV IgG antibodies [11 of which (7%) were weak positive]. Slaughterhouse workers: 26/69 (38%) HEV IgM positive [6 of it (9%) scored weak positive], and also 26/69 (38%) proved HEV IgG positive [7 of it (10%) were weak positive].

Conclusion: The preliminary results demonstrate a high seroprevalence of HEV antibodies in occupational groups with a high exposure to swine. It will be interesting to see the results of the other professions under investigation.

SESSION 12 (Poster Session)

Models of Disease Surveillance, Detection and Reporting

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

12.001 Epidemic Modelling Using Cellular Automata

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The damage caused by epidemics of infectious diseases to mankind is well established in history. With the recent outbreak of Chikungunya (after 32 years) and Dengue in Indian subcontinent, the monitoring of outbreaks is gaining paramount importance for government and health

officials.The modelling of a phenomenon such as virus infected through a population will help us understand, predict and ultimately control that phenomenon's behaviour.

We study Susceptible, Infective and Removable (SIR) model using Cellular Automata (CA). Our model not only trivially implement many parameters found in other models, but also provide a useful tool for the graphical visualisation of epidemic's spread. We calibrate the model for a finite population overlaid on a grid of fixed size. Each cell is involved in k contacts, where k is computed based on cell population and contact rate of individuals per day.The probability of exposure with infectivity rate decides the transmission of infection. The mixing pattern of population are varied over different proportions of global and local interactions. Using von Neumann and Moore's neibourhoods, the cell interactions are simulated for number of iterations.

Using 2-dimensional and 3-dimensional graphs, we study the diffusion rate of infected area,densities of infected individuals and virus morbidity.The criteria for epidemic spread like susteptible population size, homogeneity of population density and infection incubation time are also considered for effective implementation.

From the model we are able to identify the epidemic persistence and neighbourhood saturation. The greatest infection velocity is identified. Moreover, factors like leap frogging and infilling are noticed. It is also explicitly demonstrated that due to immigration, the infectious disease remains endemic. The results indicate that although disease prevalence is independent of the spatial domain, the disease infection rate is lowered with higher number of local interactions. Because of the decisive factors of epidemic spread are known, containment measures and medical service can be initiated.

12.002 A Model to Estimate the Risk of Introduction of an Emerging Respiratory Infection Such as SARS in Europe

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Background: In the context of increasing international travels, the SARS epidemic emphasized the need for better preparedness against the risks of introduction and secondary dissemination of an emerging infectious disease such as SARS or influenza in a country. We developed an individual based simulation model to provide a real time estimate of the risk to import such diseases by air travel from an endemic area to a European region.

Method: We used the 2003 SARS data in Beijing (China) chosen as a departure area and we used Frankfurt (Germany) as a destination, based on international travel data. Initial infected cases by date of infection were back-calculated from the epidemic curve using the distribution of the incubation period. To estimate the risk at a day t, we considered the contribution of every individual infected prior to t and likely to travel from Beijing to Frankfurt on day t. Every case had been assigned a probability to travel, to transmit the infection during the flight and to be detected by border control at arrival. The risk was quantified as the probability that at least one infected person arrived to Frankfurt. It was estimated by summarising over N simulations. In a scenario S1 we assigned the same probability to travel to all infected persons and in a scenario S2, infected health care workers had ten times more chance to travel than others. We considered three alternatives on border screening: no screening, sensitivity of 14% (from literature) or a sensitivity of 95%.

Results: Given a travel rate of 1 per 10,000 potential travellers in Beijing and no border screening, the risk estimate per day of entry in Frankfurt reached 4.6% in S1 and 16.6% in S2. With border screening sensitivity of 14% and 95%, the risk reached 4.7% and 3.3% respectively under S1. It reached 15.9% and 12.2% respectively under scenario S2.

Conclusions: This approach provided quantitative measure of the timely risk of importing SARS infection, using the 2003 data. It can be applied to an ongoing SARS-like or influenza outbreak provided the data availability. Its limitations are linked to uncertainty on epidemiological parameters, mainly in the beginning of an outbreak, to under-reporting of cases in the endemic region and to the unavailability of information on travel patterns of passengers. However this method can help decision makers to select appropriate public health measures in the country of arrival.

12.003 Does the Effectiveness of Interventions Depend on the Influenza Pandemic Profile?

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Background: Little research has focused on examining different profiles of spatial and temporal influenza pandemic diffusion, and exploring how the impact of different preventive and control measures varies with these profiles.

Methods: An improved version of a deterministic compartmental model incorporating diffusion by air travel between 52 world major cities and six different intervention strategies was used to simulate pandemic spread. First, using the Latin Hypercube Sampling (LHS) scheme, we sampled the parameters related to the characteristics of the pandemic, performed 1,000 simulations, and applied clustering methods to identify typical influenza pandemic profiles. Second, a multivariate Sensitivity Analysis (SA) on this set of simulations determined which input parameters had the greatest influence on the profiles. Third, we sampled the seventeen input parameters related to the control measures using the same sampling method and, then for each pandemic profile, we studied the independent and relative effects of each control measure by calculating Partial Rank Correlation Coefficients (PRCC). We considered PRCC greater than 0.4 as indicating a significant effect.

Results: We identified 5 different profiles, determined by two key parameters: the rate of transmission and the proportion of susceptible individuals in the initial population (PRCC > 0.70). Regardless of the profile, restricting air travel was of little effect (PRCC < 0.10 for all output variables), and early introduction of each control measure was the most important factor to reduce the number of people infected. Conversely, the effectiveness of other control measures seemed to vary depending on the pandemic profile. In the case of a short and massive pandemic, all intervention parameters were important (theoretical efficacy of each measure, coverage), whereas in case of a progressive and long lasting pandemic, only the date of introduction was correlated with the outcome variables.

Conclusions: Our study highlights the great variation in possible profiles of an influenza pandemic. Moreover, we showed that the effectiveness of each control measure varied depending on the pandemic profile.

12.004 A Canadian Early Event Detection Project: Multi-Source Syndromic Surveillance in the Winnipeg Region Health Authority

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Background: Early event detection is recognized as a potential prevention and mitigation strategy for both intentional and unintentional disease events. Although several real-time syndromic systems have been developed in North America, few opportunities exist to comprehensively test and evaluate them. This opportunity arose in developing the Canadian Network for Public Health Intelligence (CNPHI). Within this network is "CEWS" (Canadian Early Warning System), an event detection system that utilises data from several sources, providing public health officials with trend and alert information to enhance current local surveillance.

Methods: Set in the Canadian city of Winnipeg, Manitoba, this collaboration with Winnipeg Region Health Authority (WRHA) investigates three syndromic sources of data provided on a daily basis: "Health Links —Info Santé" (nurse telephone advice); "E-Triage", an electronic triage tool used in Winnipeg's seven emergency departments (ED); and aggregate over-the-counter (OTC) non-prescription pharmacy sales. OTC sales data are fed directly into CEWS; all other data (de-identified) are

collected via IBM's Healthcare Collaborative Network (HCN) from WRHA's secure data warehouse. Evaluation of CEWS focussed on surveillance of acute respiratory and gastrointestinal events, including: i) temporal comparisons of data; ii) ED chart review determining accuracy of E-triage chief complaints (iii) simulation study testing algorithms with disease events of varying scales; (iv) prospectively investigating CEW's usefulness from a regional health perspective.

Results: Common patterns reflecting weather and season were seen between all data sources. E-triage and Healthlinks usage was distributed differently among syndrome categories. The chart review showed strong agreement between discharge diagnosis and presenting chief complaint. Sensitivity, specificity and days to detection for various algorithms changed significantly when thresholds were altered.

Conclusion: Overall, CEWS shows promise, but our results reflect the need for continued research with syndromic data in contributing to a valuable public health surveillance tool.

12.005 Seasonal Variation of Influenza Health Effects in Hong Kong

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Background: Influenza has been associated with hospitalization and mortality of cardio-respiratory diseases, both in temperate and subtropical/tropical regions. In spite of the well-known seasonality of influenza circulation in human population, it is highly plausible that the effects of influenza also exhibit a seasonal variation because of seasonal emergence of new virus strains and seasonal variation of host susceptibility. We assessed the seasonal pattern of influenza effects.

Methods: We fitted a time-varying Poisson regression model to the weekly counts of hospitalization and mortality in Hong Kong during year 1996 to 2002. The effects of influenza were measured by excess risks per inter-quartile range increase of proportion of isolates positive for influenza (12.3% in our data) in weekly specimens for making diagnosis in the Virology Department of the Queen Mary Hospital.

Results: The estimated excess risks of influenza-associated hospitalization for acute respiratory disease, pneumonia and influenza, chronic obstructive pulmonary disease and cerebrovascular disease showed a significant seasonal pattern ($p < 0.05$), characterized with one peak in the winter and another in the late spring or early summer. Similar two-peak patterns were also found statistically significant ($p < 0.05$) in the excess risks of influenza-associated mortality on chronic obstructive pulmonary disease, cerebrovascular disease and ischemic heart disease, but not on acute respiratory disease, nor on pneumonia and influenza. Such seasonal patterns were further confirmed by a model using the interaction terms of influenza isolation proportions by dummy variables for four seasons (winter, spring, summer and autumn).

Conclusion: The effects of influenza on hospitalization and mortality vary seasonally with a two-peak pattern, although such a pattern is likely only applied to the subtropical/tropical regions. There is a need to promote the public health awareness to influenza outbreaks in the warm spring and summer, especially in subtropical/tropical regions.

12.006 Is Physical Exercise Beneficial or Harmful to the Health Impact of Influenza on Mortality for the Older Population?

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Background: Exercise is in general beneficial for health. However, overexercise may weaken host immune response to infectious diseases especially in the older people. We assessed the role of moderate exercise and frequent exercise, in modifying the risk of influenza-associated mortality in the older population.

Methods: Data on exercise were extracted from the LIMOR (Lifestyle and Mortality) Study, in which relatives of Hong Kong Chinese (n=24,053) who died at 65 years or older in 1998 were interviewed and information about lifestyles of the deceased ten years before death were obtained. The deceased subjects were categorized as never-exercise (less than once a month), moderate exercise (once a month to 4 times per week), and frequent exercise (more than 4 times per week). Proportions of positive influenza isolates (Flu-proportion) in weekly specimens were derived from the Virology Department of the Queen Mary Hospital. Influenza epidemic (Epidemic) was defined as a period when weekly number of positive influenza isolates > 4% of annual total for two or more consecutive weeks. Difference in excess risks (ERs) of mortality associated with influenza between exercise and never exercise groups were estimated by case-only logistic regression.

Results: Relative to never-exercisers, the ER of all cause mortality per 10% increase of Flu-proportion was 6.2% (95% CI 3.3 - 9.0%) lower in moderate exercisers; and was 2.4% (0.1 - 4.7%) higher in frequent exercisers; while during Epidemic (compared to non-Epidemic) the ER was 30% (19.9 - 38.8%) lower in the moderate exercisers and was 11.5% (2.1 - 21.9%) higher in the frequent exercisers. Similar ER differences for cardio-respiratory diseases were observed, and they were insensitive to analysis by stratified potential confounding factors.

Conclusion: In older people, moderate exercise may reduce the risk of death associated with influenza, but frequent exercise on the other hand could increase the risk of death during the period when influenza activity is high.

12.007 Risk Assessment of the Introduction of HP-H5N1 Avian Influenza in Spain

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Highly pathogenic H5N1 subtype avian influenza virus is world-wide distributed and recent outbreaks have occurred in European countries. Migratory birds largely contribute to its worldwide spread and together with illegal importation of live birds constitute the main ways of entrance into a country. However, all ways of entrance must be thoroughly studied and a risk assessment is necessary to identify the critical areas and species where the infection is prone to happen, both to prevent animal health as well as important economic losses derived from a possible incursion of the disease in a susceptible population.

Following a Covella-Merkhofer risk model, a risk assessment has been conducted taking into account domestic and wild birds' census and farm distribution taking into account type of production for the domestic animals; spatio-temporal wild birds' movements and behaviour; imports and information on seizures.

Susceptible populations are composed mainly by aquatic birds although all birds could suffer an infection. Therefore, areas rich in wetlands with high density of outdoor reared poultry where contact with wild birds is favoured will be hot-spots for the introduction of disease by wild birds. Overall, the outdoor poultry census in Spain is estimated to be less than 4%, and the largest wetland has less than 20.000 birds and about 40.000 Ha, which is much less than other large European wetlands. However, Spanish wetlands are rich in biodiversity, which is a point to take into consideration.

Poultry imports are mainly from other European countries, where trade is highly regulated and illegal importation is infrequent. Nevertheless, some attention should be given to smuggled products especially from Asiatic countries which happen from time to time mainly at sea ports, especially taking into account the endemic avian influenza situation Asiatic countries suffer since 2005.

Results will be represented in a map using ArcGis 9.0 and probability distributions will be calculated with 'Risk' software.

12.008 Routine Cluster Surveillance for Geographically Irregular Disease Outbreaks

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Background: Many disease cluster surveillance methods employ a circular search window to identify the location of a new disease outbreak. Recent research in geographic disease surveillance has advanced cluster search methodology in order to discover outbreaks that occur in irregular shapes, such as along rivers or around water sources.

Methods: We describe and employ a method that is sensitive to finding clusters of varying shape and scale. This method is particularly well suited for routine surveillance when the dimensions of potential clusters are not known a priori. We illustrate the method on a 6-year dataset of persons infected with E. Coli O157:H7.

Results: Our results show that a flexible cluster search framework (i.e., a framework robust at different scales and shapes) is ideal for identifying geographic clusters of disease in a routine monitoring system, particularly when the system covers a large geographic area.

Conclusion: Future work on heuristic modifications of this approach hold promise for increasing the speed and effectiveness of this and similar approaches.

12.009 Chagas Disease Surveillance in Legal Amazon with Enface in Environmental Analysis

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The transmission of Chagas Disease (CD) in the Amazon entails singularities that force the adoption of a model of surveillance that is distinct to that proposed for the original CD risk area in Brazil. There are no vectors that colonize the household and, as a result, there is no household transmission of the infection to humans. The transmission mechanisms known include: Oral transmission; extra-domiciliary vectorial transmission and household or peri-domiciliary vectorial transmission without colonization by the vector. On the other hand, it should be considered that CD was not recognized as a public health problem in the region. Because of these conditions and circumstances, the following activities are proposed to be permanently introduced in the Legal Amazon: detection of cases to be essentially supported by Malaria Surveillance, structured and exercised extensively and regularly in the region, through hemoscopic tests; identification and mapping of environmental markers, based on the recognition of preferential ecotopes of the different species of vectors prevalent in the Amazon and investigation of situations where there are evidences or even suspicion of incipient domiciliation by some vectors. To environmental studies of cases will be use the technology and structure of SIPAM (Amazon Protection System), The cases will be mapping by geo and the ecotopes of triatomines will be stratifies of vegetation degrees. The variables: vegetation original, geologic aspects, dismastment, human occupation, climate, hydrographic aspects, activities of the agricultures will be considered for analyses. After this the maps will be compared and define which the parameters of risk will be consider to another areas

12.010 Internet-Based Surveillance of Influenza-Like Illness (ILI) More Uniform Across European Countries

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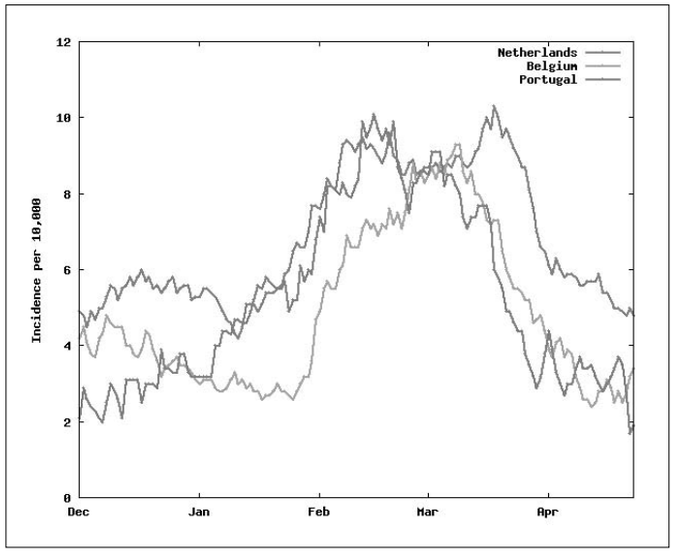
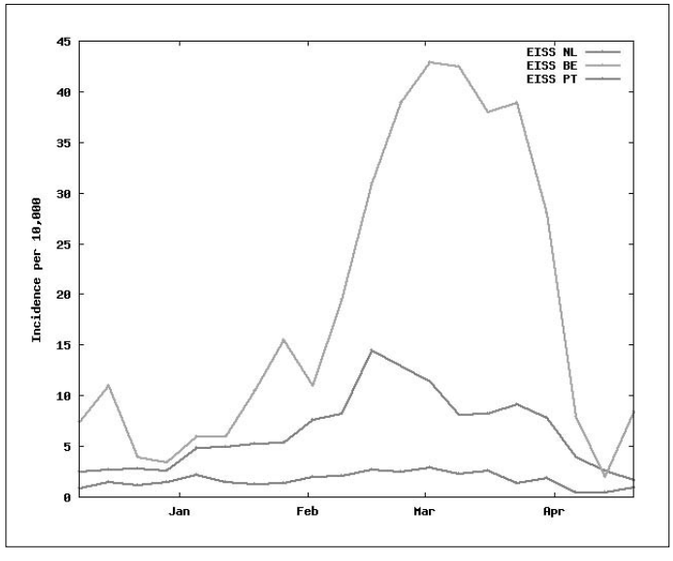
Background: The project Gripenet monitors the influenza-like illness (ILI) activity with the aid of volunteers via the Internet. In contrast with the tradition surveillance system by GPs, in Gripenet the data is received directly from the participants. The Netherlands and Belgium participate since 2003/2004 in Gripenet and Portugal since 2005/2006.

Methods: Any Internet user can become a participant by completing an application form with various questions about themselves on http://www.griepmeting.nl for The Netherlands and Belgium, and http://www.gripenet.pt for Portugal, respectively. Each participant weekly reports any symptoms they have experienced in the preceding week. Based on a strict case definition for ILI, a daily incidence curve is determined.

Results: In 2005/2006 Gripenet had 38240 participants from The Netherlands, Belgium and Portugal. The Gripenet data are compared to the data from the GPs as gathered by the European Influenza Surveillance Scheme (EISS). The incidence curves from Gripenet and EISS are qualitatively equal; rise, peak and decline of ILI activity occur at equal times. Quantitatively however, according to Gripenet the incidence in the three countries are comparable, while according to EISS there is an order of magnitude difference in the incidence between the three countries.





Conclusion: The Internet-based Gripenet is a valuable monitoring system alongside the traditional GP-based surveillance system. While EISS provides an established surveillance system that is validated by laboratory tests, Gripenet provides results which are better comparable across different countries. Gripenet provides the incidence curves earlier and the websites of Gripenet are considered a valuable information channel for influenza. Extending the project to more European countries would provide valuable information about the spatial spread of ILI throughout Europe.

12.011 Bacterial Meningitis in Children in 2005 in South Africa

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Background: Bacterial meningitis is a serious disease common in children. We aim to report the epidemiology of meningitis caused by Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae in children <5 years in South Africa.

Methods: Meningitis was defined as the isolation of one of the pathogens from cerebrospinal fluid as reported to a national laboratory-based surveillance programme.

Results: Meningitis occurred in 36% (1771/4911) of cases of invasive disease, 36% (593/1658) in children <5 years, 304 (51%) in Gauteng province. **National:** Incidence rates for each pathogen showed highest burden of disease in infants <1 year: 24/100 000 population for pneumococcal meningitis, 7/100 000 for meningococcal meningitis, and 3/100 000 for H. influenzae meningitis. H. influenzae (Hib) was confirmed in 24/34 (70%). Outcome was available for 265/307 (86%) of cases at sentinel sites: case fatality rate (CFR) for pneumococcal meningitis was 56/154 (36%), for meningococcal meningitis 5/82 (6%) and for H. influenzae meningitis 29 (10%). 56% (194/345) of pneumococcal meningitis cases were caused by serotypes contained in the 7-valent vaccine, and 60% (90/149) of meningococcal cases were caused by serogroup W135. **Gauteng:** Incidence rates in infants <1 year: 62/100 000 for pneumococcal meningitis, 22/100 000 for meningococcal meningitis and 6/100 000 for H. influenzae meningitis (of which 11/16, 69% were Hib). Outcome was captured in 91% (138/151) of cases from sentinel sites. Pneumococcal meningitis CFR was 41% (32/78), meningococcal meningitis 1/51 (2%) and 11% (1/9) for H. influenzae meningitis. None (0/13) of the HIV-seronegative children with pneumococcal meningitis died, while 21/37 (57%) of HIV-seropositive children died (P=0.001).

Conclusions: Highest rates of disease were in infants <1 year. Lower rates nationally may reflect differences in accessibility to healthcare, specimen-taking practices and reporting. CFR was highest for pneumococcal meningitis and associated with HIV-co-infection. The 7-valent pneumococcal vaccine could potentially prevent more than 60% of disease.

12.012 Evaluation of DENGUEINFO as Early Detection and Dengue Reporting System Tool in Indonesia

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Background: Dengue poses a growing public health problem in Indonesia. From 2000 to 2005 the number of dengue cases increased almost three-fold from 33,443 to 95,274 and spread to communities that had never reported infections. Active disease and vector surveillance for early detection and response is at the cornerstone of a global strategy to control epidemic dengue. The Indonesian Ministry of Health and US Naval Medical Research Unit No. 2 developed an integrated laboratory, epidemiology and health information surveillance system called DengueInfo. DengeuInfo is designed to facilitate standardized dengue case and vector data collection at the village level and regularly communicate that data for analysis by dengue control authorities.

Methods: Using a methodology called System Development Life Cycle, the project team assessed data collection and reporting needs and capabilities at the district level and developed complementary software, standardized data collection tools, and reporting guidelines. Data was collected through interviews and onsite reviews of resources and practices at 16 district health authorities and 8 provincial hospitals in 8 provinces. Software was developed using Microsoft Access 2003.

Results: There was no uniformity in the clinical and demographic data collected by district health offices. Information collected from districts was inconsistently mailed on a monthly-basis to provincial dengue authorities before being forwarded to the national arbovirus surveillance unit. Forms collecting standardized demographic, clinical and laboratory data on cases were developed. Forms will be faxed by hospitals within 24 hours to the district health office and manually data entered into the software. Data can be analyzed onsite and will be emailed weekly to the provincial health office. The provincial health office subsequently will email district data to the national arbovirus surveillance unit. Data can be analyzed graphically 1) to detect developing epidemics, and 2) to assess disease and transmission trends, particularly following control activities. DengueInfo has been deployed in 16 district health offices, and dengue control officers have been trained.

Conclusion: DengueInfo may provide a simple but robust surveillance tool for timely monitoring and analysis of local dengue transmission in Indonesia. The next step in this project is to conduct an assessment of the efficacy of this pilot surveillance system to control dengue in the 8 provinces.

12.013 Dengue Outbreak in Western Province of Sri Lanka in 2004

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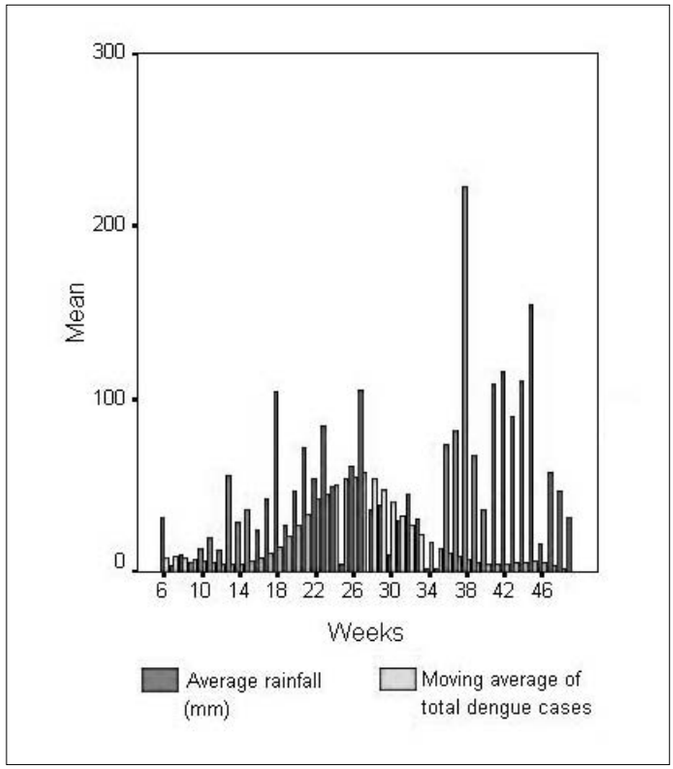
Background: Dengue is an important mosquito-borne viral disease affecting humans. Sri Lanka has experienced several outbreaks of dengue in the past few decades, the last being during April–August 2004.

Objective: To identify high risk areas by mapping incident cases of dengue reported from all Medical Officer of Health (MOH) areas of the Western Province of Sri Lanka during 2004 and to correlate the incidence of dengue with rainfall patterns in different areas

Methods: All dengue cases notified from the Western Province during 2004 to the Epidemiological Unit were identified by MOH areas on a weekly basis. Incidence data were entered into a computer database and



mapped using ARCVIEW. Rainfall data were obtained from the Meteorology Department.

Results: Serial weekly maps depicting incident dengue cases in 2004 and the moving averages by MOH units were developed. The Wattala MOH area had the highest incidence of dengue cases (4.1 per 1000 population), followed by Nugegoda (3.7 per 1000 population) and Panadura (3.0 per 1000 population) MOH areas.

Kalutara, Panadura and Mahara MOH areas showed a high correlation between the number of dengue cases reported and the average rainfall, following a 4 week lag period. In these 3 MOH areas, 38%, 34% and 19% of the variability of incident cases respectively could be explained by the rainfall. Figure 1 shows the correlation of average rainfall with the moving averages of total dengue cases reported.

Conclusion: The method helped to identify MOH areas at high risk of dengue outbreaks. Incident dengue cases correlated with rainfall having a lag priod of 4 weeks. This information is useful in initiating and implementing control activities to minimize the occurrence of future outbreaks.

12.014 Long-Term Spatio-Temporal Patterns of Rodent Cycles and HFRS in Finland

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Background: The incidence of Nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome, caused by the Puumala hantavirus, is very high in Finland, reaching up to 50/100 000 in epidemic years. The host species, bank vole Myodes (Clethrionomys) glareolus is characterized by pronounced 3–4 year population cycles in Finland, but there are spatio-temporal changes in geographic coverage of the cycles. These changes are clearly reflected in the human epidemiology of NE. We analyse here the patterns from 1988 to 2006.

Methods: Rodent cycles have been monitored twice a year at permanent study sites around Finland. NE is a notifiable disease in Finland.

Results: Basically the human epidemiology follows the local bank vole cycle. However, a drastic change in the human epidemiology has been seen recently. Since the late 1980s until the late 1990s, there were around 1000 (800–1100) human cases annually, and the yearly variation was not large at national level. However, a change took place in 1999 when a record of 2305 cases was reached, in 2002 a new record of 2603, and in 2005 again 2524 human cases were diagnosed. In addition, the numbers of human cases have been higher than earlier also in the between-peak years.

Conclusions: There are two clear trends in the Finnish data. The long-term increasing trend is apparently based on better awareness and diagnostics of the disease. However, the most important feature is that the human epidemiology has changed from a rather stable national multiannual pattern to a strongly cyclic one. The change in NE dynamics is obviously due to the change in the geographic synchrony of vole fluctuations. In the southern half on Finland, where most people live and most human NE data come from, a vole cycle usually lasts for three years. From the 1980s until the late 1990s, the geographic coverage of a vole peak was not as extensive as presently. Earlier a vole peak occurred simultaneously in western and south-central Finland, and a year later in eastern Finland. In the late 1990s a change in the geographic synchrony took place, and since then the vole peak has occurred in the same year through most of the southern half of Finland. As a result, the number of human cases that earlier were dispersed over two or three years in geographically different areas, now occur in the same year in the whole area. We emphasize that the understanding of NE epidemiology is not possible without the background data on rodent host dynamics.

12.015 Epidemiologic and Molecular Trends in Norovirus Infections, 2002 to 2005, Germany

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Background: Noroviruses (NoV) are a common cause of acute viral gastroenteritis with high incidences in the colder season. The pathogen is responsible for large outbreaks in settings where many individuals live together. We assessed the trends of NoV incidence and outbreaks and the role of new virus variants in Germany from 2002 to 2005.

Methods: In Germany, NoV infections are notifiable to the local health authorities by the diagnosing laboratory. All cases with typical clinical symptoms who are epidemiologically linked to laboratory confirmed cases are also notified. The data are managed and analysed in a central database at the Robert Koch Institute (RKI). In addition, viruses from a subsample of all outbreaks are characterised by PCR and sequence analysis in order to monitor strains circulating in the population.

Results: Between 2002 and 2005, the number of annually reported NoV cases ranged from about 42.000 cases (2003) to 65.000 cases (2004). The highest incidences occurred in the age groups <5 years and ≥70 years. The number of outbreaks (≥ 5 epidemiologically linked cases) varied from 1.019 (comprising 26.000 cases) in 2003 to 1.570 outbreaks (41.000 cases) in 2004. A very high norovirus activity with a relatively early peak in December was observed in the winter seasons 2002/2003 and 2004/2005. Characteristic for both seasons was a high number of large outbreaks in residential institutions for the elderly, hospitals and in nurseries. NoV sequence analysis showed that in these seasons one particular virus strain predominated: winter 2002/3 NoV genogroup (GG) II.4 2002 (new Grimsby-like), winter 2004/5 NoV GG II.4 (Jamboree-like) whereas in the winter seasons with lower NoV activity different strains co-circulated. Preliminary analyses of the current NoV situation (december 2006) in Germany indicates a similar trend as in the high activity seasons.

Conclusions: The surveillance data in combination with the molecular data allow to efficiently detect and monitor NoV trends. The fact that in winter seasons with one predominating, newly introduced NoV strain unusually high NoV incidences and large outbreaks are observed could be due to different factors (eg, low population immunity levels, high virulence or environmental stability of NoV strains). The timely detection and forecast of high NoV activity seasons may help to rapidly diagnose and contain outbreaks in large settings by adequate control measures.

12.016 Active Influenza Surveillance in South Africa

J. McAnerney, L. Blumberg, C. Cohen, T. Besselaar. NICD, Johannesburg, South Africa

Background: Virological surveillance is the foundation on which influenza surveillance systems are built. The global influenza surveillance network established by the WHO provides information on epidemiological trends and circulating strains. Surveillance is a critical component of monitoring genetic drift of circulating strains, and in the selection of appropriate strains for inclusion in the vaccine.

Methods: An active sentinel influenza surveillance programme, 'Viral Watch' was started in 1984. From time to time changes were made and the centres numbered between 12 and 20 over the next 21 years. The network was expanded in 2005 to 85 predominantly general practitioners at 65 sites in Gauteng. During 2006 a further 46 sites in the Western Cape, Eastern Cape and KwaZulu/Natal were added. A throat swab, brief demographic data, and clinical history are collected from patients of all age groups, with acute respiratory tract infections of recent onset and submitted for isolation of virus.

Results: Since its inception the Viral Watch has provided up to 75% of specimens and up to 100% of influenza isolates at the NICD. The isolation rate (42.5% in 2006) for these active surveillance centres has been significantly higher than that of routine specimens (6.8%). Influenza activity was primarily confined to the winter months during the 23 years, isolates being made mainly during the months June to August with the average week of onset being week 22. In 2006, the season started early (April) and a total of 554 influenza isolates were made, the majority (496) of which were influenza A. Influenza A isolates were further identified as A/New Caledonia/20/99 (H1N1)-like (6) and A A/Wisconsin/67/05 (H3N2)-like (424). Most (47) of the influenza B isolates were identified as B/Malaysia/2506/04-like.

It is of interest to note that 43% of the isolates from the Western Cape were influenza B, compared to <10% for the other 3 provinces.

Conclusions: This programme which provides specimens and epidemiological data throughout the year has been extremely valuable in monitoring the timing of the influenza season, and providing information on circulating strains for inclusion in the Southern Hemisphere vaccine.

12.017 Travel Medicine with an Evidence Base?

C.A. Redman[1], K. Smith[1], F. Genasi[1], A. Sanchez-Vivar[1], K.E. Joyce[1], H. Marlborough[2]. [1]Health Protection Scotland, Glasgow, Scotland, UK; [2]University of Glasgow, Glasgow, Scotland, UK

Background: Health Protection Scotland (HPS)maintains TRAVAX, a database that covers travel health advice on 250 destinations and 94 infectious and non-infectious health risks. The importance of TRAVAX is highlighted by its widespread use: over 4200 subscribing individuals and organisations across the UK, with approximately three quarters of a million log-ins per year. It is easily accessible and is designed as a tool to provide advice to travellers on pre-travel vaccines, anti-malarial prophylaxis and health risks while travelling, as well as guidance on the diagnosis and treatment of illness in returning travellers. The content of TRAVAX is based on monitoring of travel related literature, surveillance, outbreak data and expert advice. While TRAVAX is based on an accumulation of information, a proportion of this information has not been critically appraised and therefore lacks the robust framework that lies behind evidence-based medicine. The purpose of the pilot project was to develop a methodology for making TRAVAX an evidence-based tool.

Methods: The research topic, 'Safety of Hepatitis A vaccines in pregnant women', was used as a case study to explore and appraise the current evidence base. The approach involved the application of methods developed by SIGN and NICE in the context of travel medicine.

Results: No evidence was identified to suggest that Hepatitis A vaccine was unsafe in pregnant travellers. The process did, however, highlight certain weaknesses within HPS, in relation to systematic literature searching and critical appraisal of evidence. The SIGN/NICE methodologies employed were felt to be limited in certain aspects pertaining to travel medicine. Moreover, expert knowledge, via the TRAVAX Advisory Board, was found to be critical in facilitating the process. As a whole the development process was both time consuming and resource intensive.

Conclusion: While rigorous methodologies developed by SIGN and NICE can provide a useful framework for evidence based practice, this blueprint must be adapted through application of context, experience and initiative to take into account the nuances of travel medicine. The project was successful in its outcome at the pilot stage and provides a springboard for further development.

12.018 Using an Intelligent Database for the Early Detection of Outbreaks

J.A. Brown. Consultant, National Library of Medicine, Tacoma, WA, USA

Background: A relational database has many features that could make it useful as a decision-support system for health professionals involved in the surveillance of outbreaks. A relational database can store an unlimited amount of data. Most computer-users are familiar with queries, a relatively simple and straightforward method of "zooming-in" on information. An "intelligent database" could be designed to include only information useful for the early detection of outbreaks and to classify that information in a comprehensive and systematic way.

Methods: An intelligent database was envisioned as a map of the knowledge domain of all diseases that could present as outbreaks. The content would include emerging infectious diseases, bioterrorism, chemical weapons, occupational diseases, foodborne illnesses, zoonoses, and other communicable diseases. The system would include these features: 1.) All information will be comprehensively collected by a physician; 2.) All information will be systematically indexed using categories and a vocabulary that is structured and unambiguous; 3.) All information will be stored in a computer-based relational database with a graphical user interface that will enable users to sort and filter hundreds of records instantaneously.

Results: The prototype application was developed in Microsoft Access. All information is bi-directional, i.e., the user can see all the symptoms associated with a disease or see all diseases associated with a symptom. The same structured vocabulary is used both to display information about a disease and to query the database. Each disease profile shows initial symptoms, incubation period, signs and symptoms, and associated high-risk activities. For infectious diseases, the application shows where the disease occurs in the world; how it is diagnosed in the laboratory; its source from patients, water, soil, or animals; the route of entry; and the insect vectors and animal reservoirs. Of the 201 diseases, 156 are infectious diseases. Queries by one or more criteria are available for 135 findings, 17 syndromes, 102 jobs, 101 activities, 39 epidemiological factors, and 16 regions of the world.

Conclusion: A relational database is an effective tool to develop and update a decision-support system for outbreaks. Such a database could help medical and public health professionals participate in a surveillance system for the early detection of outbreaks.

12.019 Emergence of a New Biological Risk Culture by Sharing the Bio Terrorism's Preparedness Response Plans After 2001 and the Threat of the New Outbreaks (SRAS, AH5N1, Chikungunya)

S. Segovia-Kueny. French Governmental Administration, Paris, France

After September 2001, and the anthrax dramatic event in the United States, the face of the bio terrorism dramatically changed. A new phase in bio terrorism occurred, targeting innocent citizens, journalists, highest governmental organizations (White House, Parliament), postal service, all over the country. It triggered a huge amount of media, psychological, political, economical consequences. In one word it was the beginning of a new form of hyper bio terrorism geographically everywhere, blind and deadly.

After the initial upset, began the resilience period when a lot of person wanted to know how to protect themselves, what their country was doing and learning more about the bio terrorism threat. At the same time, all states were preparing or improving their bioterrorism preparedness response plans. Almost all the related papers were published on web sites and initiated a new 'biological risk culture.'

More than 5 years after these events, the process continues with an increased awareness of the biological terrorism and of the biological natural risk. The SRAS outbreak in 2003, the pandemic avian flu threat with the first human cases since the end of 2003, and the outbreak of chikungunya in the Indian ocean region since 2005, participated to this awareness.

Worldwide biological preparedness responses disseminated by internet, in universities, in companies have started to change the face of the biological risk management. Now activity continuity plan have been elaborated to response to the specific biological risks. What will happen in the future? What will be the impact of this new risk culture, in the occurrence of a new biological events?

12.020 Economic Impact of Dengue

H. Taliberti, P. Zucchi. Universidade Federal de São Paulo, São Paulo, Brazil

Background: Why should one study the cost of dengue epidemics? The disease covers an area where 3.5 million people live and it is present in 100 subtropical and tropical countries. It is one of the biggest problems of public health. The factors considered as favorable to the epidemic expansion are the demographic, social and climate changes; the appearance of new and virulent types, with higher transmission capacity and circulation of several serotypes in one same region and the deficiencies in public health policies, in vector control programs and in the epidemiological surveillance.

Method: Electronic database of published works on epidemic costs and control programs that show economic results was the source to research and evaluate the cost and the economic impact of dengue in countries affected by the disease since the 70's.

Results: In Puerto Rico, the total costs were between US$6.0 to US$15.6 millions, from which 7.8% to 20% was spent with epidemic control. In Cuba, the total cost was US$10.5 millions. In Nicaragua, the hospitalization costs were US$130 per day, considered very high taking in account the Gross National Product (GNP) per capita of US$469 per year. In French Guiana, the total costs in 1994 were estimated in US$150.000. In Brazil, the cost of the mosquito eradication program represented 13.5% of the tourist budget in 2001, in Fortaleza, a tourist city in the country. In Venezuela, the total costs were US$1.3 millions. In Thailand, the cost per family was from US$23 to US$61 per day. In Singapore, in 2005, they spent from US$31.5 to US$34.5 millions, if the mean age of the patient who died, was 52 and 42, respectively. In Malaysia, the hospitalization cost was US$718.14 per case. In Australia, the annual cost was US$15 millions.

Conclusion: There are few studies. They focused isolated epidemics and observe the direct costs and not the real burden of the disease that includes days off work of patients and children's parents who catch it and significant loss in strong currency incomes. Tourism sector is particularly affected, due to the decrease of the demand during the epidemic period, with consequent exchange values loss.

12.021 Tracing Severe Avian Influenza A H5N1 Virus Outbreaks by Climate Changes and Wild Bird's Migration

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Background: Since the end of 2003, highly pathogenic avian influenza viruses (HPAI) H5N1 have caused many outbreaks in poultries and wild birds from the East Asia and have spread to at least 48 countries. All countries that have experienced HPAI H5N1 outbreaks have taken stringent measures to try to curb the spread of this virus. However, dead birds carrying the H5N1 virus continue to be reported in most of Asia, Europe and Africa. What has both triggered the sudden changes occurring in many outbreaks and initiated its global spread remains unclear.

Methods: We tracked the dates and locations of HAPI H5N1 outbreaks in Eurasia between July 2005 and June 2006, and analyzed meteorological data collected from the website of the United States National Climate Data Center (NCDC). In addition, the migrations routes of wild birds were also collected from World Organization for Animal Health (OIE). Finally, we used Geographic Information System(GIS) to convert all data into georeference scale,and made the temporal and spatial correlation analysis to distinguish the relationship among viruses, climate, wild birds.

Results: We found that temperatures and wind flows on first day of each selected H5N1 outbreaks, the arrivals of colder air from higher latitude region may be the main cause of the spread. Outbreaks of H5N1 from Turkey to France between November 2005 and March 2006 were found to have the most striking temperature drops. And the trend of wild bird's migration was from the east to the west. Both the climate changes and wild bird's migrations seemed to have the similar influence on the spreading of H5N1.

Conclusion: Our findings imply that regional weather monitoring may serve as an integral part of a bird flu surveillance system. Using GIS facilitates to explore the correlations among the spatial and temporal patterns of climate change, outbreaks occurrence and wild bird's migration.

12.022 MeaslesNetIndia—A New Model of Measles Surveillance Network in India

N. Wairagkar. National Institute of Virology, Pune, India

Background: Measles is the most important cause of childhood morbidity and mortality throughout the world. Molecular surveillance in measles control and elimination programs is an extremely powerful tool for assessing transmission pathways, monitoring measles control measures, and monitoring the success of elimination strategies. Though measles is endemic in India, countrywide measles surveillance is yet to be established because of involvement in Polio eradication efforts.

Methods: In order to fulfill this gap, National Institute of Virology, Pune initiated a new model for Measles Surveillance in India. MeaslesNetIndia was established in 2005 consisting of 16 Sentinel centers, geographically spread all over India. These centers include Research Institutes, Medical colleges and other Sentinel practitioners. This network was established with objective of mapping circulating measles genotypes in various parts of India. The strategy includes investigations of measles outbreaks, confirming serologically and collection of samples for virus isolation and PRC- sequencing (N and H genes).

Results: MeaslesNetIndia has investigated many measles outbreaks in 9 states of India. All these outbreaks were serologically confirmed. Representative specimens (n= 157) were collected for serology and virus isolations. 35 N gene and 12 H gene sequences were obtained. Measles genotypes D4 and D8 predominate majority of the states. MeaslesNetIndia detected a new genotype D7 for the first time. Epidemiology data generated by the network indicated changing trends in Measles epidemiology. This will be discussed in the paper.

Conclusion: MeaslesNetIndia is a new model initiative involving various agencies for measles surveillance in absence of countrywide network.

SESSION 13 (Poster Session)
Emerging Disease Detection

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

13.001 Occurrence of *Vibrio cholerae* Serogroups Other than O1 and O139 in Austria

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Of the approximately 206 Vibrio cholerae (V. cholerae) serogroups that exist, only O1 and O139 are associated with the clinical syndrome of cholera, and can cause large epidemics.

Organisms of V. cholerae serogroups other than O1 and O139 have been associated with sporadic cases of foodborne outbreaks of gastroenteritis, but have not spread in epidemic form. They have been associated with diarrheal illness and also, rarely, isolated from patients (usually immunocompromised hosts) with septicemic disease. The non-O1/non-O139 V. cholerae is found in aquatic environments worldwide, particularly in mildly brackish waters where they constitute indigenous flora. We describe 12 cases of manifest infections and 1 case of asymptomatic infection due to non-O1/non-O139 V. cholerae, documented in Austria in the years 2000 till 2005. Twelve patients (8 years till 65 years old; 7 male) had symptomatic infection: diarrhea x 5, otitis x 6, septicemia once. All 5 patients who acquired their infection abroad, suffered from diarrheal illness. The 8 persons without travel history outside of Austria had otitis media (n=4) or otitis externa (n=2); the lethal case of septicemia affected a fisherman with underlying malignancy. One isolate was from an asymptomatic child. Detailed data on travel history inside Austria was available for 5 of these 8 patients: all 5 had visited or lived near the largest lake of Austria. The concentration of salt of this western-most steppe lake in Europe is approximately one-twentieth of that of sea water. Why otitis and not diarrheal illness is the dominating manifestation of non-O1/non-O139 infection acquired in Austria remains to be elucidated. We hypothesize that diarrheal illness due to Vibrio cholerae serogroups other than O1 and O139 acquired in Austria may be simply unrecognized by the standard operating procedures employed in clinical microbiology laboratories. Testing for Vibrio cholerae is not considered necessary for domestically acquired diarrhea. Only in patients who acquired diarrhea abroad, physicians sometimes do consider cholera as a differential diagnosis, thereby prompting the laboratory to use thiosulfate citrate bile salt sucrose (TCBS) agar plates. Without using selective media for stool specimens, diarrhea caused by non-O1/non-O139 V.

cholerae simply remains unrecognized. Samples tested for otitis, contrarily, yield growth of Vibrio cholerae serogroups other than O1 and O139 on regularly used blood agar plates.

13.002 New Adenovirus Species (Human Adenovirus G) Found in Patient Presenting with Gastroenteritis

M. Jones. United States Air Force, Travis AFB 94535, USA

Background: An unidentified agent was cultured in primary monkey cells at the Los Angeles County Public Health Department from five stool specimens from an outbreak of gastroenteritis at a convalescent home in Los Angeles County in February 2003. We wanted to determine whether or not a virus was associated with this outbreak.

Methods: Electron microscopy and an adenovirus specific monoclonal antibody confirmed this agent to be an adenovirus. Using the sequence independent viral nucleic acid amplification method DNase-SISPA, we identified several nucleotide sequences with a high homology to human adenovirus 41 and simian adenovirus 1 (SAdV-1). A colorimetric neutralization test utilizing an anti-SAdV-1 antibody, was used to determine that this virus was serologically different than SAdV-1. A PCR assay was designed to detect HAdV-52 to determine prevalence in norovirus negative stool samples. The prevalence study population included 125 individual stool samples from 25 separate gastroenteritis outbreaks and 86 stool samples from healthy asymptomatic patients.

Results: Genomic sequencing and phylogenetic analysis of HAdV-52, which is 34,250 bp in length, confirmed that this new adenovirus was so divergent from the known human adenoviruses that it was not only a new type but represented a new species (Human adenovirus G). Phylogenetic analysis also confirmed that HAdV-52 is not a recombinant of the known adenoviruses. HAdV-52 is genetically similar to other adenoviruses, yet serologically different than SAdV-1. In a retrospective clinical study, this new virus was detected by PCR in one additional patient from a separate norovirus negative gastroenteritis outbreak. We were also able to detect HAdV-52 from tissue culture samples that were individually inoculated with stool from the other 4 members of the original outbreak via PCR.

Conclusion: This study suggests that HAdV-52 may be one of many agents causing gastroenteritis of unknown etiology.

13.003 Syndromic Surveillance in Malaysia— Why Bother!

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Background: Notification of health event based on syndromes has long been a practise in Malaysia. The main objective of these syndromic surveillance systems was to complement the disease based notification system thus, enabling rapid response to disease outbreaks without being delayed by laboratory confirmation.

The emergency department and medical wards in hospitals were requested to notify severe cases of unknown etiology to the nearest district health office within 24hours of admission. Health-events with potentially high fatality rate, unusual or unexpected, is spreading rapidly or suspected zoonotic in origin were set as criteria under this syndromic surveillance. A study was conducted to assess the level of usage of the system and to identify reasons for non-performance.

Methods: A retrospective record search from ward admissions was carried out for 2 months in 2 hospitals in Y2005. Selection of cases was based upon ward diagnosis. Self administered questionnaires were given to the attending physicians.

Results: Out of 817 admissions in hospital A, 8 (0.98%) cases fulfilled the criteria but none were notified while in Hospital B, 4 (0.01%) of out 300 admissions with no notification. 41% of the physicians admitted being aware of the system and recalled being briefed. 17.6% noted treating cases that fit the criteria but did not notify since the laboratory facilities were adequate to identify cause. 40% was not sure where to notify and none knew that they need to notify within 24hours. 40% of them had seen posters on syndromic notification being displayed. 50.5% of the physicians felt that the syndromes were broad and non-specific with overlapping of syndromes where multi-organs were involved.

Conclusion: The system was poorly accepted due to laboratory dependent attitude among physicians in hospitals with good laboratory facilities. Much effort is needed to convince physicians of the importance of the system for outbreak response.

13.004 Serological Evidences of Hantavirus Infection in Human Population Lien-chiang County, Taiwan

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Background: Hantavirus, a rodent-borne virus, is the identified agent of the human diseases hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). In Lien-Chiang County, Taiwan, earlier study indicated the presence of hantavirus antibody-positive rats, but there are rarely evidence of human infection according the National Infectious Disease Surveillance System (NIDSS). To explore the discrepancy between actual infections in residents and the NIDSS database, we undertook a prospective serological survey in residents aged 20 years or above from Jun 2005 to Jun 2006 in Lien-Chiang County.

Methods: Two serum samples were obtained from residents who voluntarily joined the health screening program in Jun 2005 and Jun 2006 respectively. Serum samples were tested for anti-hantavirus IgG using commercial ELISA kits. Samples found positive by ELISA were tested by an indirect immunofluorescence assay (IFA) to identify the serotype. For susceptible individuals identified from the first serological survey in Jun 2005, a second serological study was conducted on Jun 2006 to determine the status of seroconversion of hantavirus antibody. Cases who were not reported to NIDSS despite having sought medical advice and treatment for related symptoms between Jun 2005 and Jun 2006 were analyzed.

Results: Of the 1145 participating residents in the first serological survey, 101 (8.8 %) were positive for anti-hantavirus IgG by ELISA. There was significant difference among the positive cases in terms of geographical distribution ($p<0.05$) but not in terms of sex and age (Table 1).

Variable	No. of seronegative cases (%)	No. of seropositive cases (%)	P-value
Gender			0.934†
Male	502(91.1%)	49(8.9%)	
Female	542(91.2%)	52(8.8%)	
Age(y)			0.373※
20~29	6(0.6%)	0(0.0%)	
30~39	242(23.2%)	19(18.8%)	
40~49	354(33.9%)	35(34.7%)	
50~59	242(23.2%)	27(26.7%)	
60~69	102(9.8%)	12(11.9%)	
70~79	75(7.2%)	4(4.0%)	
80~89	21(2.0%)	4(4.0%)	
90~99	2(0.2%)	0(0.0%)	
Geographic distribution			0.000†✓
Jieshou Village[a]	371(35.5%)	54(53.5%)	
Renai Village[a]	99(9.5%)	14(13.9%)	
Fueo Village[a]	118(11.3%)	12(11.9%)	
Nioujiao Village[a]	147(14.1%)	10(9.9%)	
Matzu Village[b]	107(10.2%)	3(3.0%)	
Cingshuei Village[b]	97(9.3%)	2(2.0%)	
Jinsha Village[b]	25(2.4%)	2(2.0%)	
Tangci Village[b]	12(1.1%)	2(2.0%)	
Jhuluo Village[b]	20(1.9%)	1(1.0%)	
Shiwei Village[b]	22(2.1%)	1(1.0%)	
Tianwo Village[c]	4(0.4%)	0(0.0%)	
Qingfan Village[c]	5(0.5%)	0(0.0%)	
Daping Village[c]	9(0.9%)	0(0.0%)	
Lehua Village[c]	1(0.1%)	0(0.0%)	
Xichou Village[c]	2(0.2%)	0(0.0%)	
Houwo Village[c]	3(0.3%)	0(0.0%)	
Fuzheng Village[c]	1(0.1%)	0(0.0%)	
Qiaozai Village[c]	1(0.1%)	0(0.0%)	

[a] High infection areas [c] Non infection areas ※Indepent t-test
[b] Low infection areas † Pearson's chi-square test ✓Significant difference

Of the 1145 original participants, 725 have been properly followed up, and positive seroconversion of anti-hantavirus IgG has been detected in 3 residents (0.4%). The serotype for all 104 positive cases is Seoul. However, from 1 Jun 2005 to 30 Jun 2006, data from NIDSS revealed no reporting of suspect cases, and a review of medical records of those with positive seroconversion found no clinical symptoms indicative of HFRS.

Conclusion: The findings indicate the presence of hantavirus infections in residents of Lien-Chiang County and suggest that infection with serotype Seoul hantavirus might be asymptomatic. Moreover, existing surveillance system seems to underestimate hantavirus infections in the county, where transmission of hantavirus appears to be ongoing.

13.005 Detection of Human Metapneumovirus in Children Hospitalized for Acute Respiratory Tract Infection in Jeddah, Saudi Arabia

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Background: Human metapneumovirus (hMPV) is a newly recognized paramyxovirus associated with respiratory tract disease (RTD) mainly in infants and children.

Objectives: To evaluate the incidence of hMPV infection in children hospitalized with RTD and to analyze the virologic and clinical features of hMPV infection.

Material and Methods: Real time Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect N gene of hMPV in 282 nasopharyngeal aspirates that were collected over the period from December 2004 to June 2006, and were also tested for other common respiratory viruses using indirect immunofluorescent assay.

Results: Twenty six samples (26/282 (9.2%) were positive for hMPV, most of them detected during winter months. Respiratory Syncetial virus was detected in 99/282 (35.1%) specimens. Co-infection was detected in 3/26 (11.5%) specimens. 19/26 (73%) of cases were aged 4–12 months whereas 66/99 (66.6%) RSV cases are mostly during the first 6 months of life

Culture was performed on limited number of hMPV positive specimens and 3 isolates were obtained. Sequence analysis was done in a reference lab for 10 cases 9 cases were type A and one case type B

Conclusions: hMPV was detected in children with respiratory tract infection, mainly in winter months, most of children are aged 4–12 months. Both type A and B were detected but mostly type A.

13.006 Survival of Severe Lassa Fever In Frankfurt 2006

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We report the clinical and virological course, treatment and outcome of a 70 years-old patient with severe Lassa-Fever (LF) from Sierra Leone recently treated at Frankfurt University Hospital. The specialized intensive care isolation unit provides working under BSL 4 conditions in case of infectious diseases emergencies.

The patient travelled from Freetown to Frankfurt via Brussels airport by a commercial flight. He was admitted to another hospital with a 5 days history of fever, cognitive impairment and paraplegia. LF was initially suspected by detection anti-Lassa Virus (LASV)-IgM and -IgG on day 14 in serum. Confirmatory LASV-RT-PCR from the same material revealed a positive signal 24 hours later.

On admission to our unit on day 16 of disease the patient was mechanically ventilated and treated with vasopressors due to severe systemic capillary leak syndrome and patterns of septic shock. Elevated liver enzymes, renal failure, pleural effusions and generalised edema suggested multiorgan involvement in severe LF.

Intravenous ribavirin, broadspectrum antibacterial and fungal agents were given. Daily measurements of LASV load from urine (maximum 4,7 x 106 cop/ml), plasma (9,5 x 106 cop/ml) as well as CSF (4,2 x 104 cop/ml), bronchial secretions (6,5 x 106 cop/ml) and pleural fluid (6 x 105 cop/ml) were carried out and first became negative except for urine on day 28 of illness. Immunological host response appeared to support viral clearance from blood and other compartments. After 93 days of treatment the patient could be transferred to a rehabilitation center with minor neurological impairment.

No secondary cases occurred. Surveillance strategies for contacts and techniques for prevention of nosocomial infections will be presented.

This case demonstrates that even severe LF cases can be successfully and safely treated if effective antiviral treatment and supportive care is provided. Beside ribavirin administration, the main strategy must include the risk-reduction of complications from long term intensive care treatment and prevention of nosocomial spread.

13.007 Tuberculosis in the Czech Republic—Geographical Analysis of Laboratory Notifications 2001–2005

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Notification of tuberculosis in the Czech Republic is done in two systems—Register of Tuberculosis, based on physicians reporting and Information System of Bacillary Tuberculosis (ISBT), based on laboratory reporting.The data are merged using a unique identification number. We aimed to determine the incidence of cases with tuberculosis and cases with mycobacteria other than M. tuberculosis (MOTT).

The ISBT notified 30796 positive records from more than 264000 requests to 41 mycobacteriology laboratories during the period 2001-2005. We applied standard statistical procedures including direct and indirect standardisation and thematic mapping.

During five years the ISBT notified 3976 individuals with bacteriologically proved TB (average country incidence $7.8/10^5$). This makes the Czech Republic a low endemicity country. The age and sex distribution of cases is also typical for a low endemicity country. Higher incidence is in men aged 40-60 years and in higher age groups. In comparison the highest incidence in women is seen in the over 60 years category. The age distribution of migrants is different with highest incidence observed in younger age groups (30-49) in both sexes. This reflects a younger population of migrants. Men are carriers of bacteriological positivity for a longer period than women. MDR TB cases are still very rare; 2% of cases, but more frequent in foreigners (RR=2,4). Geographical distribution of male and female cases differs slightly in pulmonary TB and more in extrapulmonary TB. Lorenzo curves show that half of the cases occur in one third of the population. The incidence of mycobacterioses due to MOTT is one tenth of that of TB incidence. High resistance to antituberculotics is seen in M. avium cases. Data from the ISBT are used to validate the physicians based Register of Tuberculosis and they provide additional information on the quality of the bacteriological diagnostics of TB.

13.008 Determination of Relationship Between Tuberculosis and Vitamin D3 Deficiency Among Hospitalized Patients in Razi Hospital Ahvaz, Iran (2004–2005)

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Background: Previous vitamin D3 deficiency has effect on the induction and development of patients infected with M. tuberculosis to tuberculosis.

Methods and Patients: In this case-control study, 45 patients with tuberculosis were selected. Forty-five age, sex, and season-matched volunteers without past medical history of tuberculosis or chronic cough were selected. All patients and control groups were evaluated by measurement the level of vitamin D3 by RIA (radioimmunoassay) method. All results were analyzed by t-test and analyze and variance in spss11/1.

Results: The mean and SD of the level of vitamin D3 were (M=12.25, SD=9.98) and (M= 24.68, SD=19.22) (P=0) among patients and control,

respectively. Thirty nine (86.66%) patients and 26 (57.77%) controls had level of vitamin D3 below 20ng/ml. Twenty eight (62.22%) patients had tuberculosis in warm seasons, (summer, P=0/007) and (fall, P=0/02) and (winter, P=0/08).

Conclusion: There was significant association between previous vitamin D3 deficiency and tuberculosis. Also, tuberculosis frequently occurred in warm seasons.

13.009 Three Cases of Paralytic Poliomyelitis Associated with the Sabin Type 3 Revertant Strains of Poliovirus in Bulgaria

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Background: The use of oral polio vaccine (OPV) is associated with some rare adverse events, including the appearance of cases of vaccine-associated paralytic poliomyelitis (VAPP). A low but constant risk of VAPP exists in the countries like Bulgaria using OPV. We report 3 cases of VAPP (two contact and one recipient type) associated with Sabin type 3 poliovirus that occurred in spring of 2006 in Bourgas region, Bulgaria.

Methods: Clinical investigation of two 55-days-old unvaccinated brothers-twins, who were in contact with vaccinees and one 4-month-old infant who had received first dose OPV 37 days before the onset of illness, with AFP was performed. Laboratory diagnosis was based on virus isolation from stool specimens in cell cultures. The ITD and genome sequencing of poliovirus isolates were performed according to the WHO guidelines.

Results: Clinical and virological features of 3 cases of VAPP are presented. Sabin-like type 3 poliovirus was isolated from stool specimens of the 3 patients. Sequencing of different regions of the genome revealed a U-to-C point back mutation at (nt) 472 that is known to correlate with neurovirulence, as well as single mutations in the VP1/2A region. By third patient was proved a presence of a type 3 Sabin with a recombination in the 3D region with type 1 Sabin and 3 silent mutations in this 3D Sabin 1 region.

Conclusion: The clinical symptoms, EMG and residual paralysis 60 days after the onset of AFP, that are typical of paralytic poliomyelitis; the contact with OPV recipients in first two cases and administration of first dose OPV in third case; the isolation of Sabin-like type 3 strains with mutations correlated with neurovirulence are evidence in support of VAPP diagnosis in all 3 cases of AFP. The presence in the isolate of recombination between different polio serotypes and the presence of mutations confirm the findings that poliovirus can accumulate mutations during the replications in humans, especially if there is no protection against polio, and can produce vaccine derived virus that have characteristic of neurovirulence and transmissibility similar to wild viruses.

13.010 High Prevalence of IgG Antibodies to Ebola Virus in Pygmy Population in Watsa Region, DR Congo

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Background: The natural history of Ebola virus (EBOV) remains unknown. Many sero-prevalence studies have suggested that circulation of Ebola virus in human population is common in sub-Saharan Africa; a recent study in Gabon and the Republic of Congo has found EBOV genome segments amplifiable by PCR and anti-EBOV IgG in fruit bats. The pygmy population living in Watsa region have been reported to be more exposed to supposed risk factors (e.g. hunting and butchering of bush meat, entry into caves) for filovirus primary infection compared to general population. We therefore carried out a study in this population to determine Ebola seroprevalence and possible risks factors.

Method: Watsa region is situated in the North East of the DRC. The population is about 180,000, including 4,000 pygmies. The region was the scene of major outbreaks of Marburg hemorrhagic fever in 1994 and 1998–2000. Volunteer participants (N=300) aged 10 years or above were interviewed about possible risk factor for transmission of EBOV including exposures to rats, bats, monkeys and entry into caves. Venous blood was taken and tested for IgG antibodies to EBOV by an enzyme-linked immunosorbent assay. We used $\chi 2$-tests and Fisher's exact test for the comparison of proportions and the t-student test to compare means.

Results: The prevalence of anti-Ebola IgG in the pygmy population was 18.7% (exact binomial 95% CI: 14.4-23.5%). Antibody-positives pygmies were significantly older than antibody-negative participants (means ± standard error: 35.9 ± 14.7 vs 31.3 ± 14.5, p= .03). The seroprevalence in the age group above 60 years was significantly higher than in younger age groups (34.8% vs. 17.3%. p = .04). Although a large majority reported exposures supposed to be risk factors for the primary transmission of filoviruses, no association was found with transmission regarding the exposures to rats, bats, monkeys and entry into caves.

Conclusion: The seroprevalence of Ebola IgG in pygmies in Watsa region is among the highest ever found in any population on the basis of ELISA testing. However, it remains unclear which exposures exactly lead to this high seroprevalence.

13.011 European Network for the Diagnostics of 'Imported' Viral Diseases (ENIVD)

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In the last decades we became aware that many viruses like Ebola, and Nipah and recently SARS passed the species barrier and cause fatal diseases in humans with close contact with animals. Through the imported Yellow Fever, Lassa and SARS cases to Europe it was obvious that these dangerous infections could be imported to Europe from endemic regions in a very short time.

In several meetings scientists from laboratories working in the field of diagnostics of "imported" viral diseases in the nearly all European countries have started to build up a network to improve the diagnostics of "imported" viral infections and have worked out objectives to be addressed in this collaboration:

1. Build a network of European laboratories working on diagnostics of "imported", rare and emerging viral infections. Provide mutual help in the exchange of diagnostic samples, i.e. sera, viruses, methods, and information in order to improve diagnostics

2. Identify those viral infections more likely to be imported and co-ordinate the objectives and identify laboratories, capable and willing to perform the rapid diag-nostics (<24h) of an acute case, suspected to be infected with a viral haemorrhagic fever.

3. Work out recommendations for standardisation and quality control in diagnostics laboratories involved in the diagnostics of such diseases.

4. Identify and operate standard assays according to defined quality control criteria.

5. Optimise limited resources by exchanging reagents, methodologies, and expertise.

6. Encourage regular contact within the network through meetings, exchange and training of laboratory personnel.

7. Open the network for members of other European laboratories.

8. Organise and co-ordinate international activities with the "Surveillance network group" or other national like CDC or international organisations WHO and PAHO.

For further information visit the websites: www.enivd.de; www.enivd.org The ENIVD is presently funded in part by the DG SANCO (SPC.2002396) under the program AIDS and other communicable diseases.

13.012 Compensatory Mutations, Antibiotic Resistance to Rifampicine and the Population Genetics of Adaptive Evolution in M. tuberculosis Isolated from Iran

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Background: Resistance to rifampin in M. tuberculosis strains is usually caused by the point mutations in the rpoB gene encoding the subunit of the DNA-dependent RNA polymerase, which is a target of the drug. describe multiple rpoB mutants for M. tuberculosis, rifampin-resistant strains harbouring mutations in different codons of rpoB Double, triple, and quadruple mutations in M. tuberculosis clinical isolates were reported in studies conducted throughout the world.

Methods: 42 Rif-R strains were identified (standard methods+PCR) and their 411 bp fragments of rpo B gene were amplified, autosequenced and analysed (MEGA and DNAMAN software).

Results: All 44 Rif-R M.tuberculosis harbored mutations in rpoB gene. It was found 74 mutations dividing in 20 types, 78,9% of all mutations were detected in codons 510, 526, 523, 531 that carried 12.51%, 16.7%, 23.6, 9.7% of isolates correspondingly. Our data represents broader spectrum of mutations in 510 codon as replacement Gln510stop (12.51 %). Mutations in codon 531 resulted in Ser531Leu replacement except one strain harbored previously undescribed TCG-TGC. Majority of tested MBT (23.6%) with mutations located at codon 523 had Gly523Ala amino acid replacement. Base substitution in codons 512, 516, 526 resulted in monotypic replacement Ser-Gly-Tyr, Asp-His, Thr-Asn, His-Tyr-Asn-Stop-Arg-Phe correspondingly. Further, the two most frequently described rpoB mutations are 531TCG-TTG (24.1%) and 526CAC-TAC (20.4%). Both are cytosineto-tymine transitions, which easily occur by spontaneous cytosine deamination to uracil.

Conclusion: for Iranian isolates are working system cytosine deamination to uracil for resistance to rifampicine.

13.013 Identification and Characterization of New Genomic Fragments of an Emerging Highly Virulent Strain of H. influenzae Causing the Brazilian Purpuric Fever (BPF) Through Novel Gene Discovery (NGD) and the Development of a Multigenome Based Microarray for Investigating the Species Diversity

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Haemophilus influenzae (Hi) is a gram negative rod shaped bacterium that lives symbiotically in the upper respiratory tract of humans. Besides the capsulated types, nontypable Hi strains are known to cause number of significant infections. Hi biogroup aegyptius (Hibae) strain cause a fatal disease known as Brazilian Purpuric Fever. We conducted comparative genomics on six Hi, one H. aegyptius and two Hibae strains utilizing microarray technology through two different approaches. In the first one we employed a 70mer based microarray representing all predicted ORFs of Hi KW20 Rd. The second approach, similar to subtractive hybridization, aimed at screening a random genomic library for discovering novel sequences with the reference genomic DNA whose sequence was already know previously. Both methods enabled us to generate a large amount of data in a high throughput fashion. The conclusions we were able to draw based on the CGH analysis, were significantly aided by taking into account the TIGR cellular functional role categories. The identification of these attributes helped comprehend relationships among the members of the species at the basic genomic level as well as explain at some extent their microbial cell biology and the observed phenotypes, especially with regard to virulence.

Recognizing and understanding the links between the phenotypic traits and the genetic background has paramount epidemiological and clinical importance. CGH has been shown to be a useful tool for screening strains for their genetic content. However, there is a major limitation when CGH is conducted on a microarray based on a single reference genome. CGH results report which genes are present or absent relative to the genome. Hence the information about novel genetic content that the query strain possesses remains obscure. We report here the construction of the first Hi multigenome microarray containing ca. 4600 genomic markers. Genomes of 20 diverse Hi strains were screened utilizing the species microarray. Brazilian Purpuric Fever isolates, which did not cluster together by previous MLST analysis, appeared to do so by gene content analysis through CGH. The results obtained by employing the species microarray provided comprehensive information about the genomic size and content of uncharacterized strains. The trees generated by CGH, in general, do not reproduce the phylogeny of a species in terms of vertical evolution, but instead represents the overall relatedness of genomes to one another and provides an assessment about the species genome evolution.

13.014 To Guarantee Safety of Blood Transfusion in Light of Newly Emerging Diseases

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Background: To date blood donor screening relies almost exclusively on specific markers, HIV and hepatitis virus are currently tested for by ELISAs and more recently developed viral nucleic acid amplification (NAT). However, all these strategies cannot handle newly emerging infectious agents, because their prior identification is necessary for development of specific assays.

Methods: In Austria, additional non-specific testing for acute virus infections is performed nationwide by screening for elevated neopterin concentrations since 1996. Neopterin is released by human monocyte-derived macrophages and dendritic cells upon stimulation with interferon-ì which is released during Th1-type immune response.

Results: Increased neopterin concentrations are detectable in the blood of patients suffering from acute infections with viruses, e.g., HIV, EBV, and rubella virus, intracellular bacteria, e.g., M. tuberculosis, and also in autoimmune diseases and malignancy. The 98th percentile of neopterin concentrations is used as a cut-off and thus, 2 percent donor-loss is tolerated. Significantly higher frequencies of acute CMV, EBV and parvovirus B19 infections were found in the group of donations with elevated neopterin, which were subsequently excluded from donation, as compared to those with lower levels (all $p < 0.01$). Moreover, in HCV seropositive donors with elevated neopterin also the frequency of pcr-reactive specimens was significantly higher. It is assumed that neopterin screening should be able to detect a wide range of acute virus infections. Thus, also newly emerging virus infections should be detectable by elevated neopterin concentrations. In fact, recent studies show highly elevated neopterin concentrations in patients suffering from acute SARS and dengue virus infection.

Conclusions: Some 25 years ago, the newly recognized AIDS-epidemic has shown the limitation of current strategies to guarantee virus safety of blood donations. With the current strategy, blood transfusion cannot escape from newly emerging and/or recognized infections as long as specific testing is solely performed. Additional non-specific screening using immune response markers like neopterin could represent a valid option to reduce this risk. Moreover, neopterin screening may

represent a suitable umbrella against the transmission of infections in areas with low economic abilities, where a broad range of virus infections is simply not being tested for because of non-affordable costs.

13.015 The Role of Electron Microscopy in the National Surveillance Programme of Austria

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Since recent years people are aware of the potential threat posed by bioterrorism involving the deliberate release of viral and bacterial agents in centres of population. The recent emergence of severe infections (e.g. SARS, H5N1-Avian Influenza) underlines also the need of consequent professional reaction. For these reasons, countries have drawn up concepts to defend emerging diseases by rapid and successful identification of the microbial pathogens. In Austria, the Electron Microscopic Laboratory of the AGES was designated from the government in 2003 as one of the front line EM laboratories involved in pox diagnosis; the Austrian EM lab is also mentioned in the list of the worldwide geographically representative EM labs as diagnostic centres for emerging infectious agents (P. Hazelton, G. Gelderblom 2003: Emerging Infectious Diseases 9 (3)).

Electron microscopic diagnosis is uniquely situated for rapid identification of infectious diseases by pattern recognition i.e. information on size and particle morphology within half an hour. The catch-all nature of this technique requires no reagents or programmes for analysis; EM can also demonstrate mixed infections with ease (only one analysis required!) even when no specific reagents are available. The demands on an analytical EM lab are versatile: 1) Methods developed for virus analysis should be proved carefully by comparing them with other front-line methods like PCR and ELISA; a validation of these methods presenting comparable data on the sensitivity, speed and reliability will be presented; improvements of the negative staining technique will be discussed; 2) Analysis should be done under quality controlled conditions; permanent training and method validation are a necessity. The EM lab of the AGES is accredited in virus diagnosis since 1999, participating regularly in the ring trials for Quality Assurance (EQA) organised by the RKI/BRD. The benefits of accreditation, after six years of experience, are described. 3) One of the major problems in EM is the missing negative or positive control in the test assembly of negative staining technique. Electron microscopists have to solve this problem rapidly since their results, especially the negative ones, lack confidence of accuracy to a certain extent. A simple technique for permanent test control used in the EM lab of the AGES since two years is presented. 4) Finally, the best analytical quality of an EM lab is of no use if it is not prepared for an emerging case. A fast outbreak surveillance and response demands a coordinated professional process management.

13.016 External Quality Assurance Programmes for the Diagnostics of Viral Diseases to Enhance the Emergency Preparedness in Europe

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Background: The threat posed by emerging and re-emerging communicable diseases as well as by the intentional release of infectious agents in a susceptible population, has been receiving considerable attention at the national and international levels. Evaluated and improved laboratory diagnostics are an important prerequisite in support of surveillance and control of emerging viral infections, and quality control measures are therefore essential.

Methods: Between 1999 and 2006 several external quality assurance programmes (EQAPs) have been established to asses the quality of serological and/or molecular diagnostics of Hanta-, Dengue-, filo-, Lassa-, orthopox-, SARS-corona-, West Nile- and tick-borne encephalitis virus infection. For the EQAPs of serological diagnostics, each participating laboratory received a proficiency panel of several freeze-dried human sera comprising positive and negative samples. For the EQAPs of molecular diagnostics, panels of prepared human plasma samples spiked with known amounts of cell-culture derived and sequence-confirmed viral nucleic acid were distributed.

Results: Here, we summarise the data generated by the mentioned recent EQAPs. A total of 134 invited expert laboratories from 52 European and non-European countries participated at least in one of these programmes. Applying proficiency criteria, the number of participating laboratories who passed the minimum requirements for successful participation ranged between 27% and 87% in the different diagnostic accuracy studies.

Conclusion: The data from the EQAPs conducted through the ENIVD provide a good overview of the diagnostics of those imported, rare and emerging viral infections that have recently become of interest (and a challenge) to expert laboratories involved in public health surveillance both within and outside of Europe. These studies revealed many points that require attention and improvement in the participating laboratories.

13.017 A Novel Neutralization Assay For Influenza H5N1

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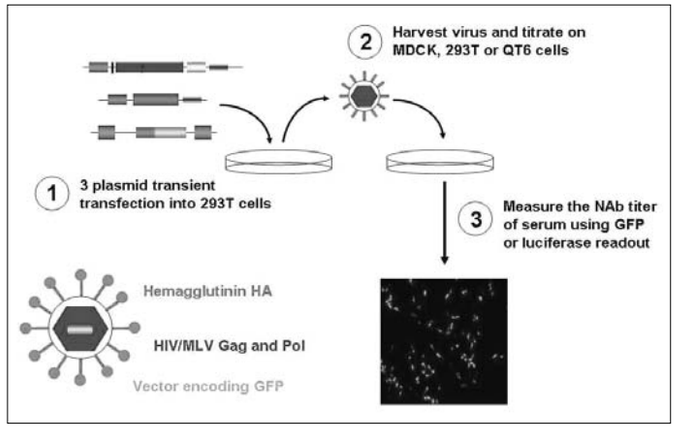
Background: The WHO has called for development of improved serological assays for influenza given that HI has been found to be insensitive for measuring responses to avian H5N1 viruses. Furthermore, current neutralization assays are laborious and require specialist facilities and expertise not always available at the front line of an outbreak. Our objective was to develop a widely applicable assay, with greater sensitivity and specificity, and with adaptation to micro-quantities of serum samples, compared to HI and microneutralization. Viral pseudotypes can be used as safe, surrogate viruses for serological assays which can be carried out in BSL2 containment.

Methods: Retroviral pseudotypes encode marker genes (GFP/luciferase/B-gal) and bear foreign viral envelopes of interest, in this case influenza H5N1 HA. The transfer of marker genes to target cells depends on the function of the envelope protein; therefore, the titer of neutralizing antibodies against the envelope can be measured by a reduction in marker gene transfer. Pseudotyped viruses were produced by three plasmid transfection of an HA-expressing plasmid, a retroviral gag-pol plasmid and a GFP vector plasmid (Fig. 1).



Results: Functional retroviral influenza H5N1 HA pseudotypes have been constructed and used to develop an in vitro microneutralization assay that is both highly sensitive and specific for influenza H5N1 neutralizing antibodies. Pseudotype NAb titres correlate well with titers obtained by HI assays and microneutralization.

Conclusion: This assay does not require the handling of live influenza H5N1 virus; it is a useful tool to determine neutralizing titres during

natural infection and for the evaluation of candidate vaccines and therapies. This influenza HA pseudotype NAb assay, with its easily interchangeable HA glycoproteins from different influenza strains and subtypes make it a powerful tool for use in surveillance and seroprevalence studies. Our results suggest that this assay is more sensitive than HI and microneutralization for the detection of antibodies to H5N1. This assay is more widely applicable and is readily adaptable to a high-throughput format.

13.018 Optical Fiber Immunosensor for the Detection of IgG Antibody to Rift Valley Fever Virus

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Introduction: Rift Valley fever virus (RVFV) causes severe outbreaks in livestock and humans and is considered as a potential biothreat agent. Recent and first outbreaks of RVF outside Africa and effects of global warming have the implication that the virus might spread further into non-endemic RVF areas since it utilizes a wide range of mosquito vectors.

Aims/Rationale: There is an increased international demand for rapid and accurate assays for confirmation and monitoring of natural outbreaks and/or bioterrorism events involving RVFV. Our aim was to develop and evaluate an optical fiber immunosensor (OFIS) for the detection of anti-RVFV IgG antibody in human sera.

Methods: The OFIS procedure was based on a sandwich ELISA. Mouse anti-RVFV antibody was immobilised on a silica fiber-glass surface activated by an argon silanization which allows for covalent binding of bio-compounds. Validation data sets derived from testing field-collected sera from Africa (n = 261) were categorized according to the results of a virus neutralization test (VNT). OFIS raw data were normalized by conversion to test sample/negative control ratios (S/N). A cut-off value at 95% accuracy level was optimized using the two-graph receiver operating characteristic analysis.

Results: At the cut-off of 2.66 S/N, of 70 VNT positive sera, all tested positive in OFIS (100% sensitivity) and of 191 VNT negative sera, all but two tested negative (specificity 98.95%). The assay was highly reproducible within and between routine runs.

Conclusions: The OFIS assay for IgG serodiagnosis of RVF is a low cost, safe, robust and highly accurate system which has the potential to be used in a portable format and thus aid early diagnosis, disease surveillance and bio-defence field monitoring.

13.019 Is the Trophic Form the Inhaled Infectious Stage of Pneumocystis jirovecii?

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Background: Low numbers of Pneumocystis jirovecii (P.jirovecii) organisms, which are undetectable using conventional microscopy can be detected using the polymerase chain reaction (PCR) in bronchoalveolar (BAL) specimens from patients with various underlying diseases and presenting alternative diagnoses of acute Pneumocystis pneumonia (PCP). These low fungal burdens are usually considered to reflect pulmonary colonization. However, the form of Pneumocystis that corresponds to amplified DNAs and that is carried by colonized patients is unknown. Our objective was to identify this form using a PCR assay, a conventionnal technique of microscopy and two immunoassays.

Methods: First, 85 symptomatic patients who underwent BALs for disease investigation were enrolled. P.jirovecii detection on BAL specimens was performed using i/ Giemsa stain, ii/ indirect immunofluorescence assay (IIFA) using an anti-cyst antibody and iii/ a PCR assay amplifying a part of the mitochondrial large subunit rRNA gene. P.jirovecii was detected by microscopy (both cysts and trophic forms) and the PCR assay in four patients who developed PCP. P.jirovecii was detected only by the PCR assay in 14 patients who were colonized by the fungus. Secondly, specimens from all patients were examined using an immuno-alkaline-phosphatase assay (IAPA) with an antibody which reacts against cysts and trophic forms of P.jirovecii. The presence of the two stages in patients developing PCP was confirmed by IAPA. Trophic forms were detected in 6 of the 14 colonized patients, whereas cyst detection was still negative.

Results: The results show that colonized patients do not harbor cysts but rather trophic forms. Conclusion: Acquisition of Pneumocystis sp. via the airborne route has been demonstrated in rodents and is assumed in humans. However, the infectious form which is inhaled is unknown. It has been suggested that pulmonary colonization was related to a small population of fungal organisms in which the growth is low or null. In colonized patients, the fungus may persist in a form indentical to that contemporary to its acquisition. In this context, our results make it possible to hypothesize that the trophic form represents the inhaled infectious stage of P.jirovecii.

13.020 Diagnosis of Mycobacterium Infection by Tb-rapid-antigen-test In Patients from Iran

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Background: Tuberculosis (TB) remains an important problem in the world, but correct TB diagnosis is often difficult. The routinely used procedures for TB diagnosis in clinical laboratory either show poor sensitivity (microscopy) or take several weeks (culture) before results obtaining.

Method: The validity of the developed test was controlled using 288 from different region of Iran sputum of suspected tuberculosis patients. Sputum samples from patients were concentrated and washed in the specific cassettes containing nitrocellulose membrane with absorbing pads above it. MBT were detected by addition anti-TB monospecific rabbit serum and gold-protein A solution. Positive samples were characterized by displaying a red-to brown colour in central area of cassettes in the contrast to the blue-purple colour in negative cases. This new rapid test was compared with the results of acid-fast staining microscopy, specific culture for TB bacilli and specific PCR amplification of TB DNA segments.

Result: Development rapid test was found to be more effective for MBT detection comparing with microscopic method in specimens. Among Iran samples, rapid test was false positive in 3 cases, false negative in 1 case, efficiency was 93.4%, specificity 92.1%, sensitivity 100%, PPV 75.0%, and NPV 98.6%. Ziehl-Neelsen microscopy was false positive in 3 cases, false negative in 3 cases, efficiency was 93.0%, specificity 98.7%, sensitivity 66.7%, PPV 76.9%, NPV 96.2%. Sensitivity of rapid test was 65.5%, sensitivity of Ziehl-Neelsen microscopy 43.1%, efficiency of rapid test was 85.1% for Belarusian and 93.4% for Iranian strains(P>0.05).Therefore, we observed 25% more sensitivity of rapid test compared with microscopy for Mycobacterium tuberculosis detection.

13.021 Comparison of the Viral Cultures and the Reverse Transcription-PCR Assays for Rapid Detection of Enterovirus RNA in Cerebrospinal Fluids of Patients with Neuroinfections in Bulgaria

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Background: Enteroviruses are the most common cause of aseptic meningitis and encephalitis. The rapid etiologic diagnosis of neuroinfections is essential in the management of patients. Detection of enterovirus RNA in cerebrospinal fluid (CSF) has proven to be more rapid and sensitive than viral cultures. The aim of this study was to compare the performance of the viral cultures with RT-PCR.

Methods: 88 patients with aseptic meningitis and 28 patients with meningoencephalitis, ages 6 months–75 years hospitalized in different regions in Bulgaria during 2006 were included in this study. A total of 106

stool specimens and 67 CSF samples were tested for enteroviruses by virus isolation and identification on cell cultures using the standard technique. 31 CSF specimens were investigated by RT-PCR using panenterovirus primers from 5-UTR of viral genome.

Results: Viral cultures were positive in 11 (12,50%) patients with aseptic meningitis and 2 (7,14%) patients with meningoencephalitis. Different enterovirus serotypes were isolated from stool specimens. ECHO13 was the most commonly identified serotype (38,46% of all isolates), followed by ECHO14 (23,08%), ECHO6 (15,39%), ECHO2 (7,69%) and Coxsackie B (7,69%). Only one CSF specimen (1,49%) was positive by viral culture, whereas 48,39% were positive by RT-PCR. Enterovirus diagnosis was achieved in 43,48% of investigated by RT-PCR CSF samples of patients with aseptic meningitis and 62,50% of those with meningoencephalitis.

Conclusion: Enteroviruses will continue to play an important role in the human infectious pathology. PCR is more rapid and sensitive than viral culture for the detection of enteroviruses in CSF that has diagnostic value. PCR may in the future reduce the length of hospitalization and use of unnecessary antibiotics in patients with enterovirus infections.

13.022 All Human Borna Disease Virus Sequences Published To-date Turned Out to Be Laboratory Artefacts

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Background: Since in 1995 the first human Borna disease virus (BDV) sequences had been described, a contribution of BDV to psychiatric illness was hypothesized. In subsequent years many reports about detection of BDV nucleic acid in human PBMC, serum or brain followed worldwide. Because of failure to link Borna disease (BD) cases in animals and BDV-positive psychiatric patients epidemiologically, the human BD issue led to controversial discussions and remained unresolved over the years.

Methods: In order to clarify the existence of human BD, we examined in total 193 single human BDV sequences, which represent all human-derived BDV sequences available to-date. These sequences were investigated by BLAST search, comparative alignment and phylogenetic analyses. Not only the most important BDV genes p40 and p24 were analysed, but also p10, p18, p57 and p180 gene regions, if available.

Results: All to-date published human specimen-derived BDV sequences are identical or highly similar to BDV laboratory strains or to other BDV samples handled in the laboratories which reported the human strains. We identified the following sources of contamination:

1. Obvious contamination of the human sample with a BDV laboratory strain, clone or other BDV handled in the corresponding laboratory
2. Mixed contamination with different contaminants in one sample
3. Mixed contaminations in different PCRs with different contaminants.

Furthermore, greater sequence variations of putative human BDVs turned out to be sequence errors due to lack of manual correction of sequence data or failure in the sequencing process.

Conclusion: Our analyses clearly demonstrate that all to-date published putative human BDV sequences are laboratory artefacts. They neither represent geographical sequence variations which can be observed in animal-derived BDV sequences nor form own branches of human BDVs. Especially the BDV sequences obtained from outside the central European endemic regions should represent higher sequence variations, as it was demonstrated for the BDV sequence of a horse from Styria, Austria. In contrast, human BDV sequences cluster together with sequences of BDV laboratory strains and other BDVs used in the laboratories which reported the human BDVs.

Consequently, our study does not provide evidence for the existence of human BDV infections and associated psychiatric disorders.

13.023 Genomic Comparison of Usutu Virus that Emerged in Austria in 2001 with a South African Strain, Isolated in 1958, and Usutu Viruses that Emerged in 2005/2006 in Hungary, Switzerland and Italy

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Background: During summer 2001, a die-off of Eurasian Blackbirds (Turdus merula) and birds of other species was observed in and around Vienna, Austria, resembling the beginning of the West Nile virus (WNV) epidemic in the United States in 1999. A virus was isolated from the birds which was identified as Usutu virus (USUV), a mosquito-borne flavivirus of the Japanese encephalitis virus (JEV) group, which previously was reported only from sub-Saharan Africa. The virus managed to overwinter in Austria and caused outbreaks also in subsequent years. In 2005 USUV-associated bird mortality was observed in Budapest, Hungary, and in 2006 in Zurich, Switzerland, and in Northern Italy.

Methods: The complete genome sequences of USUV which emerged in Austria in 2001 and of the USUV reference strain SAAR-1776, which was isolated in 1958 from mosquitoes in South Africa, were determined, as well as partial sequences of USUVs which emerged in 2005 in Budapest, and in 2006 in Zurich and in Northern Italy. The sequences were compared to each other, and phylogenetic trees were constructed displaying the genetic relationships of USUV with other flaviviruses.

Results: The Austrian and South African isolates exhibited 97% nucleotide and 99% amino acid identity. When comparing USUV with other JEV serogroup viruses, the closest lineage was Murray Valley encephalitis virus (nt: 73%, aa: 82%) followed by JEV (nt: 71%, aa: 81%) and WNV (nt: 68%, aa: 75%). Comparison of the genomes showed that the conserved structural elements and putative enzyme motifs were homologous in the two USUV strains and the JEV serogroup. The partial sequences of the Hungarian, Swiss and Italian USUVs proved to be 99% identical to each other and to the Austrian strain. A similarly small mutation rate has been observed in Austrian isolates from different years (2001–2005).

Conclusion: The USUV strains currently circulating in central Europe seem to be highly related to each other and are probably the result of a single introduction and subsequent dispersal—although it remains unknown how the virus suddenly appeared in 2005/2006 in Budapest, Zurich and Northern Italy.

13.024 An Amperometric Biosensor Based on T7 Phage-Displayed B-Cell Linear Epitope for the Detection of Anti-West Nile Virus IgG

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Background: Mosquito-borne members of the genus Flavivirus in the family Flaviviridae provide some of the most important examples of emerging infectious diseases. In particular, West Nile Virus (WNV) is widely distributed throughout the world and was unexpectedly introduced

in North America in 1999 where the rapidity and extent of its spread exceeded all expectations and most predictions. Nowadays, the main WNV antigen used for serodiagnosis of the infection is based on major viral recombinant proteins but exhibits a natural cross-reactivity with antibodies induced by other Flavivirus infections. Moreover, current methodologies for the diagnosis of West Nile associated diseases are either time consuming or lack sensitivity.

Methods: In order to increase the specificity, a B-cell linear epitope derived from the domain III of the West Nile virus envelop protein was cloned into a T7 phage display system and used as antigen. The sensitivity was enhanced by a photochemical grafting of the genetically engineered bacteriophages on an electrogenerated polymeric film of pyrrole-benzophenone and tris(bipyridine pyrrole) ruthenium (II). The resulting electrodes were applied to the amperometric detection of anti-WNV antibody via secondary peroxidase-labeled antibody, hydrogen peroxide and hydroquinone as enzyme substrate.

Results: The WNV phage ELISA was able to reach 100% specificity and 67% sensitivity when tested with both WNV/Dengue infected sera and compared to a commercial kit. When associated to the amperometric biosensor platform, the system could detect a titer as low as 1:1,000,000 of anti-WNV antibodies after 1h of total procedure.

Conclusion:T7 phage-displayed B-cell linear epitopes show a great potential to replace native viral antigen or recombinant proteins. It is safe, easy to produce and the round-shape conformation of T7 bacteriophages is enhancing the binding of target antibodies to their respective epitope. Coupled to an amperometric biosensor, these biorecognition entities are paving the way for a new generation of highly accurate and sensitive diagnostic tests.

13.025 Recombinant ELISA for the Detection of Antibody to Rift Valley Fever Virus in Humans

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Rift Valley fever (RVF) is a significant global threat to humans and livestock and a potential biothreat agent. Current serological techniques require manipulation of live virus either during the production of antigen stocks and/or when the tests are carried out. The objective of this study was to validate an indirect ELISA based on recombinant nucleocapsid protein of RVF virus for the detection of specific IgG antibody in human sera. Validation data sets derived from testing field-collected sera from Africa were cathegorized according to the results of a virus neutralisation test. A cut-off value at 95% accuracy level was optimised using the misclassification cost term option of the TG-ROC anlysis.

There was no evidence for excessive intra- and inter-plate variation within and between routine runs of the assay. The ELISA sensitivity was 99.65% and specificity 99.86%.

The ELISA reported here is safe, robust and highly accurate. Therefore, it has the potential to replace traditional diagnostic methods which pose risks necessitating their use being restricted to high containment facilities outside RVF endemic areas.

13.026 The Rapid Detection of Influenza A and B Sub-Types, Including Avian: Using a Single-Tube LATE-PCR Assay in a Portable, Point-of-Care System

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Background: Brandeis University in collaboration with Smiths Detection is developing a highly informative in vitro diagnostic point-of-care system that will detect and discriminate between influenza subtypes, including highly pathogenic H5N1.

Methods: Our approach utilizes novel technologies for sample preparation and amplification, as well as a new portable device. Reverse transcription of RNA templates and amplification of the cDNA are both achieved using Linear-After-The-Exponential, LATE-PCR, in a closed-tube. LATE-PCR is an advanced form of asymmetric PCR which generates single-stranded products of each amplicon. The final multiplex assay is designed to simultaneously generate up to three single-stranded amplicons for each of the possible viral subtypes and a positive control. The assay is quantitative over seven orders of magnitude and is sensitive down to fewer than 10 target molecules. Novel fluorescent probes permit Quantitative End-Point Analysis, rather than real-time analysis. In addition, this assay is highly reproducible because it uses special chemistries to suppress mis-priming.

Results: The LATE-PCR influenza assay has the capacity to distinguish 15 different possible outcomes in a single closed-tube. In addition, because LATE-PCR generates single-stranded amplicons, each of the amplified products can be sent to the laboratory for immediate sequencing by a simple Dilute-'N'-Go procedure in order to characterize new variations of specific sub-types. Specific aspects of assay results will be presented.

Conclusion: The LATE-PCR influenza assay meets the requirements of being both specific and sensitive, while rapidly detecting both current and new varieties of influenza A and B sub-types. Similar LATE-PCR assays are under development for other viral and bacterial pathogens.

13.027 Monitoring of IgG and IgM Responses in Patients Infected with Crimean-Congo Haemorrhagic Fever Virus

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Introduction: There is an increase in international demand for validated and standardised ELISA for serodiagnosis of CCHF.

Aim: To validate IgG-sandwich and IgM-capture ELISAs using immunoreagents developed in-house.

Sera (n=881)collected at diffrent times after onset of disease symptoms from CCHF patients (n=177)and from CCHF negative subjects (n=40), were used to determine ELISA diagnostic accuracy.

All negative subjects tested negative in both ELISAs. Seroconversion was detectable by IgM ELISA between days 4-8 and by IgG ELISA between days 4-10 after onset of symptoms. IgM-capture ELISA sensitivity was 100% in patients bled 8 days to 14 weeks, and IgG-sandwich ELISA sensitivity was 100% in patients bled 10 days to 19 weeks after disease onset.

Results: Demonstrate that the ELISAs reported here are highly accurate diagnostic tools which will be useful for timely implementation of CCHF barrier-nursing and clinical management measures.

13.028 Development of Rapid Field Diagnostics for Identification, Control and Management of Haemorrhagic Fever Outbreaks (EU FP6-INCO-DEV3, VHF Diagnostics)

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Background: To improve the early detection of Viral Haemorrhagic Fever (VHF) cases, adequate tools are needed for the basic conditions of African hospitals. Specialised outbreak management also needs on-site tools such as viral genome detection. We will develop a) line assays (LA) for antibody detection as an easy to use frontline detection assay for health care workers in local hospitals, and b) fluorescent RT-PCR (F-RT-PCR) assays to be used by specialised mobile outbreak investigation teams. Both assays will cover Ebola-Virus (EBOV), Marburg Virus

(MARV), Crimean-Congo-Virus (CCHFV) Lassa virus (LASV), Rift Valley Fever Virus (RVFV), Yellow Fever virus (YFV) and Dengue virus 1-4 (DENV).

Methods: (i) To develop LA, purified recombinant proteins of the VHF viruses will be expressed in the in-vitro RTS-500 system and sprayed onto immunoblot strips. Validation of the LA will be achieved by using available sera in the consortium of laboratories, centralised in a repository for VHF diagnostics development. (ii) We will validate F-RT-PCRs for field use by testing their sensitivity and specificity using RNA standards and recent isolates of each aetiological agent, and patient and/or rodent samples provided by the collaborating laboratories. We will adapt the extraction of nucleic acids from blood samples to field conditions. We will develop lyophilised ready to use PCR mixes to allow field PCR without the need for refrigeration facilities.

Conclusions: (i) We expect to be able to produce the envisioned line assay and to be able to test its applicability in local hospitals in Mali and Guinea. We hope to prove that an easy to use frontline test can reduce alert time in the case of an outbreak. (ii) The integrated F-RT-PCR-toolbox may enable outbreak investigation to perform initial differential diagnostics and to follow-up patients during the containment of an outbreak.

13.029 Emergence of XDR-Tuberculosis in India

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Extensively drug-resistant (XDR) tuberculosis is new threat to AIDS care. Most of XDR-tuberculosis cases are reported to be non-treatable and carry extremely high fatality. Even though high rate of XDR-TB has been reported from USA, South Africa, Korea and Iran, there is no study from India. Therefore, we carried out a study from March 2006 to October 2006. All HIV positive patients presented with signs and symptoms of TB co-infection and referred to our laboratory were included in this study. The clinical samples were subjected to culture isolation of the causative species in BACTEC MGIT-960 (BD®, USA). All culture isolates of M. tuberculosis were first subjected to first line 4 anti-mycobacterial drug susceptibility and those having multidrug resistance were further subjected to second line drug susceptibility testing using the same BACTEC MGIT-960 system. During the study period, 46 patients suspected to have HIV-TB co-infection were included. From 22 of these (47.8%) M. tuberculosis was isolated and 10 (45.4%) of these were found to have multidrug resistance. The second line drug susceptibility results showed alarmingly high rate of XDR-TB as per the definition of CDC and WHO. Out of 10 MDR isolates 3 (30%) isolates also had extensive drug-resistance to 3 classes of second line drugs. Of the three patients (all adults) with XDR tuberculosis, one was female and two males. All the three patients died within 3 months of diagnosis of tuberculosis inspite of treatment. The prevalence of XDR-TB in MDR-TB cases has been reported 4% from USA, 19% from Latvia, 15% from South Korea and recently 23.45% in rural Kwala-Zulu, Natal, South Africa and 10.9% from Iran. Our preliminary findings suggest highest (30%) prevalence of XDR-TB from India. The figures are alarming and if steps to prevent its spread are not taken immediately, the outcome might be devastating.

13.030 Rapid Universal RT-PCR based Flavivirus Detection

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Background: Rapid detection and specific identification of emerging viral diseases has important implications for human health and enables appropriate treatment and control measures to be quickly implemented. In recent years members of the Flavivirus genus have emerged as globally significant pathogens, including the ongoing outbreak of West Nile Virus in North America and the increase in cases of dengue in Asia.

The specific diagnosis of flavivirus infections may be time consuming (over two weeks) using current methods. This paper proposes a rapid method for detection of a wide range of flaviviruses in clinical samples.

Methods: Bioinformatic studies on published flavivirus genomes revealed two highly conserved regions in the NS5 gene (the viral RNA polymerase). Primers were designed to these regions generating an 800bp product spanning the RNA dependant RNA polymerase domain. Using a one-step RT-PCR protocol, RNA extracted from cell cultures infected with 60 different flaviviruses were tested for amplification.

Results: An amplicon of the predicted size was generated for each of the 60 viruses tested. Further sequencing of the PCR products and limited phylogenetic analysis conformed that the correct nucleotide sequence had been amplified for each virus.

Conclusion: The novel oligonucleotide primer set developed in this study enabled the detection of all medically and veterinary significant flaviviruses in infected cultures by RT-PCR within six hours. This represents a potentially powerful tool for rapid flavivirus diagnosis.

13.031 Laboratory Diagnostics of a Zoonotic Pulmonary Capillariasis

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Background: Capillaria (Eucoleus) aerophila is a nematode of the family Trichuridae, and is closely related to C. hepatica, C. philippinensis and C. plica, only those 4 species rarely have been found in humans. We identified a human case of pulmonary capillariasis.

Methods: The Capillariids ova in human bronchial biopsy were identified by comparation with similarly eggs in naturally infected domestic cats. We also development an indirect immunofluorescent assay for human sera with histological sections of an infected cat trachea with C. aerophila, as the antigen.

Results: A 68-year old woman was presented with fatigue, cough with expectoration, fever, anal and skin pruritus and increased appetite during the past 30 days, which didn't regress after the administration of antibiotics. Bilateral reticulonodular pulmonary infiltrates, particularly in the lower right lobe were seen on CT scans. Increasing leucocytosis with eosinophilia was reported, too. Several sputum samples showed necrotic material, Charcot-Leyden crystals and destroyed eosinophils. The bronchoscopy revealed yellow-white, tumor-like lesion in the right lower lobar bronchi, which on microscopic examination of histological slides consisted of necrotic debris, eosinophils and Charcot-Leyden crystals, and several parasite eggs were found. Eggs were oval, bioperculated, unembryonated, and showed a double layer shell with radial striations in the outer shell and diameter 65x30 micrometers. No larval stages or adult parasites were found in the sputum, biopsy specimens or stool. Liver and other abdominal organs were normal on the ultrasound examination. Diagnosis was confirmed by indirect immunofluorescence test. Serum antibody titer was detected in dilution over 1:100. Albendazole was given, orally, for 21 days.

Conclusion: We identified a human case infection with the nematode Capillaria aerophila, a parasite of dogs, cats and other carnivores, on the basis of the morphological characteristics, and by positive novel indirect immunofluorescent assay. This is the first case of pulmonary capillariasis diagnosed in Serbia and probably on the Balkans.

13.032 Preliminary Estimate of the Burden of Salmonellosis in Japan

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Background: In Japan, under the Food Sanitation Law, the numbers of food poisoning and cases must be reported. Obviously this data doesn't exactly reflect the real burden of foodborne illness. In this study, we focused on Salmonella, one of the leading foodborne disease in Japan. To estimate the real burden of Salmonella, a small scale pilot study was

conducted in Miyagi Prefecture, northern part of Japan, (population of 2.36 million).

Methods: Data on laboratory-confirmed infections of Salmonella were collected from clinical laboratories in the prefecture from April 2005 to March 2006. The stool sampling rate was cited from the previous study. The physician-consultation rates were estimated by analyzing foodborne outbreak investigation data. Each factor was multiplied to the laboratory-confirmed cases.

Results: The estimated mean cases of Salmonella per 100,000 population in the Miyagi Prefecture was 32 (5%tile=15, 95%tile=69). This number could be interpreted that it could be annually 755 cases of Salmonellosis in the Miyagi prefecture (population 2.36 million), while the number of report foodborne Salmonella cases in the prefecture in 2005 was only 12. While the data on the percentage of foodborne transmission among Salmonellosis was not available in Japan, if the data from the US and UK were applied, the enormous underreporting was indicated. It was indicated that risk management activities toward reducing the risk of Salmonella should be based on the burden of illness, including both outbreak and sporadic case situations.

13.033 Specificity and Sensitivity of TB PCR in CSF of Patients with AFB Positive Smear TB Meningitis that Admitted in Imam Reza Hospital from 2002 to 2006

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Background: TB PCR in CSF is faster than AFB smear and culture for mycobactrium tuberculousis and also needs less sample volume of CSF. If the validity of this method be proven it may lead to earlier diagnosis and better management of TBM.

Method: CSF were collected prospectively from in patients with suspected meningitis from 2002–2006. TB PCR in CSF was done in the patients with positive smear for AFB and patients that were proven other diagnosis not tuberculosis meningitis (NTBM).DNA sequences amplified were 123 bp region of IS6110. insertion element which occur in multiple copies in the mycobacterial genome.

Results: Among 110 patients 47 cases were suspicious for TBM (21 definite, 12 probable and 14 possible). TB PCR in CSF was also done in 21 patients with positive AFB smear (definite). TB PCR were positive in 16 cases. So sensitivity of TB PCR in CSF was 76.1% and CI 95% (57.8-94.3). specimens from all 21 patients with NTBM such as pyogen (7) viral (7) leukemic (1).Neuro brucellosis (2) neuroburreliosis(1) normal CSF (3). Were negative by TB PCR, giving a 100% specificity. Positive predictive value was 100% and negative predictive value was 80.7 with CI 95%: (65.5–95.8).

Conclusion: According to high accuracy of TB PCR in CSF we suggest it, in addition to AFB smear as a routine diagnostic method for TBM if it is available and cost effective.

13.034 Diagnostics of Hpai H5N1 in Wild Birds During the Winter/Spring 2006 in Austria

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In Austria, the highly pathogenic avian influenza virus (HPAI) of the subtype H5N1 was first detected in two swans at the federal district of Graz on the 13th of February 2006. Since the beginning of the outbreak more than 5000 birds have been analysed at the National Reference Laboratory for Avian Influenza in Moedling at the Institute for Veterinary Disease Control (AGES). HPAI H5N1 virus was detected in a total of 119 wild birds, including 82 mute swans, 28 wild ducks, 2 geese, 1 seagull, 1 heron and 1 bald coot, and also in 3 cats and 6 chickens belonged to an animal shelter. The last case was reported on the 26th of April 2006.

Carcasses of the birds were initially sent for pathological investigation. Target organs were taken for virology and PCR detection. Suspected birds were examined additionally by histology and immunohistochemistry.

The swab or the tissue homogenate was inoculated into embrionated eggs for virus isolation. Influenza Virus was identified by Hemagglutination Inhibition Tests using subtype-specific reference sera provided by the European Reference Laboratory for Avian Influenza in Weybridge (England).

Viral RNA from the tissue homogenates and swabs was detected by a real-time RT-PCR specific for the matrix protein of Influenza A viruses, and confirmed by a H5 specific real-time RT-PCR following the protocols recommended by the European Union. Furthermore, pathotyping of H5 viruses was performed by sequencing of 280bp long fragment coding the cleavage-site of the hemagglutinin (HA) protein.

Typical pathomorphological findings in infected birds were: pancreatic and hepatic necrosis and nonpurulent encephalitis. Immunohistochemistry revealed a typical antigen distribution for acute viremia.

All H5N1 positive samples were characterised as high pathogenic AIV of the subtype H5N1 strain with the motif PQGERRRKKR/GLF. Moreover, genetic comparison of sequences determined in Austria with sequences of neighbouring countries revealed the presence of at least three different H5N1 virus strains which we called the Graz, Bodensee and Hainburg strains in our epidemiological study. The H5N1-Graz strain was first isolated in Graz and surrounding area, and later on in wild birds from the districts Bruck a/d Mur, Scheibbs and Schaerding. However, the H5N1-Bodensee virus was surprisingly not only detected at the Austrian border site of the Lake Constance, but also in Vienna and territories along the Danube River. Finally, the H5N1 sublineage Hainburg was found in one swan in Hainburg a/d Donau near the Slovakian border.

13.035 Phylogenetic Studies on Infectious Pancreatic Necrosis Virus (IPNV) Based on Partial Sequences of Segment A

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Background: The aim of this study was to investigate the genetic diversity of infectious pancreatic necrosis virus (IPNV) isoaltes in farmed fish, collected from different areas in Austria during the years 1993 to 2001. Infected fish were rainbow trout (Oncorhynchus mykiss), brown trout (Salmo trutta fario), brook trout (Salvelinus fontinalis), grayling (Thymallus thymallus) and roach (Rutilus rutilus), with different age from few months to three years. Most of the fish appeared with clinical symptoms typical for IPN. In addition, one sample from a rainbow trout originating from Italy was included.

Methods: For detection of IPNV in 10 organ pools (head kidney, spleen and heart) collected from 70 fish, a specific reverse transcription-polymerase chain reaction (RT-PCR) was used. The genomic relationships between the analyzed isolates and 39 IPNV nucleotide sequences obtained from the GenBank database were investigated using phylogenetic analyses of 499-bp genomic fragments representing a region encoding VP2 on genome segment A of IPNV.

Results: Results indicate that RT-PCR is a sensitive, rapid and reliable replacement for virus isolation in cell culture. The phylogenetic tree of the IPNV isolates exhibited two major genogroups, four subgroups and various branches. The first genogroup included only European isolates. Austrian isolates were closely related to each other (98-99% nucleotide identity). Isolates from Italy, France and Spain showed also such high degree of identity to Austrian isolates within this genogroup. In the second genogroup, IPNVs from North America, Asia and some isolates from Europe were distributed in several clusters, in general according to their geographic origins. With few exceptions, the isolates in each group showed high identity rates to each other (95–100%).

Conclusion: This study showed that the phylogenetic relationship of IPNV was dependant on the geographic origin of the isolates but independant of host species and year of isolation.

13.036 Chromobacterium violaceum Infection in a Free Ranging Howler Monkey in Costa Rica

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Chromobacterium violaceum, a gram negative bacterium, has been isolated from water and soil in tropical and subtropical regions and is as well, although rare, an opportunistic pathogen in mammals. There are numerous reports, often fatal, of chromobacteriosis in humans and non-human primates in captivity, but there is no record in free ranging primates. Here, we report infection by C. violaceum in a wild, free-ranging non-human primate. The animal, an adult male mantled howler monkey (Alouatta palliata), was captured by park rangers at Ballena Marine National Park, in south western Costa Rica and brought to the Veterinary School at Universidad Nacional de Costa Rica. The specimen presented severe skin lesion over legs, arms and tail and due to its condition was euthanized. Necropsy revealed features reported in infections by C. violaceum in mammals. Multiple abscesses in the liver and lymphoid hyperplasia were detected. The skin showed microabscesses and deep dermatitis, necrotic epithelial cells and massive presence of neutrophils. Clusters of coccoid-shaped bacteria were observed over the skin. Coagulating necrosis, hyperaemia, haemorrhage, foci areas of telangiectasia around lesions in the liver was also observed. No significant microscopic changes were noted in any other parenchymal organs examined. Liver necrotic tissue was taken aseptically, inoculated onto blood and MacConkey agar then incubated at 37 C for 24 hours. The cultures led to isolation of C. violaceum. To the best of our knowledge, this is the first case reported of this pathogen in a wild, free ranging primate. We could not ascertain the source or mechanism of infection in this case, although infection through skin micro- abrasions is suspected.

The coastal region where this case was documented is undergoing increased tourist activity and vacation home development. This raises the potential for C. violaceum to act as sepsis pathogen to susceptible individuals during leisure activities that involve contact with stagnant water, sediment and/or soil.

13.037 Clinical Evaluation of a New ID-Tag™ RVP Test for Detection of Respiratory Viruses

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Background: Historically virology laboratories have used DFA and culture to detect six or seven respiratory viruses. With the discovery of five new respiratory viruses since 2000 there is a need for new diagnostic tests to detect a wider range of respiratory viruses. We have evaluated the performance of a new ID-Tag™ RVP test that can detect twenty respiratory viruses.

Methods: NP specimens [N=227] were collected from symptomatic patients under ERB approval and were tested by the ID-Tag™ RVP test (TmBioscience Corp'n, Toronto, Canada) and conventional DFA plus culture. The RVP test is a new test for the detection of 20 respiratory viruses that uses multiplex PCR, a Universal Array of oligonucleotides (TmBioscience), and a fluidic microbead array (Luminex X-Map). The test detects Influenza A (subtypes H1, H3, and H5 Asian lineage), Influenza B, Parainfluenza types, 1,2,3,4, RSV types A and B, Adenovirus, Metapneumovirus, Rhinovirus/Enterovirus, Coronavirus 229E, OC43, NL63, HKU1,and (SARS-CoV). The ID-Tag™ RVP test was performed according to manufacturer's instructions. Briefly, viral nucleic acid was amplified by a multiplex PCR followed a multiplex Target Specific Primer Extension (TSPE) reaction and sorting of TSPE products using the Luminex X-Map system. DFA and culture were performed using MCabs and R-Mix shell vials (Diagnostic Hybrids Inc.). For discordants and RVP positives where virus targets were not tested by DFA/culture, a second PCR (unique primers) and sequencing was performed.

Results: Twenty-two of 227 specimens (9.7%) failed to give a signal for the internal control indicating extraction failure or were called equivocal for at least one target. Of the 206 specimens analysed, 172 gave concordant results; 135 were positive by both RVP and DFA/culture and 37 were negative by both tests. There were 7 RVP- DFA/culture+ and 27 RVP+ DFA/culture- discordant results. After resolution of discordants by a second PCR test, RVP had a sensitivity of 95.8% (138/144) compared with 93.7% (135/144) for DFA/culture. RVP detected an additional 26 confirmed positives including 22 Rhinovirus/Enterovirus, 1 NL63 Coronavirus, and 3 HKU1 Coronaviruses that are not routinely tested for by DFA or culture and 4 additional Influenza B positives that were missed by DFA/culture. **Conclusion:** The ID-Tag™ RVP test is more sensitive than DFA plus culture and detects an additional 12 respiratory viruses not routinely tested for by DFA and culture. Overall 22.5% additional positive specimens were detected that were either missed by DFA and culture or not tested for. The ID-Tag™ RVP test should improve the ability of hospital and public health laboratories to diagnose viral respiratory tract infections in a hospital or community outbreak situation.

13.038 A New Quantitative Realtime PCR for Chikungunya Virus Detection

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Background: A large outbreak of a mosquito-borne viral disease, Chikungunya, has begun in 2005 at La Reunion island. In few months many other countries in Indian Ocean have experienced a dramatic increase of cases. As expected, in Europe some cases in returning travellers are appearing and concerns about its spreading in south European countries, where a suitable the vector is present, have been raised. To facilitate the rapid diagnosis of this infection a new quantitative RealTime PCR method has been developed. This method, that allow to measure the kinetic of virus replication is suitable also for in vivo and in vitro pathogenic studies.

Methods: We have developed a FRET-probe-based Real-time PCR targeted to nsP1 gene. To increase sensitivity and be able to detect all Asian and African Chikungunya strains two pairs of primers, both targeting the same nsP1 fragment (size 142 bp), were designed, to recognize both viral lineages. Primers and probe were designed by using LightCycler probe designer V. 2.0 software.

Results: Sensitivity of the test, performed by probit analysys, is of 20 cp/reaction (equivalent to 4.000 cp/ml) and the linear range spans from $3x10^1$ to $3x10^8$ cp/reaction. Chikungunya viremia detected on sera from acutely infected patients ranged from $1.3x10^5$ to $6x10^8$. To validate the quantitative assay, we performed viral inhibition experiments in vitro with recombinant IFN alfa, and virus replication was measured in parallel with infectivity titration and viral RNA measurement. A dose-dependent inhibition of viral infectivity, as well as of virus RNA production, was observed.

Conclusion: The method here described is a useful tool for a rapid detection of Chikungunya virus and to monitor the viral load in infected patients involved in the ongoing outbreak. The wide linear range of the assay is consistent with in vitro studies in which evaluation of the viral replication is needed, as for testing of antivirals. We anticipate that the primer design of the method, aimed at detecting both African and Asian Chikungunya strains, could be particularly suitable for differential diagnosis of febrile syndromes in travellers.

13.039 Metallo Beta Lactamases in *Pseudomonas aeruginosa* and Acinetobacter Species—A Study from North India

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Background: With the advent of carbapenems in the 1980s, a new treatment option for serious bacterial infections was introduced. However, emergence of bacterial agents resistant to this group is being reported worldwide. Acquired metallo-beta- lactamases (MBL), in Gram negative bacteria is becoming a therapeutic challenge. Herein we report the

presence and use of various tests for detecting MBLs, in Pseudomonas aeruginosa and Acinetobacter species.

Methods: A total of 100 isolates of Pseudomonas aeruginosa and Acinetobacter species were tested for MBL production. These strains were from different specimens and included patients from critical care wards. All these isolates showed reduced susceptibility to ceftazidime and/or imipenem.

MBL detection was done by two double disc synergy tests: Imipenem-EDTA disc method of Yong et al and Ceftazidime-MPA disc method of Arakawa et al.

Results: Pseudomonas aeruginosa: MBL production was tested in 33 isolates of Pseudomonas aeruginosa and of these, 32 strains (96.97%) were found to be positive for MBL production by Ceftazidime- MPA disc method. Of these 33 isolates, 25 strains were also imipenem resistant and imipenem-EDTA disc method was found to be positive for 21 (84%) of these 25 strains.

Acinetobacter species: 67 strains of Acinetobacter species were tested for MBL production by Ceftazidime- MPA disc method and 65 of them (97.01%) were found to be positive. 47 strains were also imipenem resistant and when tested by Imipenem-EDTA disc method, they showed a positivity of 87.23% (41/47).

Conclusion: 1) Our study clearly indicates that MBL positive strains are highly prevalent in our hospital settings especially critical care wards. 2) Ceftazidime- MPA disc method was found to be highly sensitive method giving positivity as high as 97%. 3) Considering imipenem resistant isolates only, imipenem EDTA disc method showed positivity upto 87%. 4) Ten of the strains which were imipenem resistant by disc diffusion method, did not show positivity by imipenem-EDTA disc method. However, they were ceftazidime resistant and positive for MBL production by Ceftazidime- MPA disc method. Probably, these strains belong to IMP-1 category. 5) For screening both ceftazidime resistant and imipenem resistant Pseudomonas aeruginosa and Acinetobacter species, combination of both the methods for detection of MBL resistance would be beneficial.

13.040 Herpesvirus Infections of the Central Nervous System in HIV-Infected Patients

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Background: Human herpesviruses cause a spectrum of diseases that are usually self-limiting but can be reactive during immuno-suppresion and may then lead to severe diseases.

Methods: A multiplex nested PCR assay for the simultaneous detection of five human herpesviruses DNA derived from herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus-6 (HHV-6) and Epstein-Barr virus (EBV) was used in human immunodeficiency virus (HIV) infected patients with neurological disorders.

Results: We studied 77 cerebrospinal fluid (CSF) samples from 67 patients with suspected herpesvirus infection of the central nervous system (CNS). Viral nucleic acid was detected in 16 (24%) patients (6 CMV, 4 VZV, 4 EBV, 1 HSV and 1 HHV-6). None of the CSF samples presented herpesvirus co-infection in the CNS.

Conclusion: Multiplex nested PCR assay provides a rapid, sensitive and specific method to detect and differentiate herpesvirus infection of HIV infected patients with neurological disorders related to herpesvirus

13.041 The Role in Influenza Surveillance of the United States' Department of Defense Global Emerging Infections Surveillance and Response System

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Background: The United States' Department of Defense Global Emerging Infections Surveillance and Response System (US DoD-GEIS) was created in response to the June 1996 Presidential Decision Directive National Science and Technology Council-7 that stated that emerging infections threaten national and global security. The principal goal of the tri-service DoD-GEIS is the surveillance, detection of, and response to emerging infections that threaten military populations, including acute respiratory illnesses, especially influenza. This surveillance program is an important source of global information regarding the status of influenza and its impact not just to the US military but also to civilian populations worldwide.

Methods: The US DoD has approximately 9.2 million medical beneficiaries including 2.4 million active duty and reserve personnel and 120,000 healthcare workers distributed worldwide. These populations are surveyed for influenza and febrile respiratory diseases by the Air Force Institute of Operational Health (AFIOH) and the Naval Health Research Center. In addition, augmenting influenza surveillance efforts of the World Health Organization (WHO), non-U.S. civilian and military nationals are also included; these samples are largely managed by the 5 overseas DoD laboratories located in Thailand, Egypt, Kenya, Peru and Indonesia.

Results: To date, a large network of influenza surveillance sites in the US and overseas has been developed with a total of 273 sites in 56 countries, and many large-deck ships of the US Naval Fleet; 38 sites in 9 countries are to be added in 2007. Many sites are located in regions of the world where DoD has the only existing laboratory-based surveillance capacity. As one recent result of this effort, AFIOH provided a unique seed virus obtained from a DoD surveillance site that was one of three components incorporated into the 2006-7 human US influenza vaccine.

Conclusion: The DoD-GEIS network has a significant role in global influenza (and other emerging infectious disease) surveillance. Its US-based laboratories and forward-deployed laboratory platforms provide critical data which serves as a monitoring system to detect emerging pathogens, particularly as it may relate to the emergence of pandemic strains of influenza virus. The diversity of the influenza isolates obtained through this global surveillance program helps to identify disease trends and changes in strain composition worldwide. This serves as an important contribution to the development of seasonal and pandemic influenza vaccines.

13.042 Smallpox Diagnostics: Global Preparedness

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The US Centers for Disease Control and Prevention sponsored the 2nd GHSAG Smallpox Wetlab Workshop for members of the Global Health Security Action Group Laboratory Network (GHSAGLN) who have diagnostic assays for detecting variola virus. This exercise was designed to concentrate on the laboratory algorithms that each country uses to make a definitive identification of an orthopoxvirus infection, as well as, enhance inter-GHSAGLN communication. Ten participants representing 6 countries (Canada, France, Germany, Italy, Japan, Mexico) came to the CDC for 4 days to evaluate their diagnostic assays using non-infectious materials derived from variola virus that are unavailable outside the CDC. Prepared by CDC Poxvirus Program the coded standardized panel used in this exercise consisted of 40 samples of non-infectious DNA material including 4 purified variola virus strains each serially diluted (10x) from 1ng/μl to 1 fg/μl (5e^6 to 5 genomes/μl) and also vaccinia, ectromelia, camelpox, raccoonpox, varicella, herpesvirus (HSV-1), bacteria, and human and monkey cell lines as DNA controls. Each country was asked to analyze the material using the procedures routinely used by their labo-

ratory in suspected cases of human infection. The exercise mainly focused on nucleic acid test evaluation, but some availability of protein-based test evaluation was present. The techniques utilized by the participants were standard Polymerase Chain Reaction (PCR), Real-Time PCR, DNA sequencing, and immunoassays. All 6 (100%) countries performed standard PCR and/or RT-PCR against the panel and correctly identified the variola strains where most detecting at the concentration level of 1fg/μl. 3 out of 6 (50%) countries identified an orthopoxvirus in the raccoonpox sample (a North American orthopoxvirus). One country had a false positive result for variola in one of the negative controls and another country had an indeterminate result with the same control. Overall, the algorithms utilized by these countries were proficient in identifying an orthopoxvirus infection. To increase capacity building to quickly detect the occurrences of emerging disease threats (in particular, smallpox) and to increase and promote international partnerships and share resources and expertise, the WHO Collaborating Centers for Smallpox with CDC and WHO plan similar exercises with broader global participation.

13.043 Between In-vitro and In-vivo: The Use of Ex-vivo Tissues for Infection by Pathogenic Respiratory Viruses

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Background: A proper understanding of the disease severity of various viral respiratory pathogens relies on knowledge of the cell tropism and anatomical basis of infection by the pathogen. At present research is mainly directed towards using animal models, cell line cultures or retrospective analysis of human autopsy tissues to determine why emerging viral pathogens such as SARS or H5N1 lead to disease severity and mortality. Unfortunately animal models of these diseases do not mimic the human situation and cell culture models also may not faithfully recreate disease models. The use of ex vivo freshly removed tissue biopsy fragments from surgical material represents a compromise in determining cell infection and pathogenesis without risk to the patient, and also allows an assessment of potential hazard from newly emerging pathogens.

Methods: Fresh biopsies were obtained from the normal nasopharynx of 15 individuals being screened for nasopharyngeal carcinoma and 6 biopsies were obtained from healthy volunteers. In addition, fragments of tonsillar or adenoidal tissue were obtained from patients undergoing elective tonsillectomy and adenoidectomy. The biopsies or tissue fragments of the latter were excess to the requirements of clinical diagnosis. The tissues were immediately placed into culture medium (F-12K nutrient mixture with L-glutamine, and antibiotics) and infected with influenza A viruses of subtypes H5N1, H3N2 or H1N1 within three hours of collection.

Results: We found that H3N2 and H5N1 viruses readily infected epithelial tissues of the nasopharynx, adenoid and tonsil. No viral antigen detected in mock infected tissues, or tissues infected with UV-inactivated H5N1. Productive viral infection in the tissue fragments from the nasopharynx was demonstrated by increasing viral yield in culture supernatants and viral antigen was detectable from 16- 72 hours after infection.

We also carried out parallel experiments using ex-vivo cultures of lung tissue. We identified viral antigen in ex-vivo infected lung tissues and dual labeling of these tissues showed that the virus infected cells were pneumocytes and alveolar macrophages. Increasing viral yield in culture supernatants provided evidence of productive viral replication. Viral antigen was also readily detected in H1N1 infected lung tissues.

Conclusion: The use of ex-vivo biopsies from the upper and lower respiratory tract is a feasible and safe method for determining respiratory viral pathogenesis in emerging infections and should be utilized more in research laboratories that have access to clinical material.

13.044 Meningococcal Disease: A Challenge to the Brazilian Surveillance

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Background: Meningitis has a worldwide distribution and epidemiological expression depends on many factors including the etiological agent, crowd people and social economic conditions. The World Health Organization (WHO) considers that one million cases occur each year and two thousand people die. In Brazil the meningococcal disease is in the obligatoriness notification list since 1975. The last major epidemic of disease occurred in the early 1970s and was attributed to serogroup A and C meningococci.

Methods: This is a descriptive study based on time series. The following definitions for confirmed cases of invasive meningococcal disease were considered: Confirmed cases of invasive meningococcal disease were defined as a clinically compatible illness with at least one of the following-isolation of Neisseria meningitidis from an otherwise sterile body site, or detection of gram-negative intracellular diplococci in cerebrospinal fluid (CSF) or petechiae. We evaluated using data reported to the National Notifiable Disease Information System (SINAN), during 1986-2006 until 43rd epidemiological week.

Results: In Brazil, bacterial meningitis represented 45% of the total cases confirmed in the surveillance system between 1986 and 2006. The meningococcal disease represented 38% of the cases in this period. The children < five years of age have higher risk of getting ill, but an ascendant line could be observed in older groups of age. The meningococcal disease occurs at an annual rate of 2,3 cases per 100.000 population with periods of increase activity being observed each 10-15 years. The proportion of isolated meningoccoci serogroup serogroup C related with the total strains of meningococcal disease is increasing in the last four years (7,9% in 2000 to 15,9% in 2005) and serogroup B has decreased 34% (22,8% in 2000 to 15% in 2005). The coefficient of incidence cases of serogroup C in children < five years of age is increasing when compared with the incidence of serogroup B in the Northeast and the Center Regions and we can observe the increasing trend line. In the Southeast Region the serogroup C is just the most important.

Conclusions: Clinicians need to be alerted to the presence of the disease in the community and the increasing incidence in regions with high demographic density. The surveillance system must improve the alert mechanism of detection of outbreaks to detect ongoing outbreaks and try to reduce the high rate of fatality associated with serogroup C.

13.045 The PMES Technique Allows for Accurate Analysis of Complexity and Diversity of Viral Quasi-Species

R. Kaczanowski, K. Kucharczyk. Biovectis Ltd., Warszawa, Poland

Background: Viral quasispecies composition is an important feature of viral populations. However the available techniques for analysis are laborious and expensive. Most widely used is molecular cloning followed by sequencing of clones. Here we propose a technique for analysis of viral quasispecies based on separation of single stranded DNA isoforms and sequencing of separated viral variants. We used this technique for analysis of viral quasispecies changes in HCV populations in patients undergoing antiviral therapy. We demonstrate that the changes of genetic diversity and complexity within the HCV HVR1 region correlate with the EVR to PegIFN therapy.

Methods: Eight patients infected with HCV genotype 1b, undergoing Peginterferon-α-2b (PEG-intron, Shering-Plough, USA) + ribavirin (Rebetol, US. Pharmacia, USA) therapy were chosen from a large group. Four of them demonstrated EVR and four did not achieve 2 log viremia reduction at week 12 of treatment.

The genetic heterogeneity of HCV HVR1 region was analyzed with the Multitemperature SSCP (MSSCP) method followed by the isolation of ssDNA bands from poliacrylamide gel, and sequencing. This technique, (we call it PMES), gives more information about HCV quasispecies and is less time consuming when compared with molecular cloning and sequencing of the quasispecies.

Results: Patients achieving Early Virological Response (EVR) had significant decrease in genetic complexity within the HVR1 region, after 2 weeks of treatment (p=0.001). The genetic diversity reduction measured as reduction by per cent, decrease of genetic diversity within HVR1 was significant (p=0.007) after 2 weeks of treatment. At that time in most patients with EVR the genetic diversity decreased to highly homogenous population before the subsequent clearance of HCV RNA at week 12 occurred.

In contrast in the group of patients not reaching the 2log viremia reduction at the 12th week of treatment, no decrease in the genetic diversity and the number of viral strains within the HVR1 region after two weeks of treatment was observed. No significant differences in reduction of viral loads between these groups of patients in week 2nd and 12th of treatment were seen.

Conclusion: Changes of genetic diversity and complexity between week 0 and week 2 of the HCV treatment correlate with EVR. Obtained results suggest that early evaluation of diversity and complexity changes in the viral quasispecies population by the PMES method during anti-viral treatment, could be a useful tool for early identification of patients not likely to respond for the therapy.

13.046 Application of a Modified Human ELISA Kit for Diagnosis of Hydatidosis in Sheep

G.H. Hashemitabar, G.H. Razmi, A. Sharoozian. Ferdowsi University of Mashhad, Mashhad, Iran

Cystic hydatid disease (Hydatidosis) is one of the most important zoonosis that is caused by the larval stage of Echinococcus granulosus. As its diagnosis by clinical symptoms alone is difficult and confusing, but serological diagnostic techniques are used to absolutely confirm the disease. These techniques can also be used for epidemiological studies. The present study was performed with a commercial human ELISA kit for diagnosis of hydatidosis in sera collected from sheep with hydatidosis. Sera were collected from 68 cases of hydatidosis proven by inspection of hydatid infected livers and lungs of the sheep slaughtered in Mashhad abattoir and also from 11 healthy cases. Sera samples were examined by ELISA kit.

The results showed that from 68 cases of hydatidosis in sheep, 67 samples had positive adsorbance. Also from 11 healthy samples, 9 of them had negative absorbance value. The sensitivity and specificity of the test were %98/5 and %81/8 respectively. By getting these results, it can be concluded that it is possible to use human ELISA kit for diagnosis of hydatidosis in sheep.

SESSION 14 (Poster Session)
Veterinary Surveillance Systems

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

14.001 Active Surveillance of Scrapie in the EU: Comparing Apples with Bananas?

V.J. Del Rio Vilas[1], D. Bohning[2]. [1]Veterinary Laboratories Agency, Addlestone, United Kingdom; [2]University of Reading, Reading, United Kingdom

Background: To improve the detection of scrapie throughout Europe the EU dictated the implementation of two surveys in 2002: the abattoir survey (AS) and the fallen stock (FS). Countries must follow some basic criteria on methodology, as an attempt to standardise the surveys. In this study, we modelled the unobserved heterogeneity of the data, from the EU annual report, in an attempt to inform comparisons between countries.

Methods: Countries could be grouped into clusters representing the underlying subpopulations relative to the risk of scrapie between the two surveys. This cluster structure can be modelled by means of non-parametric mixture distributions. We applied this approach to a meta-analysis of the risk ratios (RR) (FS/AS) from 18 EU countries. More specifically, we chose the multi-level (ML) and the profile-likelihood (PL) models as our choice of mixture models. We conducted our analyses on 2003 and 2004 active surveillance data.

Results: The heterogeneity dropped in 2004 (tau=0.5) compared to that in 2003 (tau=1.49). The overall RR dropped from 3.3 (95% CI: 1.57, 7.08) in 2003 to 2.2 (95% CI: 1.31, 3.76) in 2004. The ML approach overestimated the number of clusters (5 in 2003, 4 in 2004) compared to the PL approach (3 clusters in 2003, 2 in 2004). This overestimation by the ML is consistent with the presence of heterogeneity in the baseline population (AS), particularly in 2003.

Conclusions: Our results show i) an overall reduction in the ability of the FS to detect scrapie relative to the AS in 2004 and ii) a reduction in the AS heterogeneity in 2004 across the EU. Variation between countries, and within countries between years, seems to come from changes in the operation of the FS. Modelling the cluster structure in the data facilitated comparisons between countries within clusters where pooled estimates can be reported.

14.002 Emerging Antibiotic Multiresistance in Seafoods as an Hazard for Human Health

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Seafoods are the most perishable to autolysis, oxidation, hydrolysis of fat and microbial spoilage. The present study focuses on studying the hygienic quality of fresh mussels which were collected from important commercial areas. Decimal dilutions of homozeinized mussels were performed in Ringers solution and spreaded in the following plate media: McConkey agar, Mannitol Salt agar, MRS agar, Columbia blood agar, Egg Yolk agar and MYP agar (Mannitol Egg Yolk Polymyxin Agar). Incubation of the plates was performed aerobically or anaerobically up to 48h at 37°C.An aliquot was heated at 80ºC for 10 min for research of spore forms. To confirm the presence of C. perfringens, the L.S. (Lactose-Sulfite) medium was used.Fresh mussels, as they are submitted to extensive handling, they seems to carry a high load of C. perfringens spore (35%) forms and E. coli (4%). All isolated C. perfringens, E. coli and S. aureus strains were tested for their antimicrobial activities in Mueller Hinton agar by applying the antibiotic discs and detected using the disk diffusion method, according to the standards by the NCCLS All C. perfringens isolated from mussels was susceptible to metronidazole. High level of resistance (50% to 60%) showed the E. coli isolates .S. aureus isolated from mussels presented a high resistance to vancomycin (45%) as well as to ampicillin (35%),gentamycin (35%), penicillin (38%) and ampicillin (36%).In Greece, multiresistance in hospitalized patients and outpatients is reported. The case of outpatients is somewhat interesting, as these patients had not received systematically antibiotics, the observation of frequent multiresistance was unexpected in this group of patients. The reasons for the resistance patterns observed may be due to the feed ingested. It is of substantial interest to note that in our country for improving the quality of animals, antibiotics are added in their food. Antibiotics could be present at high levels in animals and their products ingested by man. It is then conceivable and understoodable, the presence of multiresistance observed in most of our isolates, and as conclusion, it may not be underestimated. In light of the increasing public health importance, national monitoring and eradication program must be developed in order to control the multiresistance observed. Moreover, regulatory bacteriological standards and HACCP guidelines must be applied and be able to produce products of high bacteriological quality.

14.003 New Approaches for the Estimation of the Prevalence of Scrapie in Great Britain

V.J. Del Rio Vilas[1], D. Bohning[2]. [1]Veterinary Laboratories Agency, Weybridge, United Kingdom; [2]University of Reading, Reading, United Kingdom

Background: The under-ascertainment-adjusted holding prevalence of diseases can be estimated via multiple-list capture-recapture (CRC) methods. The traditional CRC approach, although utilising the existing surveillance streams, requires substantial overlapping between them and a favourable regulatory context for a holding to be recorded in more than one source. We explore the application of three models, still under the CRC approach but based on a single source with repeated entries, to circumvent the above constraints and estimate the number of scrapie-affected holdings not captured (n0) by the Scrapie Notifications Database (SND) in Great Britain (GB).

Methods: We estimate (n0) by means of: i) the Zelterman estimator as an alternative to the simple truncated Poisson models, ii) the Chao bias-adjusted estimator and iii) a new weighted estimator. We built up the count distribution of cases from scrapie-affected holdings (n) and are interested in finding an estimate of the unseen frequency of n0 for 2002 (n = 144), 2003 (n = 134) and 2004 (n = 151). The total size of the scrapie-affected holding population is then calculated as N = n + n0.

Results: The results (N) from the application of the three estimators, their 95% CI and the completeness or sensitivity of the SND to detect scrapie are shown in the Table below.

Year	Zelterman N	Chao N	Weighted N	95% CI Zelterman	95% CI Chao	95% CI Weighted	Sensitivity Zelterman	Sensitivity Chao	Sensitivity Weighted
2002	312	264	278	200-423	192-336	217-338	46.29	54.74	51.95
2003	230	210	216	162-297	164-255	178-254	58.47	64.08	62.20
2004	301	270	280	207-395	204-336	226-334	50.28	55.97	53.95

Conclusion: Validation of these figures can be performed by comparison with other sources of information. The application of our models to the count distribution of reported holdings, to account for similar clinical misclassification, estimated the sensitivity of the source at 44%, in the same range as the 2002 postal survey in GB (38%).

14.004 Scanning Surveillance of New & Emerging Animal Diseases in England & Wales by the Veterinary Laboratories Agency

G. David. Veterinary Laboratories Agency, Addlestone, United Kingdom

The Veterinary Laboratories Agency (VLA) is an executive agency of the UK Department for Environment & Rural Affairs (defra). It supplies defra with scanning surveillance data on the endemic & emerging disease issues (including animal welfare) associated with farmed livestock, birds and wildlife in England & Wales. The system aims to detect and characterize exotic and novel disease conditions at an early stage and provide initial risk assessment and links into a more formal risk assessment and management structure.

VLA provides laboratory diagnostic and field investigation services to practicing veterinarians who deal with farmed livestock and birds through a network of 14 Regional Laboratories, VLA Lasswade & two Surveillance Centres (Liverpool University and Royal Veterinary College). Diagnoses conforming to an agreed case definition are given a numeric code and are captured on a database FarmFile together with descriptive epidemiological data relating to that disease incident. Disease incidents where a diagnosis is not reached are also recorded by syndrome using a similar numeric system. This diagnostic data is transferred to the Veterinary Investigation Diagnosis Analysis database (VIDA), which has data going back to 1975.

Disease data are analysed by specialist groups (Species Groups) within the Agency who act as the hub of a knowledge network. This culminates in reports delivered to defra Animal Health & Welfare Directorate General (AHWDG) describing the disease status of farmed livestock, birds and wildlife with particular reference given to the emergence of new diseases or disease trends. Early detection models have been developed to identify significant trends in undiagnosed disease which might indicate the presence of a new disease. These analyses have recently been used to support GB freedom from Bluetongue.

Profiles of new & emerging diseases with a zoonotic potential are fed into a risk assessment forum via the Human Animal Risk Surveillance group (HAIRS) comprising expert human health professionals and veterinarians. Diseases identified as being high risk are referred to higher-level assessment and national policy groups.

14.005 African Swine Fever in Italy. Updated Epidemiological Situation

F. Feliziani[1], D. Aloi[2], A. Oggiano[2], C. Patta[2], G.M. De Mia[1], S. Rolesu[2], D. Rutili[3]. [1]Istituto Zooprofilatico Sperimentale dellUmbria e delle Marche, Perugia, Italy; [2]Istituto Zooprofilatico Sperimentale della Sardegna, Sassari, Italy; [3]Istituto Zooprofilatico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

The more recent epidemic weave of ASF in Sardinia started at the beginning of 2004 and lasted up October 2005. The majority of the outbreaks were in medium and small size herd, most of which are backyard pig farms, and only two involved large premises. The total number of pig involved in the outbreaks was 6,718. Almost all the secondary outbreaks occurred within the restriction zones of the index cases. The epidemic started in March 2004 and showed two peaks: the first during the period of June/July 2004 and the second during the period of May/August 2005. In most of the 14 index cases there is insufficient epidemiological information to explain the origin of the infection.

In the framework of the eradication plan of ASF, 5,267 herds were serologically examined and 72,927 animals were tested during 2005. All except one of the seropositive herds (19) were located in the Province of Nuoro. The wild boar shot during the last hunting season (2005–2006) were virologically and serologically examined: 88(2.098%) out of 4,194 animals were serologically positive. No virus positive wild boar were found.

Even if wild boar could play an important role as reservoirs of ASF virus, it seems that in recent years they have not been a critical risk factor in the spread of ASF in Sardinia.

The most important risk factor evidenced is the uncontrolled pig movement and free-ranging pigs. The environmental conditions (Mediterranean scrub)) and breeding system are coincident. The veterinary service was unable to deal with the size of ASF emergency involving a large number of medium and little size herds spread all over the regional territory.

As shown by the results of the serological surveillance there is no relationship between the low prevalence of seropositive pigs and the high incidence of ASF in the Region.

14.006 Cattle Health Surveillance in the Netherlands

L. van Wuijckhuise, C. Bartels, P. Kock. GD, Deventer, Netherlands

Introduction: In 2003 stakeholders requested GD to redesign the cattle health surveillance system. The objectives were to detect contagious non endemic diseases or new disease phenomena in time and to describe and analyse trends in cattle health. Three surveillance components were developed.

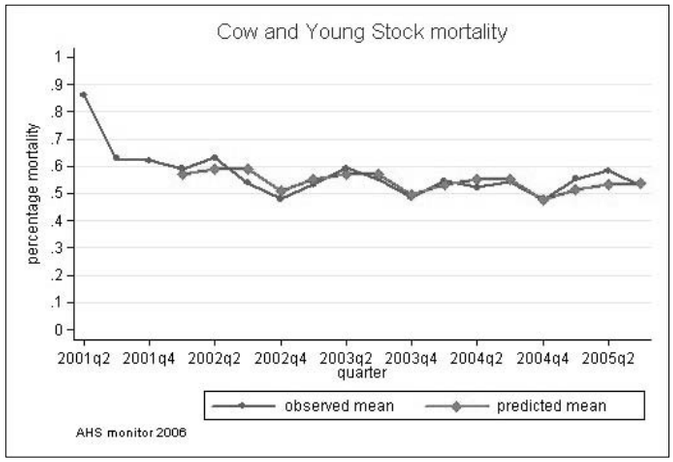
Material and Methods: Data sources: **1.** GD-Veekijker: Extracts from weekly evaluation on indications for an emerging disorder or early detection of highly contagious non endemic disease based on reports from farmers and practioners, results from laboratory submissions and post mortem examinations. **2.** Prevalence studies: Herd prevalence estimates measured every 2–4 years in non-certified herds on endemic infectious diseases like BVD-virus, BHV1, Leptospirosis, Neosporosis, Salmonellosis. **3.** Key Monitoring Indicators: on cattle health were calculated based on census data of 5 years on: culling (I&R), death on farm (Rendering plant), herd health certification for IBR, Leptospirosis, BVD-virus and Salmonellosis (GD), milk quality (Milk Control Station), herd improvement data (DCIO). This information was, where appropriate, categorized for herd type, herd size, herd production level (dairy), open/closed management, disease-free certification and region.

Results: Each quarter a full report on cattle surveillance is presented to stakeholders, including recommendations on health/disease topics that require action.

Result Examples: GD-Veekijker: **1.** Detection of bluetongue in August 2006. **2.** Detection of pithomycotoxicosis in cattle in 2005

Prevalence Studies: Prevalence in dairy herds for BVDV, BHV1, Neospora, Salmonella en L. harjo were resp. 28.3; 28.0; 22.7;8.3 and 0.01. In suckler herds resp. 34.8;not done;71.1;5.6 and 12.2.

Key Monitoring Indicators: 1. Mortality: The mean mortality % on dairy farms per quarter was 0.57%, the 90 percentile 2.0%. In Fig. 1 the observed mean is modelled against the predicted mean. **2.** Strong relation between closed farming systems and mortality: 25% lower mortality on dairy farms



Conclusions: The cattle health surveillance system is operating 3 years. Stakeholders are pleased with the system: they are informed about emerging disorders and changes in animal health trends over time. The system would benefit from international cooperation with neighbouring countries with comparable aims and systems.

14.008 Survey on diffusion of Risk Factors and Behaviors for Spreading of Cisticercus bovis in Bovine Herd of Valcamonica, Lombardy

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I. The cysticercosis is a zoonosis, cause of enteric disease in human beings infected by contaminated bovine meat consumption. The presence of cysts is usually checked with veterinary post-mortem inspection at slaugtherhouse. Since in the territory of Valcamonica-Sebino, Brescia province, an increase in cysticercosis lesions at slaughterhouse was observed, a survey was conducted aimed to study the diffusion of risk factors and behaviors in a representative sample of bovine herds.

The survey has been conducted from 2003 to 2005 on a non probabilistic sample of herds, selected according to logistical and operative possibilities of the Local Veterinary Service. A questionnaire on risk factors was administered in 651 herds. Questions focused on structural and management features facilitating passage man-animal, on human excreta and bovine slurry disposal systems, manuring technique and, making use of a visual analogical scale, on general hygienic herd condition and hygienic behavior of the personnel. Individual bovine data and details on detected cysts were gathered with a second form filled at slaughterhouse. Data have then been put in a database for the analysis.

75% of herds reared a maximum of 20 heads. Seasonal grazing was practiced in 80%. Hay and grass were mainly local. In case of presence of toilets inside the herd, 60% of them had no connection to biological holes or sewer. 97% of herds used bovine sewage for manuring. The hygienic status of 50% of herds was medium-low. In 32 % of the herds it happened to eat raw or underdone meat. 83 cases of cysticercosis were detected in animals aged between 6 months and 13 years. Cysts seemed to affect more young animals, up to 3 years. One cyst finding was mainly in animals up to 1 year and in 75% of the whole of cases; 18.64% of cases showed 2 cysts while there were few findings with 3 or more cysts (about 6%). 36% of the cysts were potentially infecting.

Herds under study are typical mountain herds with little number of reared animals and rare entry of animals of foreign origin. Well known risk factors and behaviours, like seasonal grazing, were largely present in the study area, sometimes as well consolidated rules of herd management. The disease was probably autochthon. In a similar scenario, every action aimed to educate zootechnic operators and to improve hygienic care would be desirable.

14.009 Comparative Minimum Inhibitory Concentration (MIC) and Mutant Prevention Concentration (MPC) Values of Enrofloxacin, Florfenicol, Tilmicosin and Tulathromycin Against Mannheimia haemolytica (MH) Isolates Collected from Cattle with Bovine Respiratory Disease

J.M. Blondeau, S.D. Borsos, C. Hesje, L.D. Blondeau, B.J. Blondeau. Royal University Hospital and University of Saskatchewan, Saskatoon, Canada

Background: MH is the most prevalent bacterial pathogen associated with bovine respiratory disease (BRD) and enrofloxacin (ENR), florphenicol (FL), tilmicosin (TIL) and tulathromycin (TUL) are used for therapy. MPC defines the antimicrobial drug concentration threshold blocking growth of mutant sub-populations from high density bacterial inocula. We tested clinical isolates of MH by MIC and MPC against ENR, Fl, TIL and TUL to compare in vitro potency and define the MPC potential of each agent with this pathogen.

Methods: For MIC testing, the recommended procedure of the Clinical and Laboratory Standards Institute was followed with microbroth dilution utilizing 10^5 CFU/ml tested against doubling drug dilutions in appropriate media with optimal incubation temperature and time. For MPC testing, $\geq 10^9$CFUs were applied to agar plates containing drug concentrations, incubated under optimal conditions and screened for growth at 24 and 48 hours. The lowest drug concentration preventing growth was either the MIC or MPC depending on method.

Results: Of 39 MH clinical isolates, MIC_{50} (mg/L), MIC_{90} (mg/L) MIC range (mg/L) values for ENR, FL, TIL, TUL respectively were as follows: 0.063, 0.063, 0.031-0.063; 1, 1, 0.5-2; 1, 4, 0.5-4; 1, 2, 0.5-2. MIC values for TIL and TUL were higher when incubation was in CO_2 vs O_2. The MPC_{50} (mg/L), MPC_{90} (mg/L) and MPC range (mg/L) values were as follows: 0.25, 0.5, 0.008-≥2; 4, 8, 1-8; 8, 16, 4-16; 4, 8, 2-16.

Conclusion: By MIC and MPC measurements the rank order of potency was ENR > Fl > TUL > UL. Based on published serum (ENR, FL) or pulmonary (TIL, TUL) drug concentrations, only ENR (7.5 and 12.5 mg/kg dose) had serum drug concentrations in excess of the mutant selection window for substantial durations of the dosing interval. ENR provided AUC/MPC values >22 for up to 90% of the strains.

14.010 Comparative Killing of Bovine Isolates of Mannheimia haemolytica (MH) by Enrofloxacin (ENR), Florfenicol (FL), Tilmicosin (TIL) and Tulathromycin (TUL) using the Measured Minimum Inhibitory Concentration (MIC) and Mutant Prevention Concentration (MPC) Drug Values.

J.M. Blondeau, S.D. Borsos, C. Hesje, L.D. Blondeau, B.J. Blondeau. Royal University Hospital and University of Saskatchewan, Saskatoon, Canada

Background: MH is the most prevalent bacterial pathogen associated with bovine respiratory disease and ENR, FL, TIL and TUL are used for therapy. As bacterial eradication has been shown to positively impact on clinical outcome and perhaps reduce resistance selection, we compared 4 drugs for killing clinical isolates of MH using measured MIC and MPC drug concentrations.

Methods: For MIC testing, the CLSI recommended procedure was followed using 10^5CFU/ml; for MPC testing $\geq 10^9$CFUs were applied to drug—containing agar plates with doubling drug dilutions in appropriate

media and incubation under optimal atmosphere/temperature. For kill experiments, 10^6-10^9 CFU/ml were exposed to drug and triplicate aliquots sampled at 0, 30 min, 1, 2, 3, 4, 12 and 24 hours, plated, incubated and reductions in viable counts recorded.

Results: MIC/MPC (μg/ml) values for ENR, FL, TIL, TUL against 4 MH strains respectively were: 0.016/0.125-0.5; 0.25-2/2-8; 0.5-4/4-64; 0.2-2/2-4. Exposure of 10^6-10^9 CFU/ml to MIC drug concentrations gave a +0.64 to -2.67 $\log_{10}$ reduction in viable cells by 4 hrs for FL compared to +0.56 to -0.13, +0.73 to -0.57, +0.64 to -0.35 $\log_{10}$ reduction for ENR, TIL and TUL respectively. All drugs yielded a -0.91 to -1.37 $\log_{10}$ reduction of the 10^9 CFU/ml inocula by 24 hrs. Exposure of 10^6-10^9 CFU/ml to MPC drug concentrations gave a -0.68 to -3, -0.01 to -0.2, +0.01 to -0.49 & +0.01 to -0.1 $\log_{10}$ reduction in viable cells by 30 minutes to 1 hr for ENR, FL, TIL, TUL respectively. A -0.6 to >6 $\log_{10}$ reduction was seen to the 4 drugs by 12-24 hrs with ENR > FL > TIL ≥ TUL.

Conclusion: Killing of MH strains was less efficient at MIC drug concentrations but was more complete and efficient at MPC drug concentrations with ENR > FL > TIL ≥ TUL. Dosing to achieve MPC minimizes resistance selection and ensures more efficient and rapid killing and perhaps optimizes outcome.

14.011 Austrian BSE/TSE-Screening in Cattle, Sheep and Goat 2006

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The Austrian farm animal population counts about 2.050.000 cattle, 327.000 sheep, 55.000 goat. The average live stock per farm is 23 cattle, 19 sheep and 5 goat. 960.000 cattle become older than 24 month. Screening was performed according to regulation (EU) 999/2001. Tested were all cattle aged 30 months and older and possibly sick cattle and fallen stock from 24 months at slaughter onwards. All slaughtered or fallen sheep and goat have been tested if aged 18 months or older. Cattle may have been tested at healthy slaughter at owners expense if at least 20 months of age.

Tests used for screening in cattle: Prionics Western or Prionics LIA

Tests used for screening in sheep and goat: Prionics Western SR

Sampled anatomical region for screening in cattle, sheep and goat: Obex

2 percent of the submitted data from the slaughterhouses are cross checked to supervise its accuracy.

Testing has been performed by 5 regional governemental labs.

Approximately 195.000 cattle were tested in austrian laboratories from January to November 2006 including the detection of three BSE-cases. One case in a cow from Upper Austria, another one in a cow from Tyrol and the third in a cow imported for slaughter from Slovenia.

This is very comparable to the situation of 2005 when 201.000 cattle were tested and also 3 cattle were found BSE-positive, one with swiss origin and two that were imported from Slovenia for slaughter. 2005 one additional BSE-case was found by a german laboratory in a cow originating from an austrian valley which is accessable just from Germany.

4400 sheep and 1200 goat were tested in Austria 2005, all with negative result. Numbers of sheep and goats tested in 2006 will be slightly higher than in 2005 (5600 sheep and 1600 goats tested by December 1, 2006). There are no positive results in small ruminants since the year 2000.

14.012 Concentration Dependent Killing of Mannheimia haemolytica (MH) by Enrofloxacin at the Minimum Inhibitory, Mutant Prevention, Maximum Serum and Tissue Drug Concentrations.

J.M. Blondeau, S.D. Borsos, L.D. Blondeau, B.J. Blondeau, C. Hesje. Royal University Hospital and University of Saskatchewan, Saskatoon, Canada

Background: MH is the most prevalent bacterial cause of bovine respiratory disease (BRD) and enrofloxacin (ENR) is a fluoroquinolone compound used for therapy in cattle with BRD. We were interested in determining the killing of MH by ENR and 4 different drug concentrations and against bacterial inocula ranging from $\log_{10}$ colony forming units per milliliter (cfu/ml).

Methods: For MIC testing, the CLSI recommended procedure was followed using 10^5 CFU/ml; for MPC testing ≥10^9 CFUs were applied to drug—containing agar plates with doubling drug dilutions in appropriate media and incubation under optimal atmosphere/temperature. For kill experiments, 10^6-10^9 $\log_{10}$ CFU/ml were exposed to drug and triplicate aliquots sampled at 0, 30 min, 1, 2, 3, 4, 12 and 24 hours, plated, incubated and reductions in viable counts $\log_{10}$ and % kill) recorded.

Results: Against 4 MH strains, MIC values ranged from 0.016-0.25 mg/L and MPC values ranged from 0.125-0.5 mg/L. Exposure of 10^6-10^9 cfu/ml to the MIC drug concentration for 1 hour yielded a positive growth to a <1 $\log_{10}$ reduction in viable cells (17% increase to 12% decrease). Following exposure to the MPC drug concentration for 1 hour, 1 1.1-3.1 $\log_{10}$ reduction in viable cells was seen (84-99% kill). Exposure of 10^6-10^9 cfu/ml to the maximum serum drug concentration (1.9 mg/L) for 1 hour yielded a 1.7-2.4 $\log_{10}$ reduction (96-99% killing) and similar results were seen following exposure of the maximum time drug concentration (2 mg/L)-1.7-2.3 $\log_{10}$ reduction (97.99% killing).

Conclusions: Enrofloxacin is bactericidal against MH and killing was quicker and more efficient as the drug concentration increased, thereby showing concentration dependent killing. The high density bacterial inocula used in these kill studies are more indicative of bacterial burdens during infection. Dosing to exceed the MPC reduces the likelihood for resistance selection during therapy and ensures rapid killing of high density bacterial burdens.

14.013 Risk Factors for Between-farm Transmission of Avian Influenza During the Epidemics Caused by H5N2 Subtype in Japan in 2005

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Background: Outbreaks of avian influenza (AI) in poultry have a threat of leading a worldwide pandemic. Several subtypes of AI virus are known to infect human. It is indispensable to contain and eradicate AI in early stage in order to minimize the chances to spread between farms, and possibly to human. To identify risk factors for between-farm transmission would be helpful to establish effective preventive measures, regardless of subtype of AI virus. Japan experienced H5N2 AI outbreaks in poultry in 2005, affecting 41 layer chicken farms. We conducted spatial analysis and a case control study to identify the risk factors associated with between-farm transmission during the epidemics.

Methods: One prefecture with main outbreaks of AI was selected as study area. For spatial analysis including the spatial scan test, SaTScan and ArcView ver 9.1 (ESRI) were used. For the case control study, data were collected from all chicken farms with laying hen within the movement control areas (37 case farms and 36 control farms) by interview with questionnaires. Multivariate logistic regression was conducted to assess associations with between-farm transmission using the software of JMP 6 (SAS Institute) and SPSS ver. 11.0.

Results: One spatial cluster was detected overlapping the area where chicken density was highest. Multivariate analysis identified insufficient biosecurity measures against visitors and egg containers, farm equipment sharing between farms, and the distance from the nearest case farm as significant predictors of AI infection among layer chicken farms.

Conclusion: Insufficient biosecurity measures against visitors and egg containers, farm equipment sharing between farms, and the distance from the nearest case farm were identified as risk factors associated with between-farm transmission of AI among layer chicken farms. The results will contribute to implement the efficient biosecurity measures in the poultry farms to prevent the introduction of AI virus.

14.014 The Use of PCR Methods in the Surveillance of Bluetongue in Spain, 2003–2006

M. Aguero, C. Gómez-Tejedor, **M.A. Jimenez-Clavero**. Laboratorio Central de Veterinaria, Algete, Spain

Background: Bluetongue (BT) is an arboviral disease affecting wild and domestic ruminants. It is considered an emerging disease in Europe, where in recent years different epizootics have occurred, affecting areas where this disease has either been absent for decades or never reported before. In Spain, bluetongue re-emerged after 40 years absence, first in two different incursions in 2000 and 2003 (serotypes 2 and 4, respectively), affecting the Balearic Islands, where it was subsequently eradicated, and afterwards in the mainland in 2004 (serotype 4), spreading readily from the southernmost region, to broad areas in central and south-eastern Spain, where it still remains causing new foci. Here we present our last three years experience on the surveillance of BT in Spain, with special mention to the development and application of new molecular diagnostic tools in the control of the disease.

Methods: We developed a real-time RT-PCR for detection of strains circulating in the western Mediterranean region, which was completely automated and scaled-up to yield a high-throughput method, able to analyse up to 1500 blood samples per day. This system allows monitoring of the disease spread by measuring viremia, rather than antibodies, in susceptible animals. Furthermore, we have developed a RT-PCR method targeted at the VP2 gene of BT serotype 4 for rapid serotyping and molecular epidemiology analysis. As attenuated vaccines (serotypes 2 and 4) have been applied during these epizootics, we have also developed real-time RT-PCR methods that enable to distinguish between wild-type and vaccine-derived infections from these serotypes.

Results: We have extensively used the real-time RT-PCR high throughput method for the screening of blood samples at three levels: to follow-up the spread of the disease in sentinel animals (geographic level), to diagnose the disease in each new foci (herd level), and to control animal movements (individual level). A total of 18,225, 139,787 and 25,897 blood samples have been analyzed in 2004, 2005 and 2006, respectively. In field situations, the new real-time RT-PCR protocols could differentiate between wild-type and vaccine strains with high sensitivity.

Conclusion: Molecular biology tools applied in high throughput format have proved extremely useful in the surveillance of BT epizootics in Spain. This work illustrates how this technology can assist in the surveillance of emerging infectious diseases.

SESSION 15 (Poster Session)

Outbreak Control

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

15.001 Backcalculating Infectiousness of Smallpox Relative to Disease Age

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Background: The frequency of secondary transmissions by disease-age (i.e., the time since onset of the disease) has rarely been evaluated.

Methods: This study investigated the infectiousness of smallpox relative to disease-age using a likelihood-based estimation procedure based on the observed transmission network (n = 223) and on the distribution of the incubation period (n = 379). Who infected whom information enabled us to backcalculate the infectiousness by disease-age, employing a non-parametric model for infectiousness.

Results: Between 3 and 6 days after onset of fever, which roughly corresponds to the time immediately after the onset of rash, the daily frequency of secondary transmissions was highest, yielding 20.6 % per day (95 % CI: 15.1, 26.4) of the total number of secondary transmissions. Expected cumulative frequencies of secondary transmissions before onset of fever and up to 3 days after onset of fever were 2.6 % and 23.7 %, respectively. The obtained estimates suggest that 91.1 % of all secondary transmissions occurred up to 9 days after onset of fever.

Conclusions: The obtained estimates imply that isolation could be extremely effective if performed before onset of rash and that delayed isolation of symptomatic cases could still be effective if performed within a few days after onset of rash. Since isolation of cases is critically important to control emerging infectious diseases, our new method is useful to evaluate the time until when isolation could be delayed.

15.002 Clonality of Providencia stuartii Isolates Involved in Outbreak Occurred in a Burns Unit

N. Ben Saida[1], L. Thabet[2], K. Bouselmi[3], A. Messadi[3], A. Torki[2], **J. Boukadida**[1]. [1]UR 16/02 Microbiology CHU FHached, Sousse, Tunisia; [2]Lab. Biology. Hospital A. Othmana, Tunis, Tunisia; [3]Burns Intensive Care. Hospital A. Othmana, Tunis, Tunisia

Background: We investigate an outbreak of multidrug-resistant P. stuartii that occurred in a burns unit of a hospital in Tunisia.

Methods: All isolates of P. stuartii collected during four months of 2005 from patients and from the tracheal aspirator were analysed by antibiogram and by pulsed-field gel electrophoresis (PFGE).

Results: 17 clinical isolates of P. stuartii extended-spectrum beta-lactamase producing were collected from hemoculture (n =12), vascular catheter (n= 2), skin (n= 2) and urinary probe (n= 1) of 17 patients. One other isolate P. stuartii was collected from the aspirator. The first line antibiotherapy was based on the association of two or three of "vancomycin- amikacin - imipenem". P. stuartii was isolated with other Gram-negative bacteria, namely Acinetobacter baumanii, Escherichia coli. All P. stuartii isolates were nosocomially acquired and were resistant to 10-12 antibiotics. Three different antibiotyps were identified without correlation with genotype. Two PFGE patterns were obtained with predominance of one (type A) that is observed for 15 isolates. Aspirator isolate yielded PFGE type A observed in patients isolates. The second PFGE type B was observed only for two isolates. Three patients among 17 were died. The outbreak was stopped after the stoppage of the use of aspirator. Three months after, the aspirator was reintroduced and the outbreak was beginning again after two weeks.

Conclusion: This study suggests the bi-clonality of the outbreak and transmission of epidemic P. stuartii isolates between patients. The tube of aspirator seems to be the reservoir of P. stuartii. Furthermore this study show the utility of PFGE in typing for the purpose of understanding the epidemiologic behaviour of P. stuartii and as a basis for the development of rational control strategies. The predominance of clone A suggests its stronger virulence.

15.003 Genotypic Analysis of Salmonella enterica Serovar typhi Collected During Two Successive Autumnal tTphoid Outbreaks in South Tunisia.

N. Ben Saida[1], N. Bouzouïa[2], **J. Boukadida**[1]. [1]UR 16/02 Microbiology CHU FHached, Sousse, Tunisia; [2]Infectious disease department, CHU Fattouma Bourguiba, Monastir, Tunisia

Background: Salmonella enterica serovar typhi is the etiological agent of typhoid fever, of which there are an estimated 16 million cases with 600,000 related deaths worldwide annually. The majority of infections results from consuming foods or drinks contaminated by faeces of patients or carriers.

Objective: Investigation of two successive outbreaks of typhoid fever that occurred in southeast Tunisia in autumn of 2004 and 2005.

Methods: A total of 86 isolates of S. enterica serovar typhi (76 from blood culture or stool of patients involved in both outbreaks and 10 from stool of healthy carriers), were analysed by antibiogram and pulsed-field gel electrophoresis (PFGE).

Results: All isolates of S. typhi were fully sensitive to all antibiotics tested, particularly to cotrimoxazole and ciprofloxacin. All isolates of 2004 (39 from patients and 10 from healthily carriers) appeared to be genetically identical when digested with Spe I, Avr II and Xba I and belonged to the same clone. Xba I digestion of 2005 outbreak isolates gave five different patterns with predominance of the 2004 outbreak clone. Both outbreaks were concomitant with the season of "legmi", fermented juice traditionally extracted from palm-tree.

Conclusion: PFGE with Xba was discriminatory and can be useful for routine investigation of typhoid fever. Our study suggests the monoclonality of 2004 outbreak and the multiclonality of the 2005 outbreak. The epidemic clone of S. typhi is able to persist for long period in a quiet state in the population and to give again, when the conditions become favourable, a new outbreak.

15.004 N-95 Respirator Fit-Testing of 5,200 Persons in One Hospital Over 6 months: Lessons Learned

E.S. Dade, M.S. Smith, D.R. Lucey, C.F. Feied, C.K. Wuerker, K. Myerson. Washington Hospital Center, Washington, DC, USA

Background: Our 907-bed tertiary hospital, the largest in the District of Columbia (DC), decided to offer N-95 respirator fit-testing to all full-time and part-time workers in order to enhance preparedness for SARS, tuberculosis, avian influenza, smallpox, measles, viral hemorrhagic fevers and any other natural or bioterrorist airborne-transmitted disease.

Methods: From November 1, 2003 until May 1, 2004 we partnered with a private occupational health company to offer N-95 fit-testing following the standard two-step process. First, via an optical-mark recognition questionnaire we determined if each participant could be medically cleared. Second, if this clearance was obtained then qualitative N-95 fit-testing was performed. One fit-test technician, testing four persons at a time, could test a maximum 84 persons/8-hour day. Fit-testing was offered on both day and night shifts, in large central areas and in individual smaller parts of the medical campus.

Results: 5,377 persons were medically cleared for fit-testing. 5,097 (95%) were able to be successfully fit-tested with the initial N-95 respirator. An additional 200 failed using the initial respirator but passed the fit-testing using one of two alternative N-95 respirators. Only the remaining 80 (1.5%) persons could not be fit-tested.

Conclusions: Lessons learned included: (1) by the third month of the program a directive from the hospital President was needed to increase participation in the fit-testing program; (2) Detailed information system support was essential to track what became a multi-step process from the distribution of questionnaires to the final documentation of compliance reports to department managers. (3) confidentiality concerns regarding the medical clearance questionnaires were resolved in some cases only by face-to-face interviews with an occupational health nurse; (4) time requirements ranged from 10-30 minutes for the questionnaire, and 18–35 minutes for fit-testing, including the 'fit-check' demonstration on how to self-monitor the respirator seal; (5) easy access to handwashing and drinking water were important to remove the testing solution and decrease the test-failure rate.

15.005 A Three-Year Surveillance of Nosocomial Infections by Methicillin-Resistant *Staphylococcus haemolyticus* in Newborns Revealed that Disinfectant was the Reservoir

N. Ben Saida, S. Mhalla, **J. Boukadida**. UR 16/02 Microbiology CHU FHached, Sousse, Tunisia

Background: Investigation of the acquisition of methicillin-resistant Staphylococcus haemolyticus (MRSH) infections in a neonatal intensive care unit.

Methods: From March 2004 to November 2006, infections caused by MRSH in newborns were prospectively monitored. Nurses' hands were cultured before and after hand-washing. Culture of commercial disinfectant based on chlorhexidine digluconate was done from used bottles. Pulsed-field gel electrophoresis was performed to determine genetic relatedness of strains from infants, nurses and disinfectant.

Results: During the study period, the incidence of MRSH infections has increased dramatically compared with previously years. 42 clinical isolates of MRSH (26% of the coagulase negative staphylococci in the unit) were collected from [blood culture (n = 15), vascular catheter (n =14), intra-tracheal tube (n = 11), cerebrospinal fluid (n = 2)] of newborns. Three other isolates of MRSH were cultured from the clean hands of three nurses and one isolate from disinfectant used in the unit. Other staphylococci mainly S. epidermidis and S. aureus were cultured from other disinfectant bottles. Newborns isolates were resistant to 10-14 antibiotics. Vancomycin was the main antibiotic used for infections treatment. Five clones (type A, B, C, D and E) were identified among newborns isolates with predominance of two clones (A and B). Isolate cultured from disinfectant showed PFGE profile identical to PFGE type B observed in newborns isolates. Two nurses' isolates showed PFGE type similar to types A and B. Eight newborns died.

Conclusion: This study revealed the ability of MRSH isolates to survive in the disinfectant and to conserve their ability to infect newborns probably through the hand of staff. The disinfectant system functioned as a reservoir of MRSH and other staphylococci. These results explain the increase of the incidence of MRSH and lead to regular bacteriological control of disinfectants solutions used in the unit.

15.006 Factors Influencing Influenza Vaccine Uptake Among Healthcare Workers in Greece

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Background: Influenza vaccine uptake has been generally low among health-care workers (HCWs) worldwide. Our aim was to study the impact of a campaign to increase HCWs influenza vaccination in Greek hospitals. methods: In September 2005, the Hellenic Center for Disease Control and Prevention (Athens, Greece) conducted a limited budget nation-wide campaign using written materials to promote HCWs influenza vaccination within Greek hospitals.

Results: During the 2005–2006 season, the overall HCWs vaccination rate was 16.36% (range: 0%–85.96% among hospitals). The self-reported vaccination rate during the previous season was 1.72%, which corresponds to a 9.5-fold increase. Compared with physicians, significantly less technical personnel was vaccinated, whereas administrative personnel was more likely to receive the vaccine. Among physicians, those working in Internal Medicine Departments had 2.71-fold increased vaccination rates compared with those working in Surgical Departments and 2.36-fold increased rates compared with those in Laboratory Departments. Multivariate analysis revealed the following factors associated with decreased vaccination rates: working in a hospital with > 200 beds compared with a hospital with up to 200 beds, in a hospital with an Intensive Care Unit, in a psychiatric or a dermatologic hospital compared with a general hospital. Factors associated with increased rates included working in a hospital in Northern Greece, in a paediatric or an oncologic hospital compared with a general hospital, or in a hospital located in a prefecture with avian influenza H5N1 activity.

Conclusion: In Greece, HCWs influenza vaccination rates remain low however the implementation of a nation-wide campaign had a considerable impact. Efforts should focus on hospital- and HCWs-associated factors to increase vaccination uptake.

15.007 Meta-Analysis of *Marburg* and *Ebola* Hemorrhagic Fever Outbreaks

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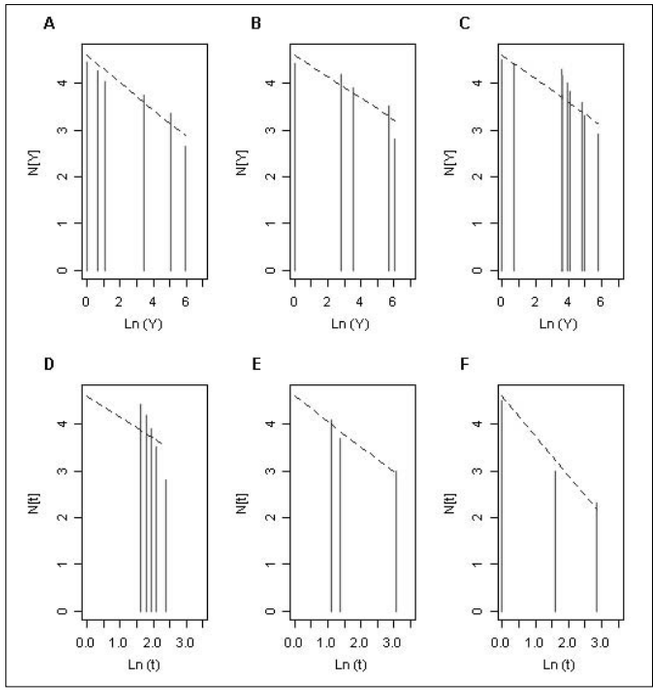
Marburg and Ebola (family: filoviridae) hemorrhagic fever re-emergence among human populations is difficult, and often impossible, to predict. Beyond supportive measures, there currently remains no treatment for patients infected with a filoviridae. Consequently, a simple probabilistic description of inter-outbreak period, outbreak fatality, and size distributions greatly enhances the risk analysis capacity. To address this, the authors conducted a meta-analysis on published filoviridae records to characterize their distributional properties. The results demonstrate that the case-fatality of Ebola sudan (EHF-S), 53 %, is lower than those of Ebola zaire (EHF-Z) and Marburg marburg (MHF) viruses, i.e., 82 % and 83 %, respectively. EHF-S, EHF-Z, and MHF outbreak size cumulative distributions appear to follow power-laws (fractals) whose scaling factors are: -0.23 ± 0.03, -0.25 ± 0.03, and -0.29 ± 0.02, respectively; likewise,



the power-law scaling factors for EHF-S, EHF-Z, and MHF inter-outbreak period cumulative distributions are: -0.54 ± 0.04, -0.86 ± 0.06, and -0.46 ± 0.11, respectively. In all cases, p-value < 0.01. Not only do case-fatality estimates assist viral pathogenicity studies but they also facilitate fatality projections for future outbreaks. Furthermore, the aforementioned scaling relations imply that future outbreaks where at least 500 individuals are infected have probabilities of 24 % (EHF-S), 21 % (EHF-Z), and 16 % (MHF); similarly, the probabilities that inter-outbreak periods span at least 10 years are 29 % (EHF-S), 14 % (EHF-Z), and 35 % (MHF). Unlike the case-fatality distribution, outbreak size and inter-outbreak period distributions are not well characterized by traditional centrality measures (e.g., mean) because the frequency of larger outbreaks (or longer inter-outbreak periods) relates to smaller, though non-zero, probabilities. Therefore, the authors conclude that the results herein presented may assist outbreak risk estimation as well as hazard mitigation.

15.008 Pseudo-Outbreak of Pulmonary Tuberculosis

N. Srivastava, A. Gram, **D. Alcid**. Saint Peters University Hospital, New Brunswick, NJ, USA

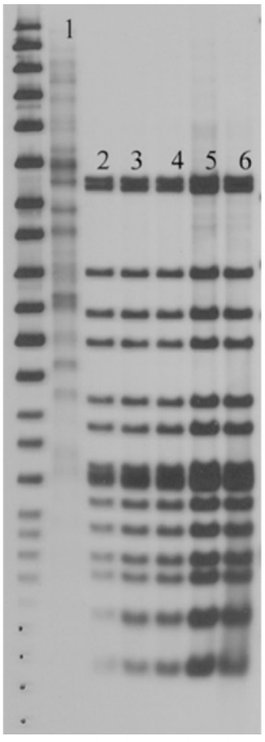
Introduction: It is well known that laboratory contamination can result in providing information that is difficult to interpret, can lead to misdiagnosis and incorrect treatment.

Case: We report 6 patients with positive sputum specimens obtained by bronchoscopy, for Mycobacterium tuberculosis. All cases were smear negative but were culture positive 4–6 weeks later. Of the 6 cases only 1 case was a true positive and the remaining 5 were false positives.

The Patient characteristics are shown in the table below (Table1).

Cases	Symptoms & Signs suggestive of TB	Indication for Bronchoscopy
1	Yes	TB symptoms
2	No	Atypical Pneumonia
3	No	Bronchiectasis
4	No	Consolidation on Chest X-ray
5	No	Pleural effusion on Chest X-ray
6	No	PPD positive

Our normal incidence of TB is 10–15 cases per year. However when 6 cases came up within a period of 1 month, the infection control department started an outbreak investigation. A chart review revealed that all patients had undergone a bronchoscopy. All but 2 patients were bronchoscoped with different instruments on 2 separate days and this was therefore unlikely to be the source of contamination. Investigation in the laboratory revealed, two different technicians had processed all the samples. All 6 samples were analyzed by PFGE and were shown to be clonal (Fig. 1).



Discussion: The present study shows that the possible contamination occurred in the laboratory. Unnecessary testing in patients with low probability of the disease should be avoided as false positive results can lead to patients getting treated for a disease they do not have. Careful specimen handling methods in the laboratory can also avoid such mistakes.

15.009 Field Evaluation of the efficacy of proprietary Repellent Formulations with IR3535® and Picaridin in the Bolivian Amazon Region

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Five proprietary repellent formulations (3 hydro-alcoholic spray solutions and 2 skin lotions) with active ingredient IR3535® (Ethyl Butylacetylaminopropionate, EBAAP) or Picaridin (Hydroxyethyl Isobutyl Piperidine Carboxylate, KBR 3023, Bayrepel®) were tested in a field study on 11 test persons for their efficacy at preventing bites. The tests were conducted in Vaca Diez Region, Bolivia, on field populations of medically important mosquitoes. Main species found were Anopheles darlingi, An. albitarsus, Aedes aegypti, Culex pedroi. The concentration of the active substances ranged from 15% to 20%. All tested samples provided lasting protection (time to first bite) over several hours: (ranging from 227.6 min to 414.4 min with a mean of approximately 350 min).

There was no significant difference in protection times between the two active substances.

Remarkably, the procetion was dependent more on the application form than on the concentration of the active: a 15% lotion showed longer protection than a 20% hydro-alcoholic spray.

15.010 Public Health Intervention Measures to Control the Spread of SARS—Modelling Outbreak Scenarios

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[2]University of Tübingen, Tübingen, Germany

Background: Respiratory pandemic infectious diseases like SARS and influenza threaten the health of people at large. Such diseases can easily be transmitted from person to person and spread globally by world wide travel. A variety of intervention measures exist to prevent and control pandemic disease spread. Measures like quarantine and isolation can efficiently interrupt disease transmission. Population based measures like closing of schools and businesses can prevent casual disease transmission by reducing contact rates in a susceptible population. However, these measures differ in their approaches and their effectiveness in reducing secondary cases. Furthermore, individual disease characteristics, like incubation period and mode of transmission, influence the efficacy of measures to limit transmission rates.

Method: Mathematical modelling was applied to estimate the effect of interventions by calculating different SARS outbreak scenarios. The current study uses a decision model to estimate the impact of intervention measures on the effective reproductive number (R_e) and the final outbreak size.

Results: The analysis showed that early case detection followed by strict isolation could exclusively control a SARS outbreak. Tracing of contacts of health care workers and of close contacts of cases had an additional effect to reduce R_e. Tracing of casual contacts and measures to decrease social interaction was predicted to be less effective in reducing the number of secondary SARS cases.

Conclusion: The current study highlights that early identification of SARS cases, by measures like contact tracing, quarantine and monitoring of vulnerable groups, is vital to limit the number of secondary cases. However, doing so transfers cases to health care facilities which makes hospital hygiene measures, like isolation and infection control important to avoid nosocomial spread. Measures to decrease social interaction, like closing schools and cancelling public gatherings have a minor impact to reduce R_e of SARS. In addition, such measures are costly and curtail social liberty. The current study points to measures suitable to prevent and control a SARS outbreak and shows how they are interconnected.

15.011 Standardized Online Classification Guiding Health Intervention for Contacts of a Case of Influenza A(HxNy) in Switzerland

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Background: Tracing, treatment or antiviral prophylaxis and quarantine of contacts of a case of Influenza A(HxNy) are important measures to prevent and control the spread at an early stage of a pandemic outbreak. Assignment of risk exposure categories is critical to guide appropriate health interventions.

Methods: In a simple model three exposure categories reflect each an overall risk of infection, which is determined by 4 key probabilities:

1. The probability of infection of the contact person depending whether the source is a patient with suspected or confirmed influenza A(HxNy)
2. The probability of infection of the contact person depending on contact intensity (spatial proximity, mode, duration and frequency of contact) with the source
3. The probability of infection depending on whether the contact person presents febrile respiratory illness or not
4. The probability of infection depending on the A(HxNy) immune status of the contact person

Each of these probabilities is estimated (0.0 -1.0). The overall risk of infection is calculated as a combined probability in a tree or matrix and is stratified into exposure categories, which in turn guide to appropriate health interventions.

Results: In Switzerland we examine the possibility to develop a tool to assign contacts to specific exposure categories. This tool should be able to integrate new findings on virulence, pathogenicity and transmission patterns of Influenza A(HxNy) during the pandemic alert periods.

Conclusion: Standardized online contact classification guiding health intervention may facilitate the implementation of an overall strategy in the management of possible contacts.

15.012 Investigation of an Outbreak of Acute Diarrheal Disease in Children Under Six Years Old in Mid-Western City Brazil, 2006

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Background: Acute diarrheal diseases (ADD) can be caused by various agents, and are an important fatality cause in children in Brazil. After an increase in ADD reporting, an investigation was conducted during August-September 2006.

Objectives: To describe cases by person, time and place; identify etiology and risk factors; implement prevention and control measures.

Methods: Last conductor a retrospective cohort study, were used standardized questionnaires to obtain information on personal characteristics and exposures. Relative risk (RR) was used as association measure. Ill were defined as resident in Mid-Western city Brazil with <6 years old with history of >1 watery stools/day between August 1st and September 18th. National Surveillance data were used to draw an ADD epi-curve from 2003–2006. Stool samples were collected for virus, parasite and bacterial tests.

Results: There was a 300% increase in ADD cases in epidemiological weeks 32(n=559)-37(n=1908), when compared to previous years. A total of 365 children were interviewed, of which 27% had ADD. Factors associated to increased risk of ADD were: contact with sick person (RR=4.8;95%CI=3.9-6.2;p<0.001), interruption in public water supply (RR=3.9;95%CI=3.1-5.0;p<0.001) and family income < 121.00 EUR (RR=4.6;95%CI=1.5-13.8;p<0,001). Persons with the following clinical features had a higher risk of being hospitalized: >5 evacuations/day (RR=3.3;95%CI=1.4-7.6;p<0.01) and >5 vomit episodes/day (RR=2.5;95%CI=1.2-5.3;p<0.03). Pathogens isolated from stool samples (n=93) were Rotavirus (20), Calicivirus (2) and Salmonella sp (1).

Conclusions: A ADD outbreak occurred in children <6 years old in Mid-Western city Brazil. Rotavirus was a more prevalence pathogenic isolated, and risk factors were mostly related to poor socio-economic conditions. Actions taken included sodium-hypochlorite distribution, health education activities and the recommendation of improvements of the water/sewage system in the area.

15.013 Building Response Teams for Infectious Diseases Emergencies in London

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Background: With the threat of deliberate release of pathogens in 2001, the Chief Medical Officer for England published a plan for responding to a smallpox incident, which included providing multidisciplinary teams to manage a suspected smallpox case. This presentation describes how the teams were created, and the lessons identified in the process.

Methods: Initial contact with potential volunteers was made by providing a training programme on the epidemiology and management of

smallpox. Volunteers were then assembled for group working to identify the tasks which teams must perform, and the required knowledge and skills. Desktop exercises, in which teams planned their response to scenarios, allowed refinement of plans and protocols, and identified gaps in knowledge and planned policies. Finally, teams progressed to field scenario exercises, in which they responded to scenarios with the support of expert facilitators. The progress and effectiveness of their response was monitored by observers. An evaluation process followed, in which teams and observers identified lessons and needs for future training and protocol development.

Results: Protocols designed at the groups working and desktop stages proved to be realistic and reasonably complete, especially in respect of patient management and local incident investigation. However, the field exercises revealed a major need for training in leadership, teamworking and communications activities. It was also clear that an ambulance team was needed to assist with maintaining clean and dirty zones and for aspects of patient care and clinical waste management.

Conclusions: Specialist teams cannot be assembled only when needed; a significant amount of teaching, training and exercising is necessary to ensure an effective multidisciplinary response.

15.014 Should Schools Be Closed During an Influenza Pandemic?

M. Eichner[1], C. Winter[2], S.O. Brockmann[2], G. Pfaff[2], H.P. Duerr[1], M. Schwehm[1]. [1]University of Tübingen, Tübingen, Germany; [2]Baden-Württemberg State Health Office, District Government, Stuttgart, Germany

Objectives: Influenza pandemic preparedness plans are currently being developed on national and international levels. Previous research has focused on the administration of antiviral drugs. Recently, social distancing measures like closing of schools have attracted attention. Using a simulation model, we investigate how combinations of pharmaceutical and social distancing measures can mitigate an influenza pandemic and determine the course of a pandemic wave.

Methods: We use the freely available planning tool InfluSim, a deterministic compartment model, which is based on a system of over 1,000 differential equations and designed to operate with an optimal combination of the competing requirements of precision, realism and generality. It allows for producing time courses and cumulative numbers of influenza cases, outpatient visits, applied antiviral treatment doses, hospitalizations, deaths and work days lost due to sickness, all of which may be associated with economic aspects.

Results: The model shows that a timely application of antiviral drugs combined with a quick implementation of contact reduction measures is required to substantially protract the peak of an influenza epidemic and reduce its height. Delays in the initiation of interventions result in much more pessimistic outcomes and can even lead to the paradoxical effect that the stockpile is depleted earlier compared to early distribution of antiviral drugs. Closing day care centres and schools generally reduces the contacts among children, but increases contact frequencies within families. Thus, it may even lead to an increase in the death toll if adults are more vulnerable than children.

Conclusion: When controlling pandemic influenza, pharmaceutical and non-pharmaceutical measures must be combined and should be implemented as soon as possible. Contact reduction measures like case isolation and social distancing must be part of mitigation strategies and have the advantage to be not limited per se. Closing of day care centres and schools may have a very limited effect on the course of a pandemic wave and should not be relied upon.

15.015 Outbreak of Acute Toxoplasmosis, Anapolis, Goias State-Brazil, 2006

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Background: Acute toxoplasmosis(AT) is a parasitic infection caused by Toxoplasma gondii. Although up to 90% of infections are asymptomatic, the severe form of the disease may cause blindness, chorioretinitis or encephalytis in neonates or immunodeficient persons. After several participants of a barbecue in Anápolis, Goías State, had suspected AT, an investigation was conducted to identify infection source, risk factors and recommend prevention and control measures.

Methods: In a retrospective cohort study, we interviewed participants about food-specific exposures. Cases were defined as persons serum reactive by enzyme immune assay(EIA) for anti-toxoplasmosis-specific IgM antibody and either IgG-seronegative or with low avidity IgG. Persons seronegative for both IgM and IgG were considered non-infected. Persons with probable immunity due to prior toxoplasmosis (i.e. IgM-positive with high avidity IgG, or IgM-negative with IgG) were excluded from the analysis.

Results: Of 706 participants, 612(87%) were interviewed and had blood samples collected; of these, 297(48%) were excluded due to evidence of prior immunity. Among 315(52%) remaining participants, 61(10%) had AT; IgG Seroprevalence was 58.3%. Attack rate was 19.4%, of which 59(97%) were symptomatic. Symptoms included malaise(88%), fever(85%), headache(81%), lymphadenopathy (78%), myalgia(68%), and arthralgia(59%). Median incubation period was eight days(range:2-27), and median symptom duration was 46 days(range:26-58). Persons consuming a traditional dish of raw beef (Kibbeh) prepared at one location had an attack rate of 19%, a 10-fold increase compared to unexposed persons (95%CI=4.2-24.9; p=0.01). Of six pregnant women, two had AT. During follow-up one infant was born with three brain calcifications.

Conclusions: Results demonstrated both a high pre-existing prevalence of toxoplasmosis in the study population, and a high attack rate of symptomatic AT among susceptibles. We initiated active surveillance, and informed the public about the risk of consuming raw or undercooked meat. Participants with ocular symptoms were evaluated by an ophthalmologist, but no chorioretinitis was identified.

SESSION 16 (Poster Session)
Emerging Vectorborne Diseases in Humans and Animals

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

16.001 Preliminary Observations on the Potential Role of Some Mammalian Species as Reservoirs of Tuscany Virus

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In some provinces of central Italy Tuscany virus in summer months is the primary cause of aseptic meningitis or meningoencephalitis. The life cycle of Tuscany virus (Bunyaviridae) to date is still based on the knowledge that transmission involves the biting of humans by midges belonging to the species of Phlebotomus. From studies conducted, neither humans during infection, nor midges in particular during the overwintering period are capable of a viral replication which ensures the endemic-

ity of the infection. Therefore the role of mammalian species involvement has been hypothesised. We have undertaken research studies to verify this assumption in the endemic area in a Tuscany province (Siena). We prioritised selection species taking into account feeding habits of vectors and sinanthropism of study species. On these basis we proceeded in studying 269 dogs and 828 sheep. The sample size in sheep was defined with an expected herd prevalence of 20% and within herd prevalence of 5%, while for dogs the within herd prevalence of sheep was applied (10% SE, 95% CI). As described for humans seroepidemiological studies, for analysis blood samples were tested by indirect immunofluorescence, as screening method, employing VERO virus infected cells. The percentage of sera giving a positive result were 8.5 (71) for sheeps and 5.2 (14) for dogs. Relating these results to finding reported in human seroepidemiological studies, in which positive sera were above 50%, these species are not to be considered as reservoirs for Tuscany virus. We are currently investigating on other mammalian and avian species.

16.002 A Change for the Worst: Dengue Virus Infection in Thailand

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Background: In Thailand, Dengue virus infection is a major cause of illness and fatality in children. In each epidemic, at least 50,000 children suffered from this disease, and at least $US 30 was spent on saving children life. However, global warming and globalization have affected the structure of the environment and human life. That affects the epidemiologic pattern of dengue disease which has a strong association with global warming. Using a surveillance data model that evaluates dengue virus infection may illustrate the variation, which will be the important data for improving the dengue virus infection surveillance system.

Methods: This operational research collected data from an annual surveillance report by the Ministry of Public Health (MoPH), Thailand from 1994 to 2005. Incident cases in 76 provinces, defined following the World Health Organization guidelines, will be reported to MoPH. Reporting case is classified into 3 groups: Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), and Dengue Shock Syndrome (DSS). Descriptive analyses were used to evaluate surveillance data.

Results: The result showed the change of dengue disease pattern of three types of illness and the population at risk. DHF was the major group, higher than 80% of the total number of case before 1998. After that the proportion lowered (50%), while DSS was still (3%) at each period of time.

After considering age patterns, the majority of dengue virus infections were children aged lower than 15 years (higher than 80%), and 80% was children age 5-9 years; however, since 1998, age the pattern changed, with the proportion of cases aged higher than 14 years dramatically increasing to 40% in 2004. Meanwhile the proportion in age group 0-4 and 5-9 has lowered to 10% and 20%, respectively.

Conclusion: This study finds the changing age patterns of dengue illness rising in number in ages higher than 14 years. Under this circumstance, Thailand seems to be faced with two groups of populations at risk, thus if the dengue program usually focuses on children, it will be insufficient. Not only a prevention program, but also surveillance systems would be adjusted to support the new population at risk of this vector borne disease.

16.003 Natural History of DENV-1 and -4 Infections: Statistical Analysis of Classic Experimental Dengue

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Background: The natural history of dengue fever involves the time from infection to onset and recovery from disease.

Methods: This study re-examined two historical experiments in the Philippines on DENV-4 (1924-25) and DENV-1 (1929-30) infections. The intrinsic incubation periods were fitted to lognormal distribution using the maximum likelihood method, and the infectious and extrinsic incubation periods were assessed by proportions of successful transmissions causing clinical dengue. Correlations between the intrinsic incubation period and other variables and univariate associations between clinical severity and serotype were also examined.

Results: Significant negative correlations were observed between the incubation period and duration of fever ($r = -0.43$, $p < 0.01$ and $r = -0.33$, $p < 0.01$, respectively). The duration of fever, which roughly corresponds to the severity, was significantly longer for DENV-1 than DENV-4 infections ($p < 0.01$). Moreover, other signs and symptoms also supported that DENV-1 infection results in a more severe clinical appearance than infection with DENV-4.

Conclusions: The historical data, which is unique in that the experimental details for primary DENV infection were provided and that the etiology was known, appeared to be extremely useful to detail the natural history of dengue. Assuming that the duration of fever reasonably reflects the severity of disease, the most likely explanation for this negative correlation is a dose-response phenomenon. Our serotype-specific findings are consistent with recent studies that suggest primary DENV-4 infection results in relatively mild illness.

16.004 Faunistic Study of Sand Flies (Diptera: Psychodidae), Vectors of Leishmaniasis in Greece

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Background: Leishmaniases are a group of infectious diseases caused by the greatest number of parasite species of all known parasitic diseases, and hence represent a special epidemiological problem worldwide. Particularly important are visceral and canine leishmaniasis which have an increasing incidence in the last years, particularly in the Mediterranean region.

During research studies related to the epidemiology of leishmaniasis and bluetongue disease in Greece, we conducted a study of sand flies as exclusive vectors of Leishmania parasites.

Methods: During the 5-year period 1999–2004, sand flies were collected yearly during the period of peak seasonal activity (15 June-31 August), at 18 sites from widely diverse areas of Greece. Outdoors, sand flies were collected by CDC miniature light traps, South African "Onderstepoort-type" light traps and sticky papers. Indoors, inside of bedrooms and living rooms, the collection of sand flies was carried out using mouth and electric aspirators.

Results: A total of 8688 sand flies (4058 males and 4630 females) were collected from all of the localities investigated. The average male/female ratio was 1:1.14. Of the collected females, 2037 (44%) were unfed, 1343 (29%) fed but not gravid, and 1250 (27%) were gravid specimens. All collected sand flies were identified to belong to ten species. The results showed that those species which are epidemiologically the most important as vectors of leishmaniasis and sand fly fever in Greece, including P. neglectus, P. perfiliewi, P. tobbi, P. similis and P. papatasi, were present in widely divergent areas of the country, accounting for 96% of all sand flies collected during this survey. Interestingly, no naturally infected sand flies with Leishmania promastigotes were foiund.

Conclusion: Compared to previous studies, noticeable fluctuations in the abundance and the distribution range of some sand fly species were found. Hence, it is necessary to continue further research of these insects, particularly in view of the very special location of Greece, between Asia and Africa to the east and Europe to the west. The presented results are significant for the control of leishmaniasis and sand fly fever in eastern Mediterranean.

16.005 Clinical Features of Dengue Infections, and Predictors of Dengue Hemorrhagic Fever in Singapore

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Background: In 2004, Singapore experienced its worst dengue outbreak in 30 years with 9,459 notified cases, of which 80% were hospitalized. The study objective was to explore the demographic, clinical and laboratory features of dengue fever (DF) and dengue haemorrhagic fever (DHF) upon presentation to hospital, and to determine predictors of DHF.

Methods: A retrospective study was conducted on laboratory-diagnosed dengue cases admitted in 2004 to Tan Tock Seng Hospital, the main dengue treatment hospital in Singapore. Demographic, clinical, and laboratory data were collected from the hospital's emergency department and standardised dengue clinical pathway during admission. Using data throughout admission, cases were classified as DF or DHF according to the World Health Organisation classification. Data on presentation to hospital was then used to determine the predictors of subsequent DHF development.

Results: The study included 2,144 laboratory-diagnosed cases. Of these, 140 (6.5%) were classified as DHF. On presentation to hospital, the mean age among DF and DHF patients were not significantly different (35.1 compared to 32.3 years respectively). DHF patients had more frequent dehydration on admission (20.7%), compared to DF patients (2.9%, $p<0.01$). DHF patients also had more co-morbid medical conditions (27.9%) compared to DF patients (10.4%, $p<0.01$). For the laboratory results, DHF patients had lower mean white cell count ($2.99x10^3/\mu L$) compared to DF patients ($3.65x10^3/\mu L$, $p<0.01$), and lower total protein counts (2.95g/dL) versus DF patients (6.32g/dL, $p<0.01$). From the multivariate analysis, males [odds ratio (OR)=1.61], dehydration (OR=6.06), white cell count (OR=0.86), total protein levels (OR=0.96), and atypical lymphocyte counts (OR=0.95) were independently and significantly associated with DHF.

Conclusions: Key routine variables collected on admission may be used to predict DHF. These variables can be used to determine the likelihood of DHF occurring during admission; and as a marker to determine the need for admission or close monitoring.

16.006 Chikungunya Virus Diagnosis In the UK

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Background: The Chikungunya outbreak in the Indian Ocean Islands in 2005-2006 has impacted on the number of cases detected in the UK. A dramatic increase in sero-positive samples from travellers to these regions was observed. Consequently this led to development of a real-time RT-PCR assay at the Health Protection Agency's Centre for Emergency Preparedness and Response.

Methods: Conserved regions of the E1 gene from published Chikungunya (CHIK) RNA sequences were identified. Primers and a dual-labelled probe were designed within this region. The real-time RT-PCR assay was then optimised on the Roche LightCycler®. A panel of clinical samples were used to test the assay alongside authenticated CHIK virus. A synthetic RNA transcript control was also designed to eliminate the need for RNA extraction from the virus stock. The sensitivity of the assay was established using this control. To investigate the phylogenetic relationships between a selection of clinical samples, the sequence of the entire E1 gene of 8 samples from travellers to Mauritius, Seychelles and India was determined.

Results: The real-time assay was found to be more sensitive than existing RT-PCR tests performed in-house and the other published real-time assay (Pastorino et al 2005), detecting as low as 20 copies of RNA transcript. The 8 E1 genes that were sequenced were all related to the Réunion outbreak strains published. However there were some nucleotide differences between the different countries of origin.

Conclusions: The new real-time RT-PCR assay was designed, optimised and implemented rapidly. The synthetic positive control has provided a reproducible standard that is also a useful indicator of sensitivity. Finally, sequencing of the E1 gene from clinical samples has confirmed that they originate from the Réunion island virus sequence.

16.007 Is Mosquito Control a Lost Cause Against Dengue, Chikungunya and Yellow Fever?

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Half a century ago, WHO/PAHO announced that Aedes aegypti, the principal urban vector of dengue, chikungunya and yellow fever, had been eradicated from 22 countries in the Americas. Fifteen years later, the mosquito had regained its abundance in many regions, and dengue became an increasingly serious public health problem. In the future, chikungunya may present a new problem, but the greatest danger is the potential for urban yellow fever.

The success and subsequent failure of the eradication campaign are attributable to the behaviour of the vector. There is now compelling evidence that female Ae. aegypti (and probably Ae. albopictus, another urban vector) distribute small numbers of eggs among many sites, and that this 'skip oviposition' is a driver for dispersal. As a result: (1) during the eradication campaign, contact with multiple oviposition sites was probably a key factor in the success of 'perifocal' treatments of infested containers with DDT, there was a high probability that females would die at these sites even if other sites had not been treated; (2) skip oviposition can prolong the gonotrophic cycle by many days. Field estimates of longevity are based on a function of the length of this cycle and the parous rate, so survival ratesa nd hence vectorial capacity are considerably higher than previously supposed; (4) dispersal promotes infestation of new breeding sites; (5) mobility of infected insects limits the epidemiological impact of 'source reduction'; (6) 'focal' treatments with insecticidal aerosols ('fogging') centered on the homes of clinical cases will not have epidemiological impact if infected insects have moved beyond the treatment area.

These factors, combined with the increasing prevalence of infested containers and the quantum leap in mobility of virus via air travel, make Ae. aegypti a formidable adversary throughout the tropics. Innovative approaches are required before effective control can become a reality.

16.008 Anaplasmosis in Belgium; A six-year surveillance

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Background: Human Granulocytic anaplasmosis (HGA) is a recently recognized tick-borne infectious disease. The causative agent of Human Granulocytic Anaplasmosis (Anaplasma phagocytophilum) is a Gram-negative obligate intracellular bacterium that invades granulocytes. Since 1995, serological evidence for HGA infection has been demonstrated in several European countries. Ixodes ticks are the main vectors for the HGA agent.

Methods: From 2000 to 2006, 420 serum samples were tested with an HGA IFA IgG and IgM antibody test kit. For IgG antibody detection a reciprocal titer of 64 was withheld as cut-off value, for IgM this was 20. A reciprocal titer higher than 64 for IgG, or higher than 20 for IgM was, according to the manufacturer's protocol, considered as positive.

Results: In total 186 samples (44.3%), out of 420, were found positive for either IgG or IgM antibodies. The 186 positive serum samples represented 143 patients. Seroconversion was observed in 5.6% (2/36), 6.1% (2/33), 9.7% (3/31), 22.2% (18/81) and 8.1% (18/223) of the patients from the years 2001 to 2005 respectivelyl. The sum for the period 2000-2005 brings the total number of confirmed cases in Belgium - according to the proposed case definition of Brouqui et al. to 43 cases

Conclusion: Our surveillance for HGA started in 2000, after we established the presence of A. phagocytophilum in Belgium. The results of this study probably still only show a tip of the iceberg; only 223 samples were

submitted in 2005 while for LB, approximately 4.000 to 8.000 samples were tested. Considering that both LB and HGA are transmitted by the same tick species, it would be warranted to test febrile ill patients with a history of tick bite for both LB and HGA. Whether or not human granulocytic anaplasmosis is a true emerging disease in our country, rather than a persisting problem, can then be established.

16.009 Brazilian Spotted Fever in the Pediatric Age-Segment in the State of São Paulo, Southeastern Brazil, 2003–2005

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Background: Brazilian Spotted Fever (BSF), a spotted-fever group rickettsiosis caused by R.rickettsii re-emerged in São Paulo (Southeastern Brazil) in 1985 and its' incidence has experienced a dramatic increase since the beginning of this century. Usually seen as an adult disease, the encroachment of urban areas in previously rural or forest areas has brought children in contact with the main tic vector and reservoir, Amblyomma cajennensis.

Objective: Describe the main characteristics of BSF in children in the state.

Patients and Methods: Retrospective analysis of surveillance records kept by the Secretaria de Saude do Estado de São Paulo. Brazilian Spotted Fever is a notifiable disease in Brazil since 2003. Records of children <18 years of age were reviewed.

Results: A total of 3,709 cases were notified in the period, 129 were confirmed. In the pediatric age segment, there were 1,717 notifications of which 38 where confirmed, 29,46 of all confirmed cases. Mean age was 9.6 years (median 9 years), 26 (68.4%) were male and 12 (31.6%). A history of tick exposure was obtained in 21 (52,5%). Main clinical aspects were: fever (95%), headache (67,5%), prostration (67,5%), myalgia (60%), abdominal pain (52,5%) exanthem (42,5%, petechiae (57,5%) hemorrhage (32,5%), shock (22,5%), shock and icterus (17,55). Case-fatality was 25%.

Conclusions: Pediatric cases represent almost a third of all confirmed cases in the state for the study period, considering that this age segment represents roughly one-third of the state's population, we can assume that, notwithstanding notification bias, incidence of BSF in children is the same as in adults. Also, as with adults, BSF in the pediatric segment is associated with a high case-fatality. BSF is far from a household name among pediatricians in Brazil therefore, much is needed to improve knowledge of the disease and guarantee prompt diagnosis and treatment, reducing the high case-fatality.

16.010 Cytotoxic activities of N-Chlorotaurine (NCT) on Leishmania infantum **and** Leishmania donovani

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Background: Due to the lack of preventive immunizations and the development of resistances against the available anti-protozoan therapeutics, there is an urgent need to develop or discover new drugs. The aim of this study was to investigate the activity of N-Chlorotaurine (NCT) against Leishmania infantum and L. donovani promastigotes and amastigotes. This substance is the main representative of long-lived oxidants produced by stimulated granulocytes and is available as sodium salt, well soluble in aqueous solutions. NCT has already been proved to be virucidal, bactericidal and fungicidal and even showed activities against Schistosoma mansoni.

Methods: Parasites were cultured in liquid culture media and effectivity tests were carried out in culture media and amino acid free media, respectively. Concentrations from 25 μM to 55 mM (1%) of NCT and different times of incubation (1 to 24 hours) were applied. Surviving and dead cells were counted by viability staining and motility assays.

Results: The parasites were highly susceptible to NCT dependent on incubation time and test media. Incubation of Leishmania infantum promastigotes with 2 mM NCT in amino acid free medium led to a 100% mortality within 1 hour. L. donovani showed lower susceptibility.

Conclusion: Since NCT is well tolerated by human tissues at concentrations up to 55 mM, it seems to be a promising candidate substance for topical anti-protozoan therapy.

16.011 E 16.011 Comparison of Tick-Borne Disease Surveillance Performance in Czech Republic and Poland Based on Data from 1999–2005

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Background: Tick-borne encephalitis (TBE) has been endemic in regions on the border of Czech Republic and Poland for over 50 years, and incidence of Lyme borreliosis (LB) has been increasing in that area since the launch of mandatory reporting in both countries in the 1990's. The aim of the present study was to compare the information recorded in both countries and assess the surveillance systems performance in bordering regions of Czech Republic and Poland.

Methods: The study area comprised comparable neighbouring regions of Czech Republic (24 districts, area 23,160 km2, population 3,381,702) and Poland (45 districts, total area 23,058 km2, population 4,961,480). The surveillance forms on TBE and LB from years 1999-2005 were used for this study. The diagnostic certainty was ascertained based on TBE and LB case definitions used in Poland. A logistic model comparing certainty of diagnosis was fitted separately for TBE and LB cases. The response variable was confirmed case status and the explanatory variables were gender, age, year of diagnosis, report of tick bite, Czech/Polish status and hospitalization status.

Results: Six hundred and sixty-four cases of TBE were recorded in Czech Republic, and 78 in Poland. A significant decrease of proportion of confirmed TBE cases by year was detected in both countries. In Czech Republic the decrease of diagnostic certainty was significantly larger than in Poland, and was accompanied by an increase in the number of reported cases from 57 in 1999 to 130 in 2005. There were 8,211 cases of LB reported in Czech Republic, and 2,259 in Poland. In Poland the diagnostic certainty increased in 2004-2005 ($p=0.0011$) to the level observed in Czech republic, which was accompanied by an important increase in number of reported cases in 2003-2005. Additionally, there was a marked decrease in proportion of hospitalized cases in Poland, from 86% in 1999 to 37% in 2005, whereas in Czech Republic the hospitalization rate remained stable at about 15%. Hospitalization was inversely related to confirmation of cases both in Poland (OR=0.68, $p<0.0001$), and in Czech Republic (OR=0.53, $p<0.0001$).

Conclusions: The comparison of data collected by Poland and Czech Republic tick-borne disease surveillance shows important differences in performance of both systems. The Czech system seems to be more specific in better diagnosing LB cases. The TBE data shows that even if southern regions of Poland are not endemic for TBE, there is a need to improve the sensitivity of the surveillance system in regions adjacent to Czech Republic.

16.012 Incidence of the Tickborne Encephalitis in the Czech Republic and in the Region of the South Bohemia

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Backround: The tickborne encephalitis, by another name the tickborne brain inflammation is an infectious viral disease. The virus is transmited

by ticks. The infection transmission is possible not only after clinging the infectious tick, but exceptionaly by intake of thermicly unprocessed milk of the parasitized animal. The occurence of parasitized ticks in the contaminated area of the Czech republic is about 1 to 5 %. The tick-borne encephalitis can result longterm health consequences.

Methods: the statistic analysis of data concerning the tickborne encephalitis incidence in the past 20 years in the Czech republic and in the region of South Bohemia.

Results: There is an increase of the tickborne encephalitis cases in the Czech republic. In 2005 there were 642 cases of this disease, that is 135 cases more than in the year before (507 in 2004). The experts noted the highest increase in the last ten years for the time being. The highest incidence is notified in the South Bohemia, where the incidence presents approximately 25 % of the total incidence in the Czech Republic and the situation seems to be more serious this year (177 cases in november 2006 compared to 166 cases in the end 2005). The parasitized ticks occure mainly in the river basin of our rivers (e.g. Vltava, Sázava, Berounka), where recreational facilities are situated. The peak incidence period of the tickborne encephalitis is since beginning of summer until the late autumn. A vertical spread of the Ixodes ricinus tick occurence changed into 1100–1200 m of altitude in the last two decades in the Czech Republic.

Conclusion: There is an increase of the tickborne encephalitis cases in the Czech Republic with the highest increase in the last ten years for the time being. The highest incidence is notified in the South Bohemia Region. The incidence period of the tickborne encephalitis is since beginning of summer until the late autumn. A vertical spread of the Ixodes ricinus tick occurence changed into 1100 - 1200 m of altitude in the last two decades in the Czech Republic.

16.013 Tick-Borne Encephalitis Virus: GAG-Binding Phenotype and Virulence

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In this work we investigated types of infection caused by different variants of TBEV.

In our work we used specific RT-PCR method with internal control that we had developed for providing a correct evidence of absence of viral RNA in a tissue sample. Besides that we used various virological and molecular biological methods, like plaque assay, experiments on mice, and affine chromatography.

We performed experiments with mouse brain-adapted strain EK-328, caused acute infection in mice, and its derivate - tick adapted variant M, caused inapparent infection. Variant M has some striking differences from parental strain: absence of hemagglutinating activity, increased binding to heparin-sepharose, lower virulence for mice upon peripheral inoculation (neuroinvasiveness). The increased binding to heparin-sepharose allowed us to conclude that variant M acquired a GAG (glycosoaminoglycan)-binding phenotype. All these properties correlate with substitutions in E protein of variant M that increase positive surface charge of the molecule in comparison with E protein of the parental strain. During propagation of variant M in PEK cells was obtained set of revertants. These revertants carried different reverse substitutions and various compensatory mutations that decreased the positive charge of the E protein.

In our studies we showed that all revertants exhibited low-binding to heparin-sepharose, even in comparison with EK-328. So, they have lost GAG-binding phenotype. Although neuroinvasiveness of revertant clones was different: low or reversed to high. This finding demonstrates the importance of the location and feature of the compensatory substitution. Besides, we showed that the revertants can cause various types of infection in mice after intraperitoneal inoculation: inapparent, acute, persistent, and acute with hemorrhagic syndrome.

We investigated binding to heparin-sepharose for 8 strains that had been isolated from nature. One of them showed increased binding. This strain, like variant M, had small-plaque phenotype and low neuroinvasiveness. And it has substitution in E protein in comparison with prototype strain that increased positive surface charge of the molecule.

So, type of infection depends on virus properties. During the reproduction of tick adapted variant in mammalian cells appears set of revertants that cause different kinds of infection. Probably type of caused disease depends on combination of revertants and their relationships during the reproduction in host organism.

16.014 Canine Leishmaniosis Spread in Croatia: Feasibilities of PCR-Based and Serological Monitoring Activities

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In the south littoral parts of Croatia canine leishmaniosis (CanL) had been recognized as a problem for the first time in the first part of 20th century. Human visceral leishmaniosis has been sporadically reported in Croatia, and as with other diseases transmitted by arthropods, it is not a major public health problem in Croatia. Since 1997 CanL in Croatia has been proven parasitologically and serologically in hundreds of symptomatic and asymptomatic dogs. Isoenzymatic analysis from 30 in vitro cultivated isolates (performed in WHO Centre for Leishmaniosis in Madrid, Spain and in Istituto Superiore di Sanità in Rome, Italy) revealed: Leishmania infantum (MON-1; MON-27; and MON -34). All the dogs visited or lived in central and south parts of Dalmatia (from the city of Trogir along the coast and city of Drnis in the hinterlands in the West, Montenegro border in the East, Bosnia and Herzegovina border in the North, the Adriatic sea in the South, as well as on middle and south Dalmatian islands). During 2005, 1776 healthy dogs sera originating from central and southern parts of Dalmatia had been serologically investigated by indirect immunofluorescence (home antigen). During the autumn in 2006, field samples taken from 200 clinically healthy dogs originating from south littoral Croatia (Dubrovnik area) were serologically tested by indirect immunofluorescence with home antigen, and a PCR-based protocol for the detection of Leishmania parasites in canine whole blood was performed. The parasite DNA had been extracted using QIAmp DNA Mini Kit® (QIAGEN). Polymerase chain reaction was performed on the highly reiterated minicircle kDNA from dogs' peripheral blood that amplified a fragment of 145 bp.In 2005, 141 (7,9%) sera among 1776 reacted positive (titre $\geq 80^{-1}$). In 2006, 16 among 200 dogs sera (8,0 %) reacted positive with titre $\geq 80^{-1}$ and the 145 bp fragment originated from kDNA was detected in 161 among 200 whole blood samples (80,5%). Since the PCR-based monitoring protocol revealed the infection prevalence, further serological and especially PCR monitoring activities among dogs in areas bordering to enzootic should be useful in identification of new positive canines before stable foci of CanL were developed.

16.015 Serological Evidence for Establishment of Herd Immunity to Usutu Virus among Wild Birds in Austria

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Background: Usutu virus (USUV), a member of the mosquito-borne clade within the genus Flavivirus, has been responsible for avian mortality in Austria since 2001. USUV activity has now been recognized during 6 consecutive years. 2005 and 2006 USUV-associated bird mortality has also been registered in Hungary, Switzerland and Italy. Intriguingly, in Austria the proportion of USUV-positives among the investigated dead

birds decreased dramatically after 2004. A possible explanation for this phenomenon might be establishment of herd immunity. To prove this hypothesis, serological examinations of susceptible wild birds were performed.

Methods: Blood samples of approximately 400 wild birds of different species were available from four consecutive years. In addition, 86 birds kept in an owl and raptor rehabilitation center within the USUV endemic area were bled at three timepoints - before, at the peak, and after the 2005 USUV transmission season - in order to identify titer dynamics and seroconversions. Antibody titers to USUV were determined using the hemagglutination inhibition test (HI) and the plaque reduction neutralisation test (PRNT). Selected sera were additionally tested for antibodies to West Nile virus and tick-borne encephalitis virus.

Results: From 48 species of wild birds, sera collected during four consecutive years (2003-2006) were analysed. While in the years 2003-2004 the proportion of seropositives was below 10 percent, the percentage of seroreactors raised to more than 50 percent in 2005 and 2006. On the other hand, almost three thirds of the owls and raptors had antibodies before the transmission season, the percentage of positives dropped to less than half at the peak of USUV transmission and raised again to more than 60 percent after the transmission season.

Conclusion: These serological data show an increasing proportion of seropositive wild birds from year to year. The owl and raptor data indicate that there had been significant viral exposure in the previous season(s), but also a number of new infections in the current season. Establishment of herd immunity is the most likely explanation for the significant decrease in USUV-associated bird mortalities in Austria during the last three years. Taken together, these data provide evidence that USUV has become a "resident" pathogen in Austria and presumably also in neighboring countries. However, its impact on the bird population will most likely become negligible due to increasing herd immunity.

16.016 Lyme Disease Caused by *Borrelia spielmanii* in Hungary

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Background: Lyme disease is the most frequent tick-borne human infection in the northern hemisphere. At least five species of the Borrelia burgdorferi sensu lato complex: B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. bissettii and B. lusitaniae have pathogenic role in human Lyme disease in Central Europe. A sixth pathogenic strain, A14S, has been isolated from a Dutch and two German patients with erythema migrans. Based on genotypic and phenotypic characteristics and specificity for a particular reservoir host (garden and hazel dormice), it has recently been described as a new species, B. spielmanii. To identify the Borrelia species occurring in Hungarian Lyme disease patients, we have started the molecular analysis of the cultured isolates.

Methods: PCR amplification of the partial osp A gene and sequence analysis of eight cultured Borrelia spp. isolates from erythema migrans lesions of Hungarian patients was undertaken.

Results: Six of the isolates were shown to be B. afzelii, one B. burgdorferi sensu stricto and one showed 99.21% similarity with the recently described B. spielmanii. The patient whose cultured borrelia revealed B. spielmanii was a 42 years old woman with a homogenous erythema migrans. The erythema was 10 cm in diameter on the front surface of the knee at the first visit. The IgM and IgG borrelia immunoblot applying B. afzelii (ACA1) antigen was negative in the serum drawn on the 7th day after the appearance of erythema migrans. The patient did not remember a tick bite and had not travelled abroad during the previous 6 months. She complained of an extremely unusual, intensive, serous nasal discharge that started 3 weeks before the appearance of erythema migrans, and of a moderate headache; both disappeared spontaneously two weeks before treatment.

Conclusions: Our observation also suggests that this species has a pathogenic role in human Lyme disease which have been described in only two previous publications.

16.017 West Nile Virus: A Decade (1997–2006) of Laboratory Based Surveillance in Romania

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A surveillance program for West Nile virus (WNv) neuro-invasive infections has been implemented in Romania following an unprecedented meningo-encephalitis epidemic in 1996. The surveillance aimed at detecting as early as possible the transmission of WNv to humans providing information to direct prevention and control activities, evaluating the impact of WNv neurological infections, identifying risk and population at risk, identifying geographic areas where interventions were necessary.

During the transmission season (May-November) paired sera and CSF samples from aseptic meningitis and encephalitis cases have been screened for WNv specific antibodies in an IgM MAC-ELISA test.

Over 10 years 3490 patients have been tested and 80 have been diagnosed with WNv acute neurological infections. 66 cases classified as confirmed, and 14 cases as probable. 17 patients presented with encephalitis, 62 with meningitis, and one with polyradiculitis. The severe clinical form (encephalitis) occurred mainly in persons over 50 years of age. There were 5 deaths (4 in elderly over 60 years old, and one in a 16 years old male). 85 % of the cases have been recorded in August and September months. 21 % cases have occurred in the area of the greater Bucharest, 38% in other urban settings, and 41% in rural settings. Cases have been distributed mainly in the plain in Southeastern Romania. The highest attack rate has been recorded in 2 districts related to Danube Delta and two of its major tributaries.

Ten years of surveillance of WNv neuroinvasive infections have documented the occurence of transmission to humans at a low level in each transmission season.

16.018 Surveillance of Lyme Borreliosis in Germany: Epidemiological and Geographical Analysis of Cases Reported from 2001 to 2006

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Background: Lyme borreliosis is the most common vector-borne infection in the northern hemisphere. Lyme borreliosis is not a notifiable disease throughout Germany, but mandatory reporting has been established in the five eastern states of Germany, where 25% of the German population live. According to the case definition for Lyme borreliosis, cases with the clinical feature of erythema migrans and laboratory confirmed early neuroborreliosis are notifiable.

Methods: In Germany surveillance started in 1991 on a voluntary basis, since 2003 notification is mandatory in the five eastern states. Notifications are transmitted electronically from local health departments to state health departments and then to the Robert Koch Institute. For case reports from Brandenburg, the unit for the geographical analysis was the community (Gemeinde, Amt), whereas in the other states the unit was the county (Landkreis, Stadtkreis).

Results: Between 2001 and 2006 a total of 19.558 cases have been reported. The incidence of Lyme borreliosis has increased in all states showing a pronounced saisonality with a peak from early summer to autumn. In Brandenburg, where surveillance started in 1991, more cases have been reported from the eastern part of the state with communities reporting regularly high numbers of cases. In the western part of Brandenburg the number of reported cases and the number of affected

communites has increased only recently. Overall the highest incidences were seen in children aged 5 to 9 years and in adults aged 60 to 69 years. In the youngest age group boys were more affected, whereas in the age groups above 15 years women were more frequently affected. The most frequent clinical presentation was the erythema migrans (97.4%). In children aged 5 to 9 years early neuroborreliosis was over-represented (12.0% and 8.5% of all cases in children and adults, respectively). 17% of children with neuroborreliosis were hospitalized, the average duration of hospital stay was 9.5 days.

Conclusion: In the early years of borreliosis surveillance underreporting of the disease has to be assumed. The data from Brandenburg possibly indicate that the annual increase in the incidence of Lyme disease might be a result of the increasing awareness of physicians and patients, and the growing acceptance of the surveillance system. Therefore the actual incidence increase seen in Eastern Germany may start to highlight the true degree of endemicity of Lyme borreliosis. In order to develop and enhance prevention strategies, nationwide surveillance should be considered.

16.019 A Clinical Outbreak of Tick-Borne Fever Due to *Anaplasma phagocytophilum* in North-Eastern Italy

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Background: Several surveys on vector born diseases in North-eastern Italy have shown a high rate of infection in the tick Ixodes ricinus and in humans. In 2005 the expected tick infection rates for Anaplasma phagocythophilum (Ap) and Borrelia burgdorferi s.l. were 30.28% and 35.74%, respectively. In summer 2006 Tick-borne Fever was diagnosed for the first time in this area in a herd of about 60 cattle. Half of them showed clinical signs as fever, anorexia, respiratory distress and a dramatic drop in milk production at the end of the summer grazing season. Animals were vaccinated against Respiratory Syncytial Virus and almost free from Infectious Bovine Rhinotracheitis and Bovine Viral Diarrhea.

Methods: The blood of 8 symptomatic animals was tested for haemoparasites both by blood smears and PCR. The pasture and surrounding areas were sampled for ticks by dragging at the end of August.

Results: In 2 smears a characteristic inclusion was seen in neutrophils and 6 cattle resulted PCR positive for Ap. All the 8 tested cattle were PCR negative for A.marginale, Babesia bigemina, B.bovis, B.divergens, B.crassa, B.major, B.motasi, B.ovis, Theileria annulata, T.buffeli, T.lestoguardi, T.mutans, T.ovis, T.taurotragi and T.velifera. The pasture, the surrounding area and the animals resulted tick-free. The environment (alpine-like area, 1200 m.a.s.l.) and the mean temperature of the summer 2006 were not favourable for the Ixodid ecology, suggesting a iatrogenic transmission within the herd.

Conclusion: Tick-borne fever has to be considered as a possible cause of clinical signs in ruminants in North-eastern Italy. Disposable needles have to be used to prevent this and other vector-borne infections.

16.020 Dengue Entomological and Virological Surveillance in Singapore

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Singapore's dengue control programme includes vector control, public education, law enforcement, research and surveillance of disease, vector and virus. Following a period of over 15 years of low dengue incidence as a consequence of the implementation of a comprehensive vector control programme in Singapore, there has been a resurgence of dengue in recent years despite the low vector population density. In the year 2005, Singapore saw an unprecedented outbreak that resulted in 14,209 reported dengue cases. This paper presents data from our dengue surveillance effort, which contributes to vector control operations and understanding of the epidemiology of the disease. Entomological surveillance shows the presence of Aedes aegypti in areas with dengue outbreaks.

Singapore is hyper-endemic, with all four dengue serotypes circulating. However, a predominant serotype is usually apparent. Through analysis of samples collected from private hospitals and general practitioners, we detected a transition from DEN-2 to DEN-1 as the predominant serotype at the end of 2003, which was followed by a huge increase in the number of cases in Singapore in 2004. The epidemic continued through 2005, when we also saw an emergence of DEN-3 at the height of the outbreak. Analysis of spatial and temporal distribution of various serotypes in Singapore revealed the emergence of DEN-3 from certain foci in Singapore.

Gene sequencing and phylogenetic analysis of the envelope gene of viruses isolated from patients, reveals that Singaporean DEN-1 and DEN-2 are contained within genotype I and the Cosmopolitan genotypes respectively. Although evidence of importation was noted, our data suggests that the serotype shift was not the result of importation of a novel virus strain, but more likely to have resulted from neutral processes rather than through specific selection. DEN-3 viruses are contained within genotype III, shared by Sri Lankan (1980–1990's) and Venezuelan (2000–2001) strains. The co-circulation of DEN-1 and DEN-3 could potentially represent a serious development, since significant pathogenesis has been associated with DEN-3 (in Bangladesh, 2000–2001), DEN-1 (in a cross sectional study in Nicaragua), or both (in Thailand, 2000–2002). Our demonstration of rapid changes in dengue serotype, particularly in light of recent research detailing the complex interactions between serotypes, highlights the importance of sustained monitoring of dengue viruses in Singapore.

16.021 Donkeys as Reservoir of Tick-Borne Zoonosis in Sicily, Italy

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Background: Rickettsia conori, Coxiella burneti are most often seen in Sicily as tick borne zoonoses pathogens, recently Anaplasma phagocytophilum has been recognized as an emerging tick borne infectious agent. This study aim is to describe the diffusion of the endemic Rickettsiosis in Italy and the role that donkeys as pathogen carrier and tick vectors. Methods In Corleone (Palermo, Sicily) in an inland mountainous territory (680-834 m a.s.l.) eleven donkeys were studied in autumn 2002, in 2003 the donkeys were 13 in winter, 11 in spring, 14 in summer and 10 in autumn and 12, winter 2004, to value the seasonal seroprevalence for R. conori, C. burneti and A.phagocytophilum. IFI tests were used for all the pathogens studied (Fuller Laboratories, USA), and PCR tests were used for A. phagocytophilum (Richter Jr. et al, 1996) and R.conori (Tzianabos T. et al, 1989). It was estimated the prevalence and ticks parasitization intensity. Results Serological prevalence show high peak in autumn 2002 for R.conori (90.9%) the other seasonal data were: Winter 2003 (46.1%), Spring 2003 (54.5%), Summer 2003 (57.1%), Autumn 2003 (60.0%) and Winter 2004 (58.3%). For A.phagocytophilum the peak was in winter 2003 (69.2%), the other seasonal data were: Autumn 2002 (36.3%), Spring 2003 (27.2%), Summer 2003 (28.5%), Autumn 2003 (10%) and Winter 2004 (33.3%). Serological tests were all negative for Coxiella burneti. PCRs assay for A.phagocytophilum were always negative, for R.conori 6.4% of the samples were positive. Ticks high peaks was in autumn (36.3-50%) and low peak in winter (0%). Parasitization intensity of ticks infestion present the high peak in spring (5 Ixodidae/infested animals). Ixodidae species founded are Rhipicephalus bursa (42.9%), Dermacentor marginatus (39%), Haemaphisalis puntata (11%) and Hyalomma marginatum (7.1%).

Conclusion: Donkeys have a role on the maintenance of the pathogens in the studied area. The results of this study show that agents of zoonoses as R.conori and A.phagocytophilum are present in the donkeys, that are probably reservoir of this pathogen and, moreover there is a considerable chance for transmission of this pathogen by other tick species.

16.022 West Nile Virus in Wild Birds, Southern France, 2004

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Background: During late summer and autumn 2004, an equine outbreak of West Nile virus (WNV) occurred in the Camargue region, a wetland area in the south of France where the virus was first reported in humans and horses in 1962 and re-emerged in 2000. We conducted epidemiological investigations on wild peridomestic birds (house sparrows Passer domesticus, tree sparrows Passer montanus and common magpies Pica pica) in order to determine whether these species were likely to be associated with WNV emergence in horses.

Methods: Plasma samples were screened for WNV immunoglobulin G using an indirect ELISA test and positive samples were confirmed by a microneutralization assay. WNV specific RT-PCR assay and virus isolation on C6/36 cells were performed on brain biopsies from two dead birds (a house sparrow and a magpie). The complete genome of both isolates was sequenced and analysed.

Results: Four birds out of 228 tested positive in serology, indicating the circulation of WNV in the peridomestic bird population. Pairwise alignment of both isolates showed that they were identical and multiple alignment with other sequences available on GenBank database revealed that they were related to WNV lineage 1 strains belonging to the European/Mediterranean/Kenyan cluster.

Conclusion: Our data indicate that WNV circulated among wild resident birds in the Camargue region before and during the 2004 equine outbreak. To our knowledge, our isolates are the first WNV avian isolates to be entirely sequenced in the western Mediterranean basin.

16.023 Lyme Disease Spirochetes in Ticks Collected During a Field Study in Central Italy (Marche Region)

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Lyme Disease is the most widespread tick borne disease in Italy, with the highest levels of prevalence recorded in North-Eastern Italy and many reports of clinical symptoms in humans.

The Pesaro-Urbino province,in the Central Italy, could be considered a risk-area for Lyme Disease because of its ecological features.However field data are not available yet, even if the disease is known to be present in neighbouring territories.

During a one year field study (Sept 2001–Oct 2002) ticks were collected from hosts as wild cervids (roe deer and fallow deer) and humans who went to the local hospital after tick-bite,as well as vegetation by dragging once a month during spring and summer 2002.

All the samples were registered and preserved in 70% ethanol. After,all ticks were classified then PCR test for the detection of Borrelia burgdorferi s.l. was performed. Pools of not engorged or semi-engorged ticks belonging to the genus Ixodes collected from the animals and from the environment and each Ixodes tick collected from humans were tested.To identify the geno-species of Borrelia burgdorferi s.l. the 5S-23S ribosomal rRNA intergenic spacer of the positive samples was amplified and then sequenced.

The most frequent species among ticks collected from animals and from humans was Ixodes ricinus 74% and 85%, respectively. This species was the most widespread among the ticks collected from environment too (56%), even though R. turanicus was well represented.

By performing the PCR test for Borrelia burgdorferi s.l. two positive pools among those collected from animals, and one single positive tick collected from human were detected, while three pools were positive among the ticks collected from environment.

Sequencing of the 5S-23S intergenic spacer has allowed to identify two different genospecies: B. burgdorferi s.s. 100 % homologous with the strain 5LM218 isolated in France and B. lusitaniae 100 % homologous with the strain PoHL1, recently involved in human infection in Portugal. Borrelia lusitaniae was identified in ticks collected from human and roe deer coming from the same area.

These are the first available data about Lyme Disease spirochetes and tick ecology in the Pesaro Urbino province. The findings on the host-tick relationships and about the genopecies involved in the cycle of Borreliosis confirm the suitable conditions for Lyme disease risk in the study area.

The results agree with the previous findings in the Mediterranean Region, although further research is needed to deepen the knowledge of the Lyme disease cycle in this area.

16.024 Epidemic Chikungunya Virus Variant from the Indian Ocean Islands Emerges in Northern India

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Background: Chikungunya virus (CHIKV) emerged in the Indian Ocean islands of the Comores, Reunion, Mayotte, Mauritius, the Seychelles and Madagascar in 2005 resulting in the infection of about 300.000 inhabitants and travelers in only one year. Beginning in March 2006 increasing numbers of CHIKV-like febrile illnesses were reported from the central parts of India, i.e. Andhra Pradesh, Maharashtra, Orissa and Karnataka states.

Methods: A German traveler presented with a typical arthralgia and fever two days after she returned from a fortnight vacation to Rajasthan, northern India. A serum sample of her was investigated by means of real-time RT-PCR, virus isolation, and nucleotide sequencing.

Results: Real-time RT-PCR results indicated an acute CHIKV infection and the virus was isolated in Vero cells. Sequencing of the entire viral genome was performed and revealed a high level of nucleotide sequence identity to CHIKV isolates from the recent epidemic in the Indian Ocean islands. Phylogenetic analyses placed our CHIKV isolate in one clade together with the 2005 and 2006 isolates from Mauritius, Reunion, and Mayotte but not into the neighborhood of any CHIKV from Asia. In detail, we found only 20 nucleotide exchanges to a recent isolate we made from a patient returning with a CHIKV infection from Mauritius, resulting in only five amino acid changes (nsP1 T128K, T376M, nsP3 S472N, and capsid P23S, V27I).

Conclusions: This is the first proof of a non-Asian CHIKV subtype occurring and causing disease in Asia. Although the excessive dimension of the 2005/2006 outbreak in the Indian Ocean islands was at least in part accounted to the naïve population affected, our results indicate that the emergence of this CHIKV subtype may rather be a result of a better viral fitness. Whether this is reflected by higher viremia in humans or an enhanced adaptation of the vector population resulting in increased transmission rates warrants further investigations. However, the occurrence of an African CHIKV in Asia further demonstrates how fast viruses can emerge and establish in places where competent vectors are prevalent.

16.025 Antibody Seroprevalence Against Arboviruses in Humans in Northern Afghanistan

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Background: Since the war in Afghanistan started data on prevalence or incidence of infectious diseases are rare. However the knowledge on the occurrence of infectious diseases is of importance as it is a prerequisite for the implementation of prophylactic measures to prevent infection.

Methods: We tested the sera of 140 residents of the northern Afghan city Kunduz for the prevalence of antibodies against flaviviruses (Dengue-, West Nile-, tick-borne encephalitis-viruses), alphaviruses (Sindbis virus), bunyaviruses (Sandfly Naples-, Sandfly Sicilian-, Crimean Congo hemorrhagic fever viruses), and hantaviruses (Puumala-, Seoul-, Hantaan viruses). For testing commercial ELISA assays,

commercial and in-house indirect immunofluorescence and neutralization tests (differentiation between West Nile virus and Japanese encephalitis virus) were used.

Results: 31/140 sera (22.1%) reacted positive against West Nile virus. By plaque reduction neutralization test specific antibodies against WNV in comparison to Japanese encephalitis virus could be confirmed. 5/140 sera (3.6 %) were found specifically positive for dengue virus. None of the sera showed specific reaction for tick-borne encephalitis. 3/140 sera (2.1%) reacted against Sindbis virus. 13/140 sera (9.2%) were positive for sandfly fever virus, subtype Naples, while all sera were negative for the Sicilian subtype of sandfly fever virus. 6/140 sera (4.2%) exhibited reactivity against Crimean Congo hemorrhagic fever virus. 8/140 sera (5.7%) showed reactivity against hantaviruses. 3/140 sera (2.1%) of them showed higher titers in immunofluorescence for Puumala virus, while one serum each (0.7%) exhibited higher titers for Seoul virus and Hantaan virus. Two sera were indeterminate for the 3 hantaviruses tested.

Conclusions: To the best of our knowledge, these are the first data available on the prevalence of antibodies against alpha-, flavi-, bunya-, and hantaviruses during the last decades. The exceptionally high prevalence rates for West Nile virus and Sandfly Naples virus imply intense virus circulation in the region tested. Except for tick borne encpehalitis virus and Japanes Encephalitis virus, antibodies against all other viruses tested were found in prevalence rates of 0.7% up to 22.1%. These results warrant further studies including isolation of arthropod-borne as well as rodent-borne viruses and the characterization of their pathogenic potential and medical importance.

16.026 West Nile Disease Surveillance: Mosquito Monitoring in Central Italy (Tuscany Region) Within the EDEN Project

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West Nile Virus (WNV) is a mosquito-borne virus belonging to the Flaviviridae family, member of the Japanese encephalitis complex. Neuropathogenic for birds, horses and humans WNV is maintained in natural cycles between birds and mosquitoes, particularly Culex species, whereas horses and humans are considered incidental hosts. In the late summer of 1998, an outbreak of equine encephalomyelitis due to West Nile Virus (WNV) occurred for the first time in Italy (Fucecchio wetlands, Tuscany Region), involving 14 race horses at nine localities in four Provinces: Firenze, Lucca, Pisa, Pistoia. So, this area was selected for the EDEN (Emerging Diseases in a changing European eNvironment) project and in particular within the subproject about the surveillance of WNV. In fact the principal objective of this subproject is to improve the understanding of the natural history of the virus and its vectors in Europe and to assess the potential for transmission under future conditions of climate and environmental change. Entomological specimens come from catches carried out fortnightly from April to October in 5 sites for a total of 29 surveys. Mosquitoes were collected using 3 different methods: bird baited traps, CDC light/CO2 traps and manual catches (mouth aspirators) into animal shelters (mainly horse boxes). Both kind of traps operated in couple, one at ground level (about 1,5 m height) and one in the canopy (3-4m of eight). Larval collections were carried out sporadically. In total 20.789 mosquito females, belonging to 11 species and 7 genera were identified. Our findings showed clearly that Culex pipiens represents the predominant species in the study area (98% of the total sample N= 20,369),in the 5 sites (range from 86% to 99.8%) and in all the samples collected by the two kinds of traps, at both the operative heights (ground and canopy). Only manual collections, carried out in the horse boxes, gave a different results, showing the prevalence of Anopheles maculipennis s.l. females (4 sites) or of Ochlerotatus caspius. The results of this entomological inquiry represent a part of the whole ongoing surveillance system, that is aimed at improving the understanding of the ecology of WNV in Italy and in Europe.

16.027 The Emergence of Bluetongue in Europe: Incriminating Vectors with Climate Envelope Models

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The spread of vector-borne diseases into new areas, commonly attributed to environmental change or increased trade and travel, could be exacerbated if indigenous vectors spread infection beyond the range of traditional vectors. This process has been implicated in the spread of bluetongue, a devastating midge-borne disease of ruminants, northward in Europe where indigenous (Palearctic) midge species have been involved in transmission beyond the range of the traditional (Afro-Asian) midge vector, Culicoides. imicola.

Combining vector and outbreak distribution data, from 12 countries affected in southern Europe (1998–2004) and 4 countries affected in North Europe this summer (2006), with fine-scale environmental data, we use environmental envelope models to analyse the differential degree of overlap between the distributions of BTV and different midge vectors to investigate:

(1) the role of the Palearctic midge vectors versus C. imicola in the spread of BTV transmission into southern Europe.

(2) the coincidence of individual species of Palearctic vectors (that differ in their competence for BTV) with northern European outbreaks.

Culicoides imicola preferred warm (annual mean 12 to 20°C), thermally stable locations that were dry in summer (< 400mm precipitation). 40% of >500 recorded outbreaks in southern Europe fall outside this species estimated environmental envelope, occurring in cooler (< 7°C annual mean), thermally more variable and wetter (up to 700mm summer precipitation) locations—conditions inhabited by the Palearctic vectors. The distribution in multivariate environmental space of BTV is also closer to that of the Palearctic vectors than it is to that of C. imicola, suggesting that they play a substantial role in transmission and have facilitated the spread of bluetongue into cooler, wetter regions of Europe. The risk to Northern Europe depends on how much and which of the distributions of the widespread, abundant Palearctic midge vectors bluetongue can occupy, perhaps determined by thermal constraints on viral replication. Future surveillance for bluetongue and for related Culicoides-borne pathogens (such as African Horse Sickness Virus and Epizootic Haemorrhagic Disease virus that have historically shared similar vectors) should aim to record and explain the distributional patterns of all potential indigenous vector groups.

16.028 Positivity of *Triatoma rubrofasciata* in an Urban Area in Sao Luis, Maranhao, Northeast of Brazil

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Background: São Luís' city, capital of Maranhão state, is not considered an endemic area for Chagas disease. Recently the program of epidemiological surveillance of the Maranhão State Secretariat of Health notified the presence of high density of triatomines in a locality of urban area of São Luís.

Objectives: To evaluate a new methodology of vector control and estimate the density and positivity of Triatoma rubrofasciata in period of January to September of 2006.

Methods: Of an universe of 5,186 houses, a sample of 1,060 houses of Fátima's neighborhood was selected by systematic way, for entomological investigation. In a period of 5 to 20 minutes after the Pirizaâ use, the capture of triatomines begun inside and outside the houses, and soon after sprinkling insecticide. The collected insects were sent to laboratory for exam of stomach content using PCR, ELISA and mass spectrophotometry techniques. The houses were located by GIS and analyzed about the space distribution of vectors.

Results: In a 1% (n=11) of examined houses were found Triatoma rubrofaciata and in 4 bugs was observed parasites. In the stomach content examined by ELISA were found blood of rodents, human and chicken.

Conclusion: Clearly, São Luís' city registered a significantly number of infected vector. It probably was related with the construction type used in the historical city of Brazil, needing therefore of being included in the control program of Chagas disease.

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16.029 Competition Between Dengue Serotypes Suppresses Replication in Vector Models

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Theoretical research has shown that within-host competition between pathogen strains can decrease the efficacy of vaccination programs, increase virulence, and enhance potential for emergence. Although this suggests that within-host competition can determine disease emergence, few empirical studies of competition among pathogen strains have been conducted; particularly in vector-borne pathogens, where investigations of competition within the arthropod vector are almost completely absent. Mosquito-borne dengue viruses offer an oppurtunistic system to address this deficiency since within-host competition among virus lineages may govern their emergence patterns for the following reasons: (i) there are phenotypically distinct lineages that co-circulate in many geographical regions and are known to coinfect vectors, and (ii) phylogenetic studies demonstrate that frequencies of particular strains often fluctuate during epidemics, and suggest that competitive displacement commonly drives epidemiological patterns. At present no method of control is available to circumvent the escalating dengue pandemic. It is crucial to quantify the impact of factors that mediate emergence to improve the efficacy of existing dengue control measures, and develop new strategies for control. Thus, we quantified effects of within-host competition between pairs antigenically-distinct dengue strains on replication. We infected mosquito cells in culture (C6/36) and live mosquitoes with different pairs using 2 methods of mixed infection: co-infection (both strains added simultaneously) and superinfection (one strain added 6 hours before the other). We compared titers from mixed and single infections to determine effects of competition on replication. We found that: 1) within-host competition between serotypes can suppress replication, 2) suppression can be asymmetric, and 3) the impact of superinfection depends upon the time lag between infections. These experiments are the first empirical investigation of the impact of within-host competition in the vector of an arthropod-borne disease. Understanding effects of competition on disease emergence will provide important insight towards the design of disease control methods.

16.030 Barriers to Emergence of Sylvatic Dengue Virus

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Dengue virus (DENV), which infects 100 million people per year, is an emerging threat to global public health. Endemic DENV circulates exclusively between humans and peridomestic Aedes mosquitoes. However, DENV also circulates in a second, sylvatic, enzootic cycle. Sylvatic viruses are believed to have emerged in four independent events resulting in the four endemic DENV serotypes (DENV-1,2,3,4), and it is critical to assess their potential for future emergence. In this study, we tested the hypothesis that sylvatic DENV lacks infectivity for either humans or peridomestic mosquitoes and that adaptation to one or both of these hosts is required to enable emergence. To assess infectivity for humans, six isolates of endemic DENV-2 and four isolates of sylvatic DENV-2 were inoculated into groups of severe combined immunodeficiency mice engrafted with human HuH-7 hepatoma cells (SCID-HuH-7 mice). Virus titer in serum was measured seven days post-infection. To assess infectivity for peridomestic mosquitoes, Ae. aegypti were fed on artificial bloodmeals containing high titers of one of four isolates of endemic DENV-2 or four isolates of sylvatic DENV-2. Titer was quantified in each mosquito body, head and saliva to measure overall infection, dissemination and potential transmission, respectively. Sylvatic DENV replicated to slightly but significantly lower titers than endemic DENV in SCID-HuH-7 mice. Sylvatic viruses infected, disseminated and entered saliva in Ae. aegypti with the same efficiency as endemic viruses. These data suggest that the initial emergence of sylvatic dengue viruses into the endemic cycle may not have required adaptation for transmission by Ae. aegypti. Moreover, although sylvatic DENV showed significantly lower replication in the SCID-HuH-7 model than endemic DENV, sylvatic DENV were highly infectious for human liver cells in this model. Thus the potential for future emergence of sylvatic dengue viruses is high.

16.031 Evidence of Bartonella henselae Transmission By Ixodes ricinus Using Artificial Skin Feeding Technique

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Background: Bartonella species are facultative intracellular bacteria associated with a number of emerging diseases in humans and animals. Bartonella henselae causes cat scratch disease as well as being increasingly associated with a number of other syndromes, particularly ocular infections and endocarditis. Cats are the main reservoir for B. henselae and its mode of transmission by cat fleas is now better understood, but new potential vectors, specially ticks belonging to Ixodes ricinus species, have been recently suspected. Considering that Bartonella sp. are emerging human pathogens and that Ixodes sp. can transmit a large spectrum of pathogen to humans, it is therefore, of high importance to determine whether Ixodes sp. are capable of transmitting human pathogenic Bartonella species. In this study, we use a membrane technique for infecting I. ricinus with B. henselae and to investigate tick transmission of the bacteria.

Methods: I. ricinus, collected by flagging pature, were artificially fed on gerbil or rabbit skin with B. henselae infected ovine blood. Ticks were allowed to molt to nymphs and adults, which were then fed with uninfected blood, and engorged females were allowed to lay eggs. Ticks were dissected and B. henselae DNA was detected by PCR on salivary glands, remaining carcasses, eggs, hatched larvae and blood used to fed potentially infected nymphs and adults.

Result: B. henselae DNA was detected in eggs laid by female adults fed on infected blood but not in larvae resulting from hatching of half of these eggs.

After the first B. henselae-infected blood meal and tick molting, B. henselae DNA was detected in 9 carcasses of nymphs (100%) and 4 carcasses of female adults (67 %) while no Bartonella DNA fragment was detected in salivary glands of either nymphs or female adults.

After the second uninfected blood meal, B. henselae DNA was detected in salivary glands of nymphs and adults as well as in blood samples removed from feeders as soon as 72h of attachment.

Conclusion: we have proved transmission of B. henselae DNA from blood to ticks, conservation of DNA through molting, and dissemination of B. henselae DNA to the salivary glands. Finally, we showed B. henselae DNA transmission from ticks to blood after 72h of feeding. These results support the argument that I. ricinus ticks are involved in transmission of B. henselae and represent a potential source of infection for persons exposed to tick bites.

SESSION 17 (Poster Session)
Detection, Surveillance and Control of Emerging Diseases in Wildlife

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

17.001 Rift Valley Fever Virus Surveillance in Kenyan Wildlife

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Background: Although seropositivity to Rift Valley fever virus has been documented in several animal species, the reservoir responsible for periodic epidemics in Kenya has not been determined. In this study, we investigated the possibility that certain wildlife species serve as this reservoir.

Methods: An ELISA was used to measure IgG levels in 183 wildlife serum samples collected by the Kenya Wildlife Service between 1999 and 2004.

Results: Of the 183 serum samples, 24.0% (44) were positive. The seroprevalence was 26.0% in buffalo (27/104), 27.2% in eland (3/11), 33.3% in hartebeest (4/12), 42.9% in gazelles (3/7), 7.7% in giraffes (1/13), 33.3% of oryx, 33.3% of hirola, and 66.7% of kongoni. All serum samples from waterbuck (5), gerenuk (1), impala (6), and topi (1) were negative.

Conclusion: The high rate of seropositivity detected in this survey clearly indicates a role of wildlife in Rift Valley fever virus epidemiology.

17.002 Gene of Type A Influenza Virus-Associated NS1BP Might be Upregulated by Body Burden Dioxin: Mechanistic and Epidemiologic Concepts

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We have shown earlier that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced HIV-1 gene expression in infected human cells thus facilitating the HIV-1 viremy. Dioxin response elements (DRE) were identified in promoter region of HIV-1 and some cancer-associated human common viruses. These and several other model and epidemiologic data allowed us to postulate about the Ah receptor (AhR)-mediated mechanism of viral transactivation by body burden TCDD.

The above mechanistic concept has been applied to NS1 protein (NS1) of type A influenza viruses (AIV) and its binding protein (NS1BP) in humans and chicken (G. gallus gallus). The data are presented here at gene, cellular, and population levels. It was shown that gene encoding the NS1BP possessed DREs (core nucleotide sequence 3' A-CGCAC 5'), two of which were identified within the promoter area. Since determining DREs at positions -7942 and -687, primers for mutational analysis were constructed.

At the cellular level, activation of NS1BP gene expression in vertebrate cells by TCDD suggests that it might contribute to AIV effects in humans and chicken. Increased NS1BP might effectively interact with NS1 thus mediating inhibitory effect of NS1 on pre-mRNA splicing. Due to interaction with the AhR, NS1BP overproduction might enhance concentration of functional AhR and AhR signaling in response to TCDD. As NS1 is abundantly expressed in AIV-infected cells, TCDD induction of NS1BP may lead to elevated ability of NS1 to diminish antiviral responses by preventing transcriptional induction of IFN-ß. Such role for NS1BP might bring insight to the fact that resistance of highly virulent H5N1 to antiviral effects of IFN-ß and TNF-alfa is directly associated with the NS1.

At the population level, information on wild birds and domestic poultry dying from H5N1 in Guangdong province of China, and Long An, Tieng Giang and Ben Tre provinces of Vietnam might relate to the fact that water and soil in these very regions are highly contaminated with dioxin-like compounds. Because human AIV NS1BP promoter contains two DRE, an extrapolation from the data on HIV-1 (1 DRE) and CMV (10 DRE) suggests that concentration of TCDD upregulating NS1BP gene should be moderately above current TCDD blood levels in general population. Thus in human subgroups slightly exposed to TCDD, its body level is a possible promotional factor for seasonal AIV outbreaks, and may strongly facilitate spreading of the H5N1 if avian flu pandemic were to occur.

17.003 The Application of Multitemperature Single-Strand Conformational Polymorphism for Detection of Minute Changes in Avian Influenza Genome Fragments

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Classical single-strand conformational polymorphism (SSCP) analysis is based on the observation that single-stranded DNA fragments attain a number of conformational forms which may be separated by electrophoresis in native polyacrylamide gels giving a characteristic pattern of electrophoretic bands. Minute sequence changes (e.g. point mutations) may have significant effect on electrophoretic pattern of single-stranded DNA. Changes of gel temperature during electrophoresis increase the sensitivity of mutation detection in PCR products; this technique was named MSSCP (where M stands for "multitemperature"). We have applied this method modified in our laboratories for characterization of influenza A cDNA fragments. A series of primers were synthesized after the comparison of the hemagglutinin and neuraminidase gene sequences of different origin. PCR reactions were run using these primers and the products were denatured. Single-stranded DNA fragments were subjected to MSSCP electrophoresis where, after silver staining, they gave characteristic ssDNA band patterns. This technique was applied to analyse hemagglutinin and neuramidase gene fragments from recent isolates of avian flu. Minor differences within a serotype were detected which makes the MSSCP technique a valuable tool for characterization of influenza variants.

17.004 Spatial Distribution of Jungle Yellow Fever (JYF) Outbreaks Outside the Amazon, Brazil, 1999–2003

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Background: The Amazon River basin is the main endemic reservoir of Yellow Fever Virus (YFV). However, YFV can spread beyond that region, causing outbreaks of human cases that are generally preceded by non-human primate epizooties. Geographical determination of epidemic areas is of paramount importance to identify outbreak causes and to recommend and implement prevention and control measures.

Objectives: To describe JYF outbreaks that happened during YFV dispersion out of the Amazon from 1999-2003, focusing on environmental characteristics of the areas.

Methods: We used data from the National Yellow Fever (YF) Surveillance and the Brazilian Institute of Geography and Statistics (IBGE). Locations were GPS-marked (UTM) or located through Dengue Surveillance maps. Landsat-7 ETM (bands 3-5) images from year 2000 were used through Datum SAD69. PAHO cartographic vector digital databases were used through SigEpi 2001. Kernel analysis was performed using Spring 4.0 (National Institute of Space Research/INPE), considering a 75-kilometer range for each locality with YF cases. Maps were finalized using MapInfo 7.0.

Results: Four JYF outbreaks occurred from 1999-2003 in non-amazonic river basins, as it follows: Tocantins/Araguaia and Paraná basins (Goiás State-GO): 64 cases; São Francisco basin: 10 cases in Bahia State (BA) and 32 in Minas Gerais State (MG); Rio Doce basin: 63 cases, also in MG. Outbreaks occurred near highways, road junctions and rivers, reaching non-forested areas. Cerrado (Brazilian Savannah), Caatinga (Brazilian Desert) and Atlantic Forest were the predominant biomes.

Conclusions: Brazilian biomes support the pathogenic complex of JYF. Although epizooties can be used as good indicators for intervention/prevention opportunities, epidemic patterns also occur in anthropized landscapes, so, dispersion prediction is not possible.

17.005 Rabies: Surveillance, Diagnosis, Eradication in Austria

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This report gives a graphic account of the ongoing and successful program to control and eliminate rabies that the National Reference Laboratory at the AGES, Institute for Veterinary Disease Control in Moedling in collaboration with the BMGF (Federal Ministry for Health and Women) has performed.

Control of rabies transmission from terrestrial animals to other terrestrial animals and humans is now a successful procedure for rabies control. The results of the last ten years surveillance of rabies in wildlife and domestic animals in Austria are presented. For this purpose the use of different diagnostic tools in ante mortem and post mortem diagnosis of rabies in animals as well as humans such as Fluorescence Antibody Test (FAT), Immunohistochemistry (IHC), Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Virus Isolation in Cell Culture has been necessary.

This report gives also an overview of the eradication as well as prevention measurements, their efficiency and side effects, and the tools to check up the efficiency of these measurements in terms of Fluorescence Antibody Virus Neutralisation Test (FAVNT), tetracycline detection in jaws, Enzyme Linked Immunosorbent Assay (ELISA) and FAT. Finally, the costs spent for surveillance, diagnosis, and eradication measurements per year are presented.

17.006 Interagency Surveillance for Highly Pathogenic Avian Influenza in Wild Birds in Utah, USA

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Background: Concerns over the potential introduction of Highly Pathogenic Avian Influenza (HPAI) H5N1 into the United States via migrating wild birds prompted the development and implementation of an early detection system for HPAI in wild birds. Implementation of this plan was undertaken by the US Department of Agriculture (USDA), Department of Interior, and state wildlife agencies. The plan utilizes veterinary diagnostic laboratories within the National Animal Health Laboratory Network (NAHLN) as screening facilities and the National Veterinary Services Laboratories (NVSL) for confirmatory testing. In Utah, biologists from USDA Wildlife Services and the Utah Division of Wildlife Resource collected samples from wild birds for testing at the Utah Veterinary Diagnostic Laboratory (UVDL).

Methods: Sampling strategies included sampling reported morbidity/mortality events, live birds, hunter-killed birds and avian cases submitted to UVDL. Field samples, predominately cloacal swabs, were pooled according to collection date and site, species and sample type. Samples were analyzed with real-time RT-PCR detecting the matrix gene. Matrix positive pools were screened for H5 and H7 subtypes. All H5 or H7 presumptive positive samples were forwarded to NVSL for confirmatory testing; RT-PCR, virus isolation, nucleic acid sequencing and pathogenicity testing as warranted.

Results: The prevalence of Avian Influenza (AI) in pooled samples from targeted migratory species in Utah during the sampling period was 17%. H5 subtype was detected in 13% of AI positive pools. AI occurred most commonly in American Green-winged Teal, Northern Shoveler, Mallard and Northern Pintail. H7 subtype was not detected. AI was not detected in non-migratory species.

Conclusion: HPAI H5N1 was not detected in Utah in 2006. Continued surveillance for HPAI will provide epidemiological data regarding wild bird reservoirs of AI.

17.007 Surveillance of Rodent-Borne Diseases in Singapore

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The aim of this project is to survey the species of commensal rodents and insectivores in Singapore, as well as selected zoonotic pathogens and ectoparasites they carry. The four pathogens tested for are Seoul hantavirus (enzyme-linked immunosorbant assay), Leptospira spp. (Polymerase Chain Reaction (PCR)), Yersinia pestis (PCR), and Rickettsia typhi (indirect immunofluorescence assay).

To date, more than 1100 rodents from different areas and types of premises around Singapore have been caught and sampled for ectoparasites and tissues. Of the 401 animals analyzed, 79% are Rattus norvegicus (Sewer rat), 13% Rattus rattus diardii (Malayan black rat), 0.5% Rattus tiomanicus (Tioman rat) and 7% Suncus murinus (Asian house shrew). Among the animals tested, 55% (253/464) were sero-positive for Seoul hantavirus, 30% (45/152) for R. typhi and 17% (19/110) were PCR-positive for pathogenic Leptospira. No animals tested positive for Y. pestis.

Preliminary results show that the sero-prevalence for hantaviruses among R. norvegicus and R. rattus diardii is similar at about 50%, but prevalence in S. murinus is only 14%. Only R. norvegicus was found to carry Leptospira. For R. typhi, R. norvegicus was three times as likely to carry the bacterium compared to R. rattus diardii. S. murinus was found to carry neither Leptospira nor R. typhi. Too few animals belonging to R. tiomanicus have been tested to draw any conclusions for any of the diseases.

As for ectoparasites, Xenopsylla cheopis (rat flea) was found on 23% of the R. norvegicus, while only on 12% of R. rattus diardii and 5% of the S. murinus. Laelaps echidnina (Spiny rat mite) was carried on 39% of R. norvegicus, 35% of R. rattus diardii and 5% of the S. murinus. Co-infection with both parasites was found on 4% of R. norvegicus and 12% of R. rattus diardii.

The sero-positivity of rodents towards hantaviruses seems to have increased in comparison to last published data in 1989 in Singapore, where 26% of commensal rodents were sero-positive. It is however, similar to levels recently reported for Jakarta, Indonesia. Since Leptospira and Rickettsia typhi in rodents have previously not been surveyed in Singapore, no comparison can be made.

Further serological and genetic testing will be performed to confer a more accurate and wholesome scenario of the prevalence of the pathogens in the rodents and insectivores in Singapore.

17.008 The Ecological Surveillance of West Nile Virus Circulation in Romania (2001–2006)

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Background: The circulation of West Nile flavivirus (WNV) takes place in cycles between mosquitoes and birds as main hosts, and the mammals including humans as tangential hosts. The surveillance of WNV circulation has two main directions: confirmation of human cases and the ecological surveillance including the investigations on domestic and wild birds, horses and mosquitoes.

Methods: Sera samples from domestic and wild birds and from horses have been screened using ELISA adequate procedures for the capture of specific IgG antibodies against WNV. Batches of 40–50 identified mosquito females from natural and human habitats have been inoculated intra-cerebrally on suckling mice for the isolation of the virus.

Results: Seroprevalence of the antibodies against WNV in 5060 domestic birds from 112 rural and urban localities of 15 districts and Bucharest had a mean value of 8.5 % (0.0 - 35.0 %), and in 1696 wild birds from 4 districts and Bucharest 17.7 % (12.8 - 38.1 %). Seroprevalence in 5278 horses in 105 localities from 5 districts and Bucharest had a mean general value of 17.7 %, and its variation was between 9.0 % in Teleorman district and 32.1 % in Tulcea district (where the virus is introduced by migrating birds in the Danube Delta). The WNV potential vectors dominated among 23 identified species on 52456 mosquitoes collected in 33 localities of 14 districts. Two WNV strains were isolated from Culex pipiens females collected in August 2002 in a focus of blocks of flats in Bucharest.

Conclusion: It has demonstrated the intensive and permanent enzootic circulation of West Nile virus on extended territory in Romania. The isolation of WNV from mosquitoes collected in Bucharest confirms the presence of the virus even in the anthropic ecosystems and the possibility of transmission to humans. The permanent surveillance of WNV circulation and the implementation of integrated mosquito control programmes in the key areas at risk in Romania are adequate decisions for public health.

Work supported by FP6 EDEN project (Contract No. 010284/2004) and VIASAN Research Programme of the Ministry of Research, Romania (Contract No. 167 / 2002).

17.009 Serological Evidence of West Nile Virus in Wild Living Passerine Birds in Slovenia

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Background: During the migration, wild living passerine birds stop-over in Slovenia. The aim of presented study was to investigate the presence of specific antibodies against West Nile virus (WNV) in sera of different species of wild living passerine birds.

Methods: A total of 351 wild living passerines were captured into mist nets during their migration in Slovenia. One hundred eighty six birds were caught at ornithological station Vrhnika (x = 91582.1 y = 445909.6) in autumn 2004 (13th to 18th of September) and 165 birds at ornithological station at Cerknisko jezero (x = 70012.4 y = 454067.8) in autumn 2005 (23rd to 26th of September), respectively. The birds were clinically examined and ringed. The birds were released after collecting 0,05 ml of the blood by puncturing the jugular vein. The blood collected in microtainer® (Becton Dickinson and Co, Franklin, Lakes, NY USA) tubes was centrifuged and sera were stored at -20μC prior the testing. The bird's sera were serologically investigated for presence of specific antibodies against West Nile virus. For the detection of specific IgG antibodies against WNV indirect imunoflorescence test (IIF) was used. The commercial goat anti-wild bird fluorescein isothiocyanate (FITC) conjugated antibodies (Benthyl Laboratories, Inc., Montgomery) were used in the test.

Results: A total of 351 blood samples were taken from 33 different species of wild living migratory passerine birds. All sampled migratory passerine birds were clinically healthy. Sampling procedures did not have any negative effect on health of the birds.

The presence of specific antibodies was detected in 3 of 186 (1.6%) passerine birds sampled in 2004 and in 7 of 165 (4.2%) passerine birds sampled in 2005, respectively. A total of 10 of 351 (2.8%) sampled birds were positive in two years period (2004 and 2005.The results are presented in Table 1.

Table 1: Passerine birds sampled and tested in years 2004 and 2005

Passerine birds sampled		Positive / Total passerine birds sampled	
		2004	2005
Tree Pipit	(*Anthus trivialis*)	–	0/1
Meadow Pipit	(*Anthus pratensis*)	–	0/1
Robin	(*Erithacus rubecula*)	0/25	0/9
Thrush Nightingale	(*Luscinia luscinia*)	0/1	–
Rufous Nightingale	(*Luscinia megarhynchos*)	–	0/1
Bluethroat	(*Luscinia svecica*)	0/1	–
Common Redstart	(*Phoenicurus phoenicurus*)	0/1	0/1
Whinchat	(*Saxicola rubetra*)	–	0/1
Blackbird	(*Turdus merula*)	0/2	0/3
Song Thrush	(*Turdus philomelos*)	–	0/1
Grasshopper Warbler	(*Locustella naevia*)	0/2	0/1
Sedge Warbler	(*Acrocephalus schoenobaenus*)	0/10	0/1
Marsh Warbler	(*Acrocephalus palustris*)	0/1	–
Reed Warbler	(*Acrocephalus scirpaceus*)	0/11	0/7
Icterine Warbler	(*Hippolais icterina*)	0/1	–
Barred Warbler	(*Sylvia nisoria*)	–	0/1
Lesser Whitethroat	(*Sylvia curruca*)	1/4	2/2
Common Whitethroat	(*Sylvia communis*)	0/3	2/4
Garden Warbler	(*Sylvia borin*)	1/27	1/18
Blackcap	(*Sylvia atricapilla*)	1/75	0/74
Chiffchaff	(*Phylloscopus collybita*)	0/1	–
Willow Warbler	(*Phylloscopus trochilus*)	0/1	–
Spotted Flycatcher	(*Muscicapa striata*)	0/1	–
Pied Flycatcher	(*Ficedula hypoleuca*)	0/2	–
Coal Tit	(*Parus ater*)	–	0/2
Blue Tit	(*Parus caeruleus*)	0/1	–
Great Tit	(*Parus major*)	0/1	0/3
Red-backed Shrike	(*Lanius collurio*)	0/1	–
Greenfinch	(*Carduelis chloris*)	–	0/9
Goldfinch	(*Carduelis carduelis*)	–	2/13
Siskin	(*Carduelis spinus*)	0/14	–
Yellowhammer	(*Emberiza citrinella*)	–	0/7
Reed Bunting	(*Emberiza schoeniclus*)	–	0/5
	33 species	**3/186**	**7/165**
TOTAL	**5/33 species positive**	**10/351**	

Conclusion: His serological investigation for WNV is the first of this kind in our country. On the basis of the collected data in this study we conclude that WNV was circulating in passerine birds captured during their migration in Slovenia. However, further systematic research is needed to illuminate the role of mosquitoes, other species of animals (horses, backyard flock birds, resident free living birds), and humans in the ecology of WNV in Slovenia.

17.010 Relationships Between Serological Diagnosis of Leptospira interrogans Serovar Bratislava in Ewes Population and Regional Factors in Mexico

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Serological data combining from a cross-sectional study were used to establish the prevalence of antibodies to Leptospira interrogans serovar bratislava infection in flocks and ewes, and the relationships between seroprevalence and climatic, environmental, and flock management in a representative area of ovine production in State of Mexico in Mexico. Also, the status of Chlamydophila abortus (Cp. abortus), Brucella ovis (B. ovis) and smooth-type of Brucella seropositivity was investigated. Sera

were collected using a stratified random sampling from 367 ewes in proportion to the ovine population in different flocks. One ewe in five from 9 flocks (in flocksize < 50 animals), one in eight from 16 flocks (flocksize 51-140 animals), and one in seventeen from 8 flocks (flocksize > 141 animals) were sampled. One hundred per cent of all flocks (one or more animals with titer- 1:100 using the microscopic agglutination test) showed evidence of leptospiral infection, 80% seropositive to Cp. abortus, and 9.5% L. bratislava-Chlamydophila abortus dual reactivity. Within-flock seroprevalence was L. bratislava 39%, and Cp. abortus 21.5%, B. ovis 3.3% and smooth-type of Brucella 7.4%. The seroprevalence of L. bratislava, Cp. abortus, smooth-type of Brucella, and Brucella ovis infection was significantly higher in ewes from valley than in other regions. General associations between regions and flock-level occurrences of Leptospira interrogans serovar bratislava revealed that seropositive-ewes from valley region were more likely than seronegatives have to be animals destined for meat-production (multivariate odds ratio [MOR], 10.7; 95% confidence interval [CI], 1.3-91.6), replacement policy from rural livestock market (MOR, 2.9; 95% CI, 1.2-7.5), cleaning of farmyard during ewes parturition labor (MOR, 3.1; 95% CI, 1.1-8.7), smooth-type of Brucella dual reactivity (MOR, 4.7; 95% CI, 1.6-13.9), and water supply from watering place (MOR, 1.3; 95% CI, 0.6-2.9). In addition, potential risk factors among ewes with abortion history from valley region were determined.

SESSION 18 (Poster Session)

Balancing Science, Surveillance and Society: Who needs to know What and When?

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

18.001 Clustering of Deaths During Influenza Vaccination Campaigns. The Role of Real-Time Monitoring of Mortality Data for Event Detection and Management

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Background: At the start of the influenza vaccination campaign in Israel in 2006, three deaths were reported from a single community within a day to a week following vaccination. The vaccination campaign was temporarily halted pending the outcome of an epidemiological investigation. It was found that all three cases were chronically ill and there was no evidence linking the vaccine to the deaths. Within several days, the vaccination campaign resumed. Nevertheless, interruption of the campaign created uncertainty which could impact on compliance with the vaccination. The aim of this study was to determine the role of real-time monitoring of mortality data in both the identification of such incidents and support for decision-making in the event that such clusters are reported.

Methods: We carried out simulations on a database of all deaths occurring in 2002 in the Tel Aviv District, which has a population of about 1.2 million. The data includes age, address and date of death. Prospective spatial analysis using SaTScan was used to detect unusual clusters of deaths both in time and place between October and December, coinciding with the period of influenza vaccination campaigns.

Results: On any particular day, we identified an average of up to ten possible active clusters, varying in size and place. Most were statistically non-significant. Since this could be due to low statistical power for small clusters, even non-statistically significant clusters may require evaluation.

Conclusions: Mortality clusters during the period of influenza vaccination campaigns can occur quite frequently. The question of which clusters require an epidemiological investigation and whether this can be a method for early detection of adverse events, remains moot. However, the use of real-time monitoring of disease and mortality together with the appropriate analyses can provide an extremely useful tool for decision-making in the event of a report of an unusual cluster during vaccination campaigns. This could be particularly important in mass vaccination or treatment campaigns following a bioterrorist attack.

18.002 A Decision Support Tool to Aid Prioritisation of Government Resources for Animal Health

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Background: New diseases and incursions of known diseases led to implementation of a strategy to enhance veterinary surveillance. A key goal was to design a prioritisation process to provide an accessible evidence base to guide animal health policy. This would define the risk and impact of different diseases (or issues), to ensure finite resources for animal health are targeted at those of most importance according to the GB Animal Health and Welfare Strategy (AHWS).

Methods: Groups of stakeholders with a wide range of perspectives were established; members attended workshops and agreed the key evidence that should inform priorities. This was assembled in 'profiles', peer reviewed, and published. Further workshops defined the key characteristics required of the analysis tool. A prototype was built in Microsoft Excel.

Results: Key characteristics identified were (i) output must be based on each disease's 'risk' and 'impact' (ii) outputs must be simple and transparent (no 'black box'), (iii) sufficient precision in the data to enable discrimination between issues, (iv) tool must be generic and (v) able to be populated and run rapidly. A score is derived for each disease from up to 10 criteria that quantify each of five features. These are the impact on each of four reasons for government intervention (RFI) defined in the GB AHWS: protect public health, protect animal welfare, protect wider society, support international trade. The fifth feature measures the 'risk and epidemiology'. A value is assigned to every option for each criterion, and each criterion has a relative weight. Values for criteria are aggregated to give a score for that disease's relative importance.

Conclusion: A prototype decision support tool has been constructed that uses standardised, peer reviewed evidence to assign a priority score to each disease, according to standardised criteria that determine how important it is with respect to the four RFIs, as specified in the GB AHWS, and according to its current level/risk of introduction. These scores enable RFI-specific prioritised lists of animal diseases or issues to be produced. Thus priorities can be identified from a 'level playing field' of evidence. The criteria and scoring system are simple enough to be understood when the Tool is explored by an intelligent layman.

18.003 Standardized Delphi Method for Prioritizing Infectious Diseases in Germany

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Introduction: Increasing budgetary constraints in the public health service warrant a prioritisation of activities for surveillance, research and health promotion. In order to assure rational allocation of resources the Robert Koch Institute (RKI), the central federal public health agency in Germany in charge of infectious diseases surveillance and control, developed a systematic procedure, with the objective to have a reproducible, standardized and transparent prioritization of pathogens that could be updated periodically and that would reduce the bias of individual expert preferences.

Methods: We initiated a delphi process in which a total of 11 epidemiologists and infectious disease specialists of 4 different disease specific and non-disease specific units of the department for infectious disease epidemiology at RKI were asked to individually rate every pathogen according to the individual assessment. Independently from this procedure each scientist was also asked to rank each of the 12 categories by

importance. In the database the computed values for each category were then multiplied by the weight and the weighted values were added to one overall priority value.

Results: The list of pathogens consisted in 85 pathogens of which 50 (59%) were potentially foodborne or zoonotic pathogens. The high priority group contained a total of 29 pathogens of which the following 10 (34%) were potentially foodborne or zoonotic pathogens (in decreasing order of priority): Campylobacter, Norovirus, Cryptosporidium, hepatitis A virus, Salmonella, pathogenic Escherichia coli, Yersinia pseudotuberculosis, Shigatoxin producing Escherichia coli, Borrelia burgdorferi, West Nile virus.

18.004 Human Health and Societal Benefits of Interventions in Animals: Towards Global Subsidiary Funding

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While the recent outbreaks have been contained with a concerted response in industrialized countries, in many developing and transition countries, the health and veterinary systems and public health surveillance are often not able to react adequately. The key for the control of zoonoses like rabies, echinococcosis, brucellosis is the focus on the animal reservoir. In this respect, ministries of health raise the issue if the public health sector really benefits from interventions in livestock. Transsectoral assessments of interventions like brucellosis mass vaccination in Mongolia or rabies vaccination of dogs in Chad consider both human and animal health sectors from a societal economic perspective. Combining the total societal benefits, the intervention in the animal sector saves cost, provides the economic argument and thus opens new approaches for the control of zoonoses in developing countries through cost contributions from multiple sectors. Approaches to global subsidiary funding is outlined.

SESSION 19 (Poster Session)
Vaccines against Emerging Diseases

Saturday, February 24, 2007
Room: Brahms/Mahler/Bruckner
11:15–12:30

19.001 Screening of Rubella among Pregnant Women in Tunisia: Necessity of Rubella Vaccination

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Background: The decision of a country to introduce rubella vaccination should be based on the level of susceptibility to rubella among women. The aim of this study is to determine the immune status to rubella and the incidence of the infection in pregnant women in our region.

Methods: We reviewed the rubella antibody test results of 8812 women attending antenatal clinics in Sousse region from January 2003 to September 2006. The districts around Sousse city have similar income, social level and medical service. Immunoglobulin G antibodies were tested for all women, Immunoglobulin M antibodies were tested for each woman with suspect acute infection or with significant variations in levels of IgG antibodies between two tests. Immunoglobulin G avidity, when done, was tested for positive IgM serology. These tests were performed using enzyme immunoassay.

Results: Overall, 15.89% of the pregnant women had no immunity to rubella; Immunoglobulin M was tested in 3.62% of women and was detected in 0.10% of them. Immunoglobulin G avidity was always high when tested.

Conclusion: Susceptibility to rubella infection among Tunisian pregnant women is higher than countries where vaccination program was introduced. The measurement of IgG avidity which can avoid aberrant abortions is not available in all laboratories. These findings indicate the necessity of rubella vaccination program in Tunisia. Given the impossibility of a very high coverage of vaccination program, a policy to immunize all children does not appear to be interesting. Screening and selective vaccination of non immune women in post-partum is recommended.

19.002 Immunogenicity and Adverse Events of the 3rd Generation Live-Attenuated Smallpox Vaccine LC16m8 in Adults

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Background: Vaccine is the most important preparedness tool against bioterrorism attack by smallpox. As the conventional 'first generation' calf-lymph vaccine used in the eradication era has a high rate of severe adverse events, the risk/benefit ratio in the pre-event vaccination is an issue of concern and a less-virulent vaccine strain has been highly expected. Although most of the stockpiles in the world are consisted of the first generation vaccine, only Japan has a stockpile consisting solely of a '3rd generation' tissue-cultured live attenuated vaccine LC16m8. LC16m8 developed in Japan has been shown its high immunogenicity and safety profiles by pre-clinical studies and vaccination program in children and is already licensed in Japan in 1970's. However, there were few reports of vaccination in adults.

Methods: In Japan, smallpox vaccination program for selected personnel was launched and ~8,000 subjects were vaccinated with LC16m8 in 2002–2006. Subjects were clinically followed up by questionnaires and physical examination on day 10–14 and day 28 after vaccination. Serum samples were evaluated by the plaque reduction neutralization test (PRNT) using LC16m8 and Dryvax for the challenge virus. Vaccinees with suspected symptoms of severe adverse events and who were severely sick were hospitalized and closely examined.

Results: The take rates among primary vaccinees and re-vaccinees were 94% and 87%, respectively. Five individuals were reported to be admitted to the hospital within 30 days after immunization and two of them with rash (allergic dermatitis, erythema multiforme) were suspected to be related to the vaccination. No other severe adverse events were reported throughout the vaccination program and VIG was not used for any vaccinee.

Conclusions: LC16m8 has shown its high immunogenicity and safety profiles in adults (n=~8,000) as well as in children (n=~100,000).

19.003 A ML29 Vaccine Candidate for Lassa Fever: Immunogenicity and Efficacy in Non-Human Primates

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Lassa fever (LF) is an endemic disease of West Africa which results in thousands of deaths each year. A ML29 vaccine candidate carrying mutated major structural proteins of Lassa virus (LASV) and RdRp and Z protein of non-pathogenic Mopeia virus (MOPV) is attenuated in guinea pigs and non-human primates. Vaccination with ML29 induced sterilizing immunity and completely protected guinea pigs against challenge with homologous and distantly-related LASV. Recently we developed a model of human LF in common marmosets (CM), Callithrix jacchus. Here we described results of immunogenicity and efficacy studies of ML29 in CM.

Immunogenicity studies included 18 animals which were subcutaneously inoculated with 1,000 or 1,000,000 PFU of ML29 and weekly necropsied over a one month period. 10 animals were allocated for challenge experiments. On day 30 after vaccination (1,000 PFU) animals were injected with a lethal dose of LASV-Josiah and observed during 33 days.

In non-vaccinated CM a single inoculation of LASV induced a fatal hepatitis with immunophenotypic alterations in liver, spleen, and lymph nodes resulting in reduction in overall numbers of CD20+, CD3+, and MHC-II antigen expression. In contrast, the ML29 immunization stimulated expression of HLA-DR,P,Q and recruitment of CD3+ cells to the hepatic parenchyma. Statistically significant elevation in numbers of CD3+ T lymphocytes and high HLA-DR expression was observed in peripheral blood. ML29-specific T cells were detected in PBMC and spleen of immunized CM by TNF-alpha ELISPOT.

All vaccinated animals survived after challenge and had no clinical manifestations of LF. Hematology and biochemical parameters in vaccinated animals were in normal ranges after challenge. LASV was not detectable in plasma samples at the end of the observation period. Immunization and challenge experiments in CM confirmed our previously results describing the high immunogenicity and efficacy of the ML29 reassortant vaccine.

19.004 Prevalence of Contraindications to Smallpox Vaccination in France in 2003

D. Bitar, D. Levy-Bruhl. InVS, Saint Maurice, France

Background: As part of the national preparedness plan against smallpox, we estimated the prevalence of contraindications to smallpox vaccination in the French adult population in 2003.

Method: A population based survey was carried out in 26 randomly selected medical centres run by the national health insurance system, which covers 80% of the population and regularly offers a free medical consultation to insurees. The first ten males and ten females presenting to the consultation in both the 18–49 years and ≥50 years age groups were selected. A standard anonymous questionnaire on health conditions representing a contraindication to smallpox vaccination among interviewed subjects and their household contacts was applied. Absolute contraindications (recent atopic dermatitis or severe immune disorders), relative (past history of atopic dermatitis or disorders of the central nervous system) and temporary contraindications (acute skin or eye infection) were studied. We also included cardiovascular risk factors.

Results: A total of 1064 persons were interviewed: 266 men and 269 women in the 18–49 years group, 263 men and 266 women in the ≥50 years group. The prevalence of all contraindications was 32% in subjects alone and 50% in subjects and their household contacts. It decreased by 1% after excluding the cardiovascular risk. Absolute contraindications were reported in 15% of subjects and 28% of subjects and their contacts. The prevalence of absolute and relative contraindications was lower in men than in women: respectively 20% vs. 30% in the 18-49 years group and 25% vs. 31% in the ≥50 years group. The most frequently reported diseases representing a contraindication were atopic dermatological conditions (48%), transitory skin or eye infections (25%) and immune deficiencies (15%).

Conclusions: A similar survey performed among children aged 1–15 years yielded comparable prevalence results. Both surveys provided useful data to refine current modelling exercises and the national smallpox vaccination strategies. Although our findings showed that in a pre-event stage one-half of the population would be exempted from vaccination, the levels of contraindications would be reconsidered in the hypothesis of an immediate threat.

19.005 Response to Hepatitis B Vaccine Among Health Care Workers with Isolated Antibody to Hepatitis B Core Antigen

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Background: The presence of isolated anti-HBc may be associated with either 1) a window phase between the appearance of anti-HBs after acute hepatitis B virus (HBV) infection, 2) remote HBV infection with loss of measurable anti-HBs, 3) occult HBV infection with undetectable HBsAg or 4) non specific, cross reacting antibody. The purpose of this study was to examine the serologic response to HB vaccination in health care workers (HCWs) with isolated anti-HBc.

Methods: The single dose of recombinant hepatitis B vaccine (20 ug of Euvax-B) was injected intramuscularly into the deltoid of HCWs at Prasat Neurological Institute. The HCWs were classified as isolated anti-HBc group and seronegative (for HBsAg, anti-HBc and anti-HBs) control group. One month post vaccination HBsAg, anti-HBs, and anti-HBc were measured by Microparticle Enzyme Immunoassay (MEIA) methods. The postvaccination anti-HBs levels and geometric (GMT) were compared between two groups.

Results: Thirty-nine HCWs with isolated anti-HBc (12 male and 27 female, mean age 47.2 years) and 20 with seronegative for HBV markers (3 male and 17 female mean age 40.4 years) were enrolled. The two groups were not significantly different in terms of sex, BMI, history of smoking and chronic disease.

The one month post vaccination, 24 (61.5%) of those isolated anti-HBc group had rising anti-HBs titer to ≥10 mIU/mL, 7 had anti-HBs <10, and 8 still had negative anti-HBs. In 4 cases who had detectable HBV DNA by PCR (occult HBV infection), all had postvaccination anti-HBs level <10.

For the postvaccination anti-HBs results in the control group, 15 (68.2%) were still seronegative, 4 had anti-HBs <10, only one had anti-HBs ≥10.

Comparing between isolated anti-HBc group and control group after age adjusted, there were statistically significant differences in GMT of postvaccination anti-HBs level (18.49±7.98 vs. 1.46±2.21, p≤0.001).

The 4 isolated anti-HBc group with detectable HBV DNA had significant different level of anti-HBs response when compared with 35 isolated anti-HBc with negative HBV DNA (GMT of change from baseline: 2.27±1.74 vs. 8.77±6.54, p=0.01).

Conclusions: The anti-HBs response of single dose of HB vaccine in HCWs with isolated anti-HBc was significantly higher than HCWs with seronegative. About 60% of the isolated anti-HBc group had anamnestic response to this single dose except for those who had occult HBV infection. A positive anti-HBs response provides evidence against chronic infection, and the vaccine might be used as one of the diagnostic tools of occult HBV infection.

19.006 Evaluation of Influenza Virus-Like Particles and Novasome Adjuvant as Candidate Vaccine for Avian Influenza

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The development of safe and effective vaccines for emerging avian influenza strains is a priority for pandemic preparedness. Adjuvants improve the efficacy of vaccines and may allow antigen sparing during a pandemic. We have previously shown that influenza virus-like particles (VLPs) comprised of HA, NA, and M1 proteins represent a candidate vaccine for H9N2 avian influenza (Pushko et al., Vaccine 23 (2005), 5751-9). In this study, we evaluated the H9N2 VLP vaccine and recombinant HA (rH9) vaccine in animal models. The H9N2 VLP vaccine protected mice and ferrets from challenge with A/Hong Kong/1073/99 (H9N2) virus. Novasome adjuvant significantly increased immunogenicity and protection. The Novasome adjuvant also improved the efficacy of the rH9 vaccine. The results have implications for the development of safe and effective vaccines for avian influenza viruses with pandemic potential.

19.007 Explicit Comparison of Smallpox Vaccines by PRNT Titer Requires Standardization of PRNT Methods

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Background: The potential threat of smallpox as a bioweapon has led to the stockpiling of current smallpox vaccine and development of new generation smallpox vaccines. The efficacy of these vaccines need to be evaluated. The Plaque Reduction Neutralization Test (PRNT) is one of the methods to evaluate the efficacy of vaccines. However, there is no gold standard PRNT method for smallpox vaccines, and a vast amount of research is currently being conducted. The objective of this study was to develop an appropriate LISTER-specific PRNT method for the LC16m8 vaccinated individuals. In this study, we focused on the factors which are thought to influence the test results.

Methods: As the reference virus (RV),three kinds of LISTER virus cultured in different cell substrates and NYCBH were used. As the test articles, vaccinia immune serum was used. For the PRNT, RV was added to serial diluted serum samples for neutralizing reaction. The mixture was inoculated onto VeroE6 cell monolayer and incubated for plaque formation. The endpoint serum neutralizing antibody titer was defined as the reciprocal of the highest dilution of the serum with a mean plaque count less than or equal to the 50% plaque reduction cutoff value for the assay.

Results: The strain of RV, cell substrate used for RV preparation, neutralization period, incubation period and calculation method influenced the results of PRNT. In particular, for the RV, it became apparent that even with the same strain, the different cell substrates used for virus preparation affected the test results. In addition, it was also confirmed that there is variation in the characteristics of RV.

Conclusions: It is important that a gold standard PRNT method is established for the evaluation of smallpox vaccines. For the LC16m8 vaccinated individuals, we were able to establish an appropriate PRNT method with the LISTER strain, which was used during the smallpox eradication. Considering the RV strain influenced the test results, I believe that similar research needs to be conducted on PRNT methods using other virus strains as the RV.

19.008 Efficacy and Safety Evaluation of Attenuated Smallpox Vaccine LC16m8

H. Yokote[1], Y. Shinmura[1], C. Nagai[1], A. Satou[1], T. Kanehara[1], T. Sasaki[1], H. Matsui[1], T. Terano[1], K. Ohkuma[1], T. Oka[1], A. Funatsu[1], M. Saijo[2], S. Morikawa[2], I. Kurane[2], T. Kurata[2], S. Hashizume[3]. [1]Kaketsuken, Kumamoto, Japan; [2]Natl. Inst. of Infectious Diseases, Tokyo, Japan; [3]Professor Emertitus, Univ. of Chiba, Chiba, Japan

Background: The attenuated smallpox vaccine LC16m8 is the sole licensed smallpox vaccine in Japan, attenuated from the parent strain Lister Elstree. A clinical evaluation was conducted on 90,000 infants during the initial development in Japan, where there were no encephalitis or other serious adverse events. In this study, we investigated the efficacy and safety of the KAKETSUKEN manufactured LC16m8 vaccine in animal models.

Methods: A mouse vaccinia-Western Reserve (WR) intranasal challenge study was conducted to evaluate the protective efficacy and investigate into the protection mechanisms of the LC16m8 vaccine. Safety evaluation of LC16m8, was conducted to compare to Lister Elstree strain in rabbit skin proliferation study, suckling mouse and monkey neurovirulence (NV) studies, SCID mouse intraperitoneal (IP) and scarification studies.

Results: In wild-type mice, LC16m8 showed a high protection against lethal challenge similar to Lister, regardless of the vaccination dose and immunizing period. In the CD4 and MHC class II-deficient mice, well protection was confirmed for LC16m8. In the LC16m8 immunized MHC class I- & II-deficient mice, a delay in the onset of symptoms caused by WR was confirmed.

In the rabbit skin proliferation study, 50% Erythema Dose (ErD_{50}) value for LC16m8 was over 10^3 higher than Lister. In the suckling mouse NV studies, there were significant differences between mean survival times for the LC16m8 and Lister groups. In the monkey NV study, 3 out of 6 Lister-inoculated monkeys died while no deaths or paralysis were observed in the LC16m8 group. In the SCID mouse IP and scarification studies, all mice that received Lister died while all mice that received LC16m8 survived during the 28 or 45 days observation.

Conclusion: The results from various animal studies show that the attenuated vaccine LC16m8 is significantly safer than the parent non-attenuated strain, Lister, and it can be suggested that LC16m8 retains the same protective efficacy as the Lister strain, which was used during the smallpox eradication campaign.

19.009 Cross-Subtype Immunity Against Avian Influenza in Humans Recently Vaccinated for the Influenza Season

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Background: Avian H5N1 influenza viruses could be transmitted to humans, resulting in severe or fatal disease. Aim of this study was to evaluate the immune cross-reactivity between human and avian (H5N1) influenza strains in healthy donors vaccinated for seasonal H1N1/H3N2 influenza A.

Methods: Health care workers wishing to receive seasonal influenza vaccination at the Spallanzani Institute were enrolled. Blood samples for the assessment of humoral and cell-mediated responses were obtained before and 30 days after vaccination. The frequency of circulating antigen-specific CD4 and/or CD8 T-cells in healthy donors enrolled in the study were analyzed by flow cytometry, using intracellular cytokine staining assay after the expansion of effector-cells in vitro. Human sera from the same donors were tested for their HA-inhibition activity against vaccine preparation and neutralization activity against H5N1 virus.

Results: Our data indicate that vaccination may boost cross-subtype cellular and/or humoral immunity against H5N1 influenza. Specifically, H5N1-specific CD4 T-cell frequency was significantly higher in pluri-vaccinated versus first flu-vaccinated donors. The main target for cross-reactivity between H5N1 and H3N2/H1N1 strains was N1. No correlation between influenza-specific CD4 T-cells and humoral response was observed, suggesting that this response was mainly CD4 T-cell-independent. Differently, CD4 T-cells may help for anti-influenza CD8 T-cell response. Furthermore, human sera from the same donors, tested for their HA-inhibition activity against vaccine preparation, showed a significant rise (73.7%) after vaccination. The same sera were tested for their H5N1 neutralization activity and the 34.2% of subjects was able to show anti-H5N1 antibody rise after seasonal influenza vaccination, showing the existence of an antibody-dependent cross-type immunity. No correlation between influenza specific CD4 T-cells and humoral responses were observed, suggesting that this type of antibody response was mainly CD4 T-cell independent.

Conclusion: In this study, we demonstrated that vaccination against seasonal influenza might induce both cellular and humoral cross-reactive immunity against H5N1 avian influenza. This cross-type immunity may represent an important component of the immune response against novel influenza A infections.

19.010 Development of a Live-attenuated, Chimeric Alphavirus-based Vaccine Against Venezuelan Equine Encephalitis Virus

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We evaluated the safety and immunogenicity of a chimeric alphavirus vaccine candidate in mice with selective immunodeficiencies. This vaccine candidate was highly attenuated in mice with deficiencies in the B and T cell compartments as well as in mice with deficient gamma-interferon responsiveness. However, different levels of protection were induced, depending upon the specific immunodeficiency. Wild type mice were protected against lethal challenge and cleared infectious virus from the CNS. In contrast to wild type immunocompetent mice, alpha/beta TCR-deficient mice developed lethal encephalitis upon challenge with VEEV, while mice deficient in gamma/delta T cells were protected from a lethal outcome. Surprisingly, a minority of vaccinated immunoglobulin heavy chain-deficient mice survived challenge, suggesting that neutralizing antibody is not absolutely required for protection. In the absence of gamma/delta T cells, previously immunized mice developed biphasic, non-lethal brain infection upon challenge with VEEV; in the acute phase (during 7 days of infection), viral loads were comparable to those of unvaccinated immunocompetent mice, which uniformly died. The clearance of infectious virus from the brain was interferon-gamma-independent. Together we provide comprehensive evidence that prolonged replication of encephalitic VEEV in the brain of pre-immunized mice is not lethal and that the presence of virus-neutralizing antibodies is beneficial but not required for survival and virus clearance from the brain. The observations described herein provide a framework in which to dissect potential intervention points and perhaps, to add prognostic markers that correlate with protection against encephalitis in vaccinated military and civilian human subjects.

19.011 Alphavirus-based Vaccines Against Rift Valley Fever Virus

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Rift Valley fever virus (RVFV) is a representative member of the Bunyaviridae family that is in continuous circulation in the livestock-raising regions of Africa. The introduction of sheep and cattle, which are not native to this continent, into intensively farmed areas, has resulted in the emergence of massive mosquito-borne epidemics. Death rates are very high for domestic animals. Moreover, the fatality rate for fetuses in pregnant livestock is 100%, and the fatality rate for newborn lambs is 90%. The virus has spread to new geographic areas, as evidenced by an extensive epidemic in Egypt in 1977. Most recently, RVFV caused a massive epidemic in sub-Saharan Africa in 1997-98 and spread across the Red Sea to Saudi Arabia and Yemen, causing devastating disease outbreaks in sheep and cattle. RVFV is also a significant human pathogen with an approximate 1% mortality. There is no treatment for humans or vaccine for the livestock that are such important amplifiers of the virus. Our goal is to create a new type of preventive recombinant vaccine against RVFV infection. This vaccine will combine the safety of inactivated and subunit vaccines and the efficacy of live attenuated vaccines and is based on Sindbis and Venezuelan equine encephalitis virus replicons and viruses expressing structural proteins of RVFV. We are developing i) the optimal strategy for expression of structural proteins of RVFV by recombinant Sindbis and VEE viruses, ii) the system for delivery of recombinant genomes into antigen-presenting cells and optimal presentation of the RVFV-specific antigens, and iii) the manufacturing procedure for the large-scale production of the recombinant viruses. Vaccine candidates that protect mice efficiently against the lethal RVFV infection will be presented.

19.012 Hepatitis A Epidemiology in Brazilian Fire Department

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In developping countries, the first contact with hepatitis A virus (HAV) occurs early in life. In recent years, the improvement on sanitation conditions in some developping areas resulted in an increase of proportion of the population that reaches adulthood without acquired immunity. In Brazil, although hepatitis A is considered an endemic disease, data published in recent investigations carried out in the State of Rio de Janeiro revealed a shift in the hepatitis A epidemiological pattern. Consequently studies must be conducted to define the seroprevalence of anti-HAV antibodies in diferent populations and a programme to vaccinate non-immune people must be created.

Aim: To find out the seroprevalence of anti-HAV antibodies in a population professionally exposed to water contact (flooding, sea and fresh water) living in Rio de Janeiro, Brazil. A secondary objective was to evaluate the cost-benefit of performing serological screening prior to vaccination.

Methods: Participants were military fireman from the State of Rio de Janeiro, that filled in a socioeconomic questionnaire. Blood specimens were obtained for anti-HAV serological test (ELISA). The results were annalysed using t-student test and 2 test, as well as a logistic regression.

Results: Two hundred and seventy eight participants were enrolled in the study. The median age of the population was 38 years and the overall seroprevalence of anti-HAV was 71.58%. The distribution of positive tests according to age groups were 15.07% in < 30 years, 47.73% in 30-39 years and 37.68% in > 40 years.

Conclusion: Young adults from Rio de Janeiro may have a low level of immunity against HAV. Professional exposition. It is cost-benefit to vaccinate with no serological screening fireman younger than 30 years old.

19.013 Design of Dengue Virus Vaccines: Analysis of the Impact of Deletion Mutations on RNA Secondary Structure

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The four serotypes of mosquito-borne dengue virus (DENV; genus Flavivirus) are the aetiological agents of dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. This positive-strand RNA virus has emerged over the past five decades, resulting in a global pandemic, and a vaccine is urgently needed. One approach to vaccine design has utilized a 30 nucleotide (nt) deletion (delta30; nt's 10477-10507 in rDEN4) in the 3' untranslated region (UTR) (Blaney, J. E., Jr., Durbin, A. P., Murphy, B. R., Whitehead, S. S. 2006. Development of a live attenuated dengue virus vaccine using reverse genetics. Viral Immunology 19, 10-32), resulting in two monovalent vaccine candidates, rDEN4delta30 and rDEN1delta30, currently in clinical trials. Puzzlingly, previous research has demonstrated that insertion of delta30 in DENV-3 does not result in attenuation. Function of the 3' UTR depends upon both primary sequence and secondary structure, thus one hypothesis for the observed variation in the phenotypic effect of the delta30 mutation is that its impact on the structure of the 3' UTR differs between DENV-3 and DENV-4 and 1. In this study, we have generated Mfold predictions of the 3' UTR of rDEN4 and rDEN3, as well as rDEN4delta30 and rDEN3delta30. Moreover, we have assessed the congruence of these predictions with data from nuclease maps of this region. The structure of the 3' UTR differs substantially between rDEN4 and rDEN3. Nuclease mapping revealed more regions of dynamically-folding structure, suggestive of structural complexity, in rDEN3 than rDEN4. The delta30 mutation substantially distorts secondary structure in both serotypes by reducing stem-loop structure. To generate additional vaccine candidates for DENV-3, more extensive deletions have been engineered into the 3' UTR (rDEN3delta31/30 and rDEN3delta86, respectively) and the effect of these mutations has also been analyzed. These mutations decrease the complexity of secondary structure, suggesting potential phenotypic effects.

SESSION 20 (Plenary Session)
Polio Eradication

Saturday, February 24, 2007
Room: Park Congress
13:30–14:15

20.001 Polio Eradication: Finishing the Job, Guaranteeing the Investment

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The polio eradication initiative is in its final phase, with intensified vaccination activities using oral polio vaccine (OPV) in all countries where polio transmission has not yet been interrupted, and in those countries that were once polio-free and have recently become re-infected. New tools have been developed and are in use to ensure interruption of the last chains of human to human polio transmission, including a monovalent type 1 oral polio vaccine (mOPV1), and surveillance is being intensified in all countries that are endemic or that have become re-infected during the past two years.

Once eradication of wild poliovirus has been confirmed, the public health benefits of routine immunization with OPV will no longer outweigh the burden of disease either due to paralysis caused by OPV (Vaccine Associated Paralytic Polio), or by outbreaks caused by circulating vaccine-derived polioviruses. The eventual cessation of OPV use in routine immunization programmes worldwide will become necessary to assure a lasting eradication of polio.

As the world moves towards polio eradication and its certification, preparations are therefore being intensified for OPV cessation, and the pre-requisites for safe OPV cessation are being put in place. Pre-requisites include destruction or bio-containment of all known poliovirus and potentially infected substances, development of an international stockpile of oral polio vaccine, ensuring a mechanism for continued global surveillance of polio after eradication has been certified, and national policies if countries decide to continue vaccinating with inactivated polio vaccine (IPV).

It is ironic that the vaccine on which the world has depended for polio eradication will itself become a risk to eradication once the transmission of wild poliovirus has been interrupted. Final preparations for the eventual global and simultaneous cessation of OPV will require the same level of international cooperation and coordination that has brought the world to the verge of polio eradication.

SESSION 21 (Parallel Session)
Late-Breaking Emerging Disease News: Selected Oral Abstract Presentations

Saturday, February 24, 2007
Room: Park Congress
14:30–16:00

21.001 Emergence of Epizootic Hemorrhagic Disease Virus (EHDV) in Cattle in Jordan and Israel

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Background: Israel and Jordan share a long border trailing along the Rift Valley (RV). Current conditions enable the movement of many potential arthropod vectors and domestic and wildlife hosts of arbo-borne diseases between Africa, the Arab peninsula, the Middle East and Europe. Since 1999, repeated outbreaks of Bovine Ephemeral Fever devastated the Israeli cattle industry causing enormous financial losses. In September 2006 a huge outbreak, took place, following a similar pattern of spread but with different clinical signs, affecting cattle in Jordan and over a 100 Israeli beef and dairy cattle herds.

Methods: In a joint effort lead by veterinarians and epidemiologists from both sides of the border, a special outbreak surveillance network was expeditiously established in the two countries. Whole blood and serum samples were collected from clinical cases and normal animals in all affected herds and a sample of non-affected cattle, sheep and goat herds. In Israel, data was collected daily by field veterinarians and stockholders to identify clinical cases and disease outbreaks.

Results: The disease was reported from the northern tip of the Dead sea up to the Lebanese border in 83 dairy and 22 beef herds, initially almost all of them along the Jordan river and the shores of the Sea of Galilee later expanding westward in the Israel valley towards the coastal plans. The morbidity rates varied from 90-100% in the RV to 1-5% in the coastal area. The main and most prominent clinical signs were a sharp reduction in milk production, anorexia, mild fever, lameness and hyperemia of the skin above the hooves. Duration of acute disease was 7 to 10 days and mortality was less than 1.5%. All sheep and goat farms in the outbreak region were free of disease signs.

Blood sampled from severely affected acute cases were tested by PCR and ELISA. All samples were negative by PCR for BTV while 4 whole blood samples were positive for EHDV and 20 serum samples were ELISA positive for EHDV.

Conclusion: An outbreak of EHDV occurred in Israel during September-November 2006 probably involving also Jordanian herds. Establishment of common Israeli Jordanian surveillance team has already started to collect data in order to unveil the questions posed by this sudden emergence of EHD in cattle. Future cross border cooperation will enable to understand the dynamics and epidemiology of these diseases in the region.

21.002 Leptospirosis in Urbanized Wild Boars, Berlin, Germany

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Background: Within the last decade, an increase in wild boar populations has been observed in urban areas of Berlin, with an estimated number of 5,000 animals in 2006. Wild boars are susceptible to Leptospira species pathogenic to humans, and may shed leptospires in urine. To assess the potential role of boars in the transmission of Leptospira to humans, we conducted a study among wild boars and municipal hunters in Berlin.

Methods: Sera collected from boars killed in urban areas of Berlin during fall and winter 2005/06, and from municipal hunters were screened with a reference panel of 12 Leptospira serovars using the microscopic agglutination test. For histological investigation, wild boar tissues were fixed in 10% formalin, embedded in paraffin, and stained according to standard protocols. Frequency of hunters contact to wild boars and use of gloves during evisceration were assessed by a standardized questionnaire. Univariate prevalence ratios (PR) and 95% confidence intervals (95%CI) were calculated.

Results: Antibodies against pathogenic leptospires were detected in 25 of 141 boar specimens (18%; 95%CI 12-25%). Leptospira interrogans serovar Pomona (24%) was the most frequent single serovar. Leptospires were detected in 3 of 10 (30%) specimens from seropositive wild boars by silver-staining. Among municipal hunters (n=84) no anti-

leptospiral antibodies were detected. Of hunters shooting >10 boars/year (n=61), 72% used gloves at least on occasion, compared to 35% of the hunters shooting <10 boars/year (n=17) (PR 2; 95%CI 1.2-3.4).

Conclusions: Our study describes a newly discovered urban focus of leptospirosis among wild boars in the city of Berlin. The high frequency of porcine Leptospira spp. serovar Pomona and the demonstration of leptospires in kidney specimens indicate that wild boars most likely act as a maintenance host of Leptospira in this urban area. In contrast to other published studies, no anti-leptospiral antibodies were found in municipal hunters, possibly due to regular glove use among highly exposed persons. However, wild boar sightings are increasingly reported from recreational public parks and outdoor swimming areas within Berlin. In these areas, recreational contact to freshwater or mud contaminated by infected boars may represent a risk for human leptospirosis.

21.003 MRSA Infection in the Equine Hospital of a Veterinary University

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Background: Already for decades MRSA are a serious problem of nosocomial infections in human medicine, which reaches a further dimension with the emergence of community MRSA. The most recent emergence of MRSA in companion animals and livestock bears a potential public threat with respect to further spread and transmission to humans. Here we report about an investigation on the emergence of MRSA infections in a veterinary university hospital.

Methods: Samples where taken specifically from equine patients with wound infections (nose, wounds), and sporadically from the nose of other equine patients. Additionally nasal swabs were taken from veterinary staff. Specimens were processed by use of selective agar medium for MRSA screening (Chromagar MRSA). Furthermore the isolates were subjected to molecular typing.

Results: The frequency of S.aureus among clinical bacterial isolates (1700 routine samples) was 35%, about 2,5 % of them were MRSA.The emergence of MRSA started in 2003 with a cluster of infections in surgery (n=11) followed by infections in orthopaedics (n=6) and in internal medicine (n=2). Typing revealed spread of one particular clone within and between these departments (spa-sequence type t0036, MLST254).After implementation of infection control measures there were only sporadic infections in surgery and in orthopaedics in 2004 (n=3), 2005 (n=2), and 2006 (n=6). Nasal colonization in horses as a potential reservoir for spreading was found in part in animals with infections but rarely (1/63) in other horses being present at the same time. Among veterinary staff 4/ 98 persons were identified as carriers (repeatedly demonstration of MRSA t036); decolonization by use of mupirocin ointment was successful. Cases of MRSA infections in veterinary staff have not been recorded.

Conclusion: MRSA can establish in a veterinary hospital, and colonization of staff is possible. As asymptomatic colonization of horses seems to be rare attention should be focussed on animals posing symptoms of infections with subsequent proper bacteriological diagnostics and appropriate treatment. Further dissemination can be prevented by targeted measures of hygiene including hand disinfection, barrier control nursing of affected animals. Regular screening of staff for nasal colonization and consequent eradication of MRSA carriage is important for prevention of both: transmission among animals, and further spread to humans.

21.004 Several Pathogens Including a New Bacillus anthracis Subgroup Were Identified from Dead Great Apes from Western and Central Africa

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Hunting and consumption of bushmeat has the potential to expose humans to a wide range of deadly pathogens, and increasing evidence suggests that viral pathogens such as STLV-1, SIV and Ebola move readily between primate species including humans. Very little information is available yet on the prevalence of pathogens in non-human primates living in their natural habitats. For a behavior research project of the Max-Planck-Institute for evolutionary anthropology over the last 20 years in the Taï National Park in Côte d'Ivoire 3 groups of free-living chimpanzees have been habituated to the presence of human beings. The groups are under permanent human observation and all animals in these groups are known individually. In a unique interdisciplinary cooperation between the Max-Planck-Institute and the Robert Koch-Institut tissue samples from dead great apes have been collected to elucidate a number of unexplained deaths.

Using urine samples, we determined an STLV-1 antibody prevalence of 70% in adult chimpanzees. With combined pathological, cytological and molecular investigations and behavior research data we identified interspecies transmission of the virus from prey monkeys to chimpanzees. We could also attribute a number of death cases to different pathogens, showing that infectious diseases have joined habitat loss and hunting as threats to the survival of the remaining wild populations of great apes. From several animals that had died from respiratory disease we could detect new strains of Str. pneumoniae. We also identified three different anthrax outbreaks responsible for the sudden death of nine wild chimpanzees in 2001/2002. This was the first description of anthrax in wild non-human primates living in a tropical rainforest, a habitat not previously known to harbor B. anthracis. In 2004/2005 we also identified B. anthracis from three chimpanzees and one gorilla found dead more than a thousand miles to the east, at the Dja Reserve, Cameroon. Viable bacteria were recovered from most tissues, and molecular and microbiological analyses showed that the B. anthracis isolates termed B. anthracis CI and CA were closely related and clearly differed from any previously described strains, forming a new subgroup well separated from B. anthracis subgroups A and B. They also showed atypical gene regulation and displayed some unusual microbiological features suggesting that B. anthracis is a species far less homogeneous than currently believed.

21.005 Emerging Chikungunya Virus in La Reunion Island 2006

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Background: Chikungunya virus emerged in the Indian Ocean at the beginning of 2005, with cases first reported from Comoros. La Reunion, a French overseas territory (pop 770 000) has been highly affected.

Methods: A mathematical model based on historical series collected by the sentinel network was developed. It provided an estimation of the cumulative number of cases with clinical signs compatible with acute chikungunya fever since the beginning of this outbreak (whether or not patients sought medical consultation). The surveillance system also included data on severe clinical presentations in patients admitted to hospital intensive care units. In addition, since the beginning of the outbreak, mortality certificates were compiled when chikungunya was mentioned as one of the diagnosis.

Results: For the period March 2005–July 2006, 266.000 cases have been estimated by the surveillance system, leading to an estimated attack rate of 35%. After a first peak of 450 cases weekly in May 2005, transmission decreased during the austral winter, before increasing again, and reaching a peak of 45 000 cases during the second week of February 2006. For the first time, neonatal transmissions (N=44) and severe chikungunya diseases (N=247) including meningitis, renal, cardiac and liver failure have been described. More than 250 death certificates mentioning 'chikungunya' as a diagnosis were notified. Crude

mortality rate in la Reunion was significantly higher during the period of high chikungunya transmission compared to previous years. Between January and September 2006, more than 800 chikungunya cases have been diagnosed in metropolitan France, most of them imported from the Indian Ocean.

Conclusion: As Aedes albopictus has been identified in France and some other European countries, the potential risk of introduction of chikungunya has to be evaluated. This leads to an urgent need for global surveillance and articulation between public health and research.

21.006 Epidemiology and Laboratory Diagnosed Cases of Crimean-Congo Haemorrhagic Fever in Southern Africa

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The widespread distribution of Crimean-Congo haemorrhagic fever (CCHF) virus and its ability to produce severe human disease with high mortality rates make this virus an important human pathogen and a worldwide public health concern.

Nucleotide sequences for southern Africa isolates plus sequnce data from other locations retrived from GenBank were analysed. Despite the potential which exists for dispersal of the virus between Africa and Eurasia, it appears that circulation of the virus is largely compartmentalized within the two land masses and its evolution is associated with particular tick vector species within specific geographic regions.

There had been 27% fatalities among the 180 cases of CCHF recorded in southern Africa from February 1981 up to the end of March 2006. The largest group of 80 (44%) cases arose from known tick bite or the squashing of ticks; 72 (40%) cases arose from known or potential contact with fresh blood or other tissues of livestock and/or ticks; 7 (4%) nosocomial infections arose from contact with blood or fomities of known CCHF patients. In 21 (12%) instances there was no direct evidence of contact with livestock or ticks, but the patients lived in or visited a rural environment where such contact was posible.

21.007 A Simple and Reliable Method to Real-Time Prediction of Influenza Outbreaks

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Background: In the present context of influenza pandemics threat, the availability of methods of real-time detection of outbreaks is crucial. A large body of work has been devoted to these methods, which define the outbreak as an increase above a historical baseline threshold, or use composite indexes in more complex approaches. Other developments also offer the ability to forecast future incidence. The historical baseline threshold is based on influenza-like illness (ILI) reporting and the characteristics of previous influenza epidemics. One of the most widely used methods, developed by Serfling, which used regression analysis to derive epidemic thresholds from weekly pneumonia and influenza deaths. This work presents a 'quick and dirty' method able to forecast the onset of the influenza outbreak with high reliability.

Methods: Twenty two years data of ILI incidence were obtained from the French "Sentinelles" network, which comprises voluntary sentinel general practitioners who update a web-accessible database with information on communicable diseases including ILI. The method uses a very simple estimator for outbreak detection: a relative variation of the influenza weekly incidence which is compared to a constant threshold value. Historical data are necessary only for optimize the threshold, but not for the detection itself.

Results: We explore different values for the constant threshold in order to find the optimal one in terms of predictive values. When compared to the Serfling based outbreak detection, the best performance of our method showed a specificity of 0.99 and a sensibility of 0.86 for a temporal window of three weeks.

Conclusion: Our work presents a real-time approach to predict the onset of influenza outbreaks up to 3 weeks ahead. Results are in good agreement with the observed predictions based on Serfling method from the French surveillance system. Implementation of this method using other national surveillance data will give us additional insight in its reliability and larger applicability.

21.008 Forecasting Sporadic Epidemics with a State-Space Framework: Applications for the Management of Marburg and Ebola Hemorrhagic Fever Outbreaks

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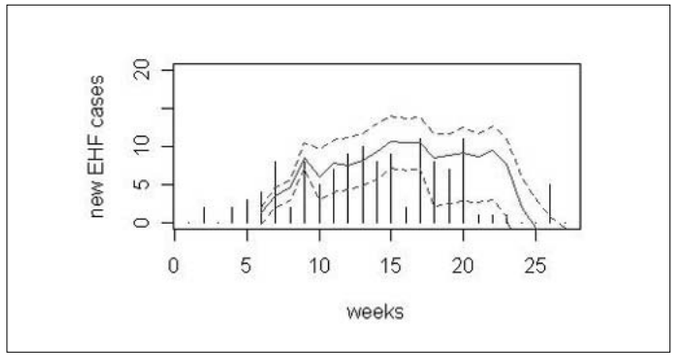
Hemorrhagic fever re-emergence (e.g., Ebola) among human populations is difficult, and often impossible, to predict, i.e., these outbreaks are: 1) typically short-lived; 2) spatially sparse and temporally non-stationary; as well as, 3) highly stochastic. Once these outbreaks commence, affected communities react vigorously while organizations rapidly mobilize to bring the outbreak under control and hence, immediately imparting changes on the outbreak evolution. Ebola and Marburg hemorrhagic fever outbreaks are so alarming that records may become available weekly, prompting interventions with similar windows. Thus, a flexible forecasting method is de rigueur to assist hazard mitigation during ongoing outbreaks. The authors employ a state-space framework with a single innovation source (which trains, conditioned on the first 3 observations, online) to retrospectively produce 2- and 3-week horizon forecasts, as well as (ordinary) bootstrap-based prediction intervals (PI), for the Ebola hemorrhagic fever (EHF) outbreak bordering the Republic of Congo & Gabon (October 2001). The results for the 2-week horizon forecasts are depicted in Fig. 1. The median absolute percentage error



(MdAPE) for 2-week horizon forecasts is 21%. Longer horizon forecasts perform comparably, albeit with larger MdAPE values. The automated forecasting (stochastic) framework employed herein neither relies on deterministic transmission models nor requires information other than the current outbreak record to produce reasonably accurate forecasts. Therefore, it may be implemented easily with minimum personnel training to assist, as well as to assess the impact of, current interventions conditioned on the predicted number of new cases. To the knowledge of the authors, this is the first successful forecast for filoviridae outbreaks with potential applications to other hemorrhagic fever outbreaks.

21.009 EUNID Consensus on Personal Protective Equipment for Highly Infectious Diseases in High Isolation Units

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Objectives: to reach an agreement on Personal Protective Equipment (PPE) necessary to manage patients with Highly Infectious Diseases (HID) admitted in High Isolation Units (HIU).

Methods: The European Network for Infectious Diseases (EUNID) is a network, co- funded by European Commission, of Member States experts in infection control and management of HID and HIU. A questionnaire was developed to evaluate what PPE are in use in each country for the management of HID patients: body protection (single or double gloves, gown, plastic apron, tyvek suit, shoe covering, surgical boots, hair covering); face protection (surgical mask, full face mask, goggles, eye glasses with lateral shield, hood with face shield, safety glasses); respiratory protection (FFP2, FFP3, filters, PAPR). EUNID partners discussed the results and achieved an agreement during a meeting. For each PPE a strength of recommendation was proposed: recommended; considered, but conferring a higher protection than that enough; considered, but conferring a lower protection; discouraged. Standard and high risk procedures were considered.

Results: 12 partners fulfilled the questionnaire. For body protection it was recommended: single pair of gloves, impermeable gown, shoe and hair covering for Orthopox, SARS, and pandemic influenza; double gloves and full body tyvek suit for VHF. For face protection it was recommended full face mask for VHF and for all HID in case of high risk procedures; goggles in the routine care of Pox, SARS, and emerging influenza. For respiratory protections it was recommended adequately fitted FFP3 mask, and HEPA filtered PAPR in case of high risk procedures, in all HID management. Procedures related to PPE removal have been indicated.

Conclusions: According to the European Legislation, EUNID stressed to give priority to collective over individual protective measures, and to workers education and training. In order to encourage adherence to PPE, the partners agreed to favour homogeneity in infection control protocols for HIU even against the risk of over protection. A precautionary principle guided the recommendations. Several unresolved issues had been highlighted, such as the limited information available on the efficacy of respirators or masks in preventing transmission, and the difficult use of some PPE that may cause difficulties in taking care of patients.

21.010 Molecular Epidemiology of Rabies: Focus on Black-backed Jackals and Domestic Dogs in Northern South Africa

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Background: While sustained parenteral dog vaccination and annual campaigns have kept dog rabies under control in the northern regions of South Africa for the past two decades, cases in black-backed jackals (Canis mesomelas) have been on the increase. The question has arisen as to whether C. mesomelas maintains rabies cycles independently of domestic dogs. The objective of this epidemiologic study was to clarify the phylogenetic relationships amongst rabies viruses isolated from the black-backed jackals and domestic dogs in this region.

Methods: In this epidemiologic investigation, the cytoplasmic domain of the glycoprotein and the non-coding G-L intergenic region of 120 canid rabies viruses recovered from the two host species from Limpopo, Mpumalanga and North-West provinces were characterised.

Results: Through phylogenetic analysis of a 592 nucleotide sequence of the rabies viruses in the study sample, five distinct viral clades were identified in this region. These clades clustered according to the geographical origin of these virus isolates. Two of these clades were composed of viruses from both canid species in the north to central Limpopo and eastern North-West areas. The other two consisted of viruses exclusively from C. mesomelas and C. familiaris respectively. The latter was found to be responsible for the human outbreak in this province. The last clade consisted of viruses primarily from domestic dogs from Mpumalanga.

Conclusion: It appears as if C. mesomelas maintains rabies cycles in the commercial farming areas adjacent to bushvelds in Limpopo and domestic dogs in areas where there are high human densities. It is then crucial to formulate wildlife rabies control strategies that are in synergy with the existing domestic animal vaccination strategies for effective control of canine rabies in South Africa.

21.011 An Alphavirus Replicon Derived Candidate Vaccine Against Rift Valley Fever Virus

M. Heise[1], A. Whitmore[1], J. Thompson[1], J. Paweska[2], K. Madric[1], L. White[1], R. Swanepoel[2], **F. Burt**[3]. [1]Carolina Vaccine Institute, Chapel Hill, NC, USA; [2]National Institute for Communicable Diseases, Johannesburg, South Africa; [3]NHLS, University of the Free State, Bloemfontein, South Africa

Background: Rift Valley fever (RVF) virus is a mosquito-transmitted virus of Africa. Humans acquire infection from mosquito bite or contact with tissues of infected livestock. Most people suffer benign febrile illness but a small proportion succumb to fatal haemorrhagic disease or encephalitis. No specific treatment is available for RVF virus and vaccination is the most promising method of protecting humans. Although live attenuated and inactivated vaccines have been used in livestock, and on a limited scale in humans, there is a need for an improved vaccine.

Methods: The correlates of protective immunity against RVFV are not completely understood, however there is good evidence that neutralizing antibody responses against the viral glycoproteins are able to mediate protection. Replicon-based vectors derived from Sindbis-group alphaviruses were developed to express RVFV glycoproteins. A mouse model was used to characterize humoral immune responses and to demonstrate protective immunity against lethal challenge. The vaccine was tested in sheep to determine if neutralizing antibody could be elicited in a target animal.

Results: Sindbis virus replicon vectors were constructed which expressed the RVFV Gn and Gc glycoproteins, plus nonstructural nsM protein, to high levels and induced antibody responses in mice as determined by RVFV specific ELISA, indirect immunofluorescence and neutralization assays. Neutralizing antibody titers ranged from 1:8 to 1:128. Vaccination provided 100% protection in the animals against either peripheral or intranasal RVFV challenge. Neutralizing antibody was demonstrated in two vaccinated sheep and an ELISA using recombinant nucleocapsid antigen was able to differentiate between natural infection and replicon vaccinated animals.

Conclusions: We demonstrated that Sindbis virus based replicon vectors efficiently express the RVFV glycoprotein and elicit protective immune responses against RVFV challenge in a mouse model and induce neutralizing antibody in sheep. The results suggest that replicon vectors show promise as potential vaccines against RVFV.

21.012 DNA Aptamers Impair Orthopoxvirus Infection

A. Kurth[1], A. Dunkhorst[1], A. Kage[2], **A. Nitsche**[1]. [1]Robert Koch-Institut, Berlin, Germany; [2]Charité Universitätsmedizin Berlin, Berlin, Germany

The potential bioterrorist use of variola and other bioengineered or natural orthopoxviruses (OPV) such as monkeypox virus as a biological weapon has stimulated efforts to develop new therapeutics for the treatment of orthopoxvirus infections.

Antisense oligonucleotides have been successfully introduced as treatment in numerous clinical studies. There are high expectations for another type of short (20-60 base), single-stranded oligonucleotides, called aptamers. In contrast to antisense drugs, aptamers are assumed to bind tightly to very specific proteins and, therefore, can also work against extracellular targets. To circumvent some of the crucial problems occurring with RNA aptamers in in vivo applications, e.g. their rapid nuclease degradation in the bloodstream and short serum half-time, we

developed DNA aptamers as a superior substitute against OPV infection.

The DNA aptamers in this study were selected in vitro by MonoLex© technology against complete vaccinia virus (ATCC #VR-1536). Starting from random sequence libraries, this technique optimizes oligonucleotides for high-affinity binding to presented vaccinia viruses. With flanking primers as part of the final aptamer they are easily detected, quantified, multiplied and linked with other molecules such as fluorescent labels. Selected aptamers were tested for their ability to impair vaccinia virus infection in Hep2 cells, using the immunofluorescence assay (IFA), real-time PCR and plaque assay. Subsequently, inhibition was determined for 6 other OPVs (vaccinia Lister Elstree and I-HDW, cowpox 81/02, callithrixpox, camelpox CP19 and mousepox ectromelia). Finally, the stability of DNA aptamers was assessed after incubation in cell culture media at 37°C for up to 28 days.

Four different aptamers were selected, cloned and manufactured. When used in a concentration of 2.5 μM, one aptamer (#91) reduced the VR-1536 titer to 27% after 24 hours and 99% after 96 hours compared to a VR-1536 infected cell culture. This result could be confirmed by real-time RT-PCR and IFA. Repeated plaque tests with 6 different OPVs revealed the lowest titer reduction of 50% on CP19, increasing to 90% for Lister Elstree after 30 hpi. There was no significant loss of the inhibitory effect when aptamer #91 was incubated in cell culture media at 37˚C for 28 days.

The aptamer #91 tested in vitro prevented Hep2 cells to produce infectious virus particles for at least 96 h. In regard to their high stability, DNA aptamers also seem promising for in vivo studies. In summary, DNA aptamers could represent a highly effective therapeutic tool to inhibit OPV infections.

SESSION 22 (Parallel Session)

The Revised International Health Regulations: What will be Different in 2007

Saturday, February 24, 2007
Room: Klimt Ballroom 2 & 3
14:30–16:00

22.001 **The New IHR: What Do They Say? What Do They Mean?**

B. Ganter. WHO, Stockholm, Sweden

22.002 **Country Perspectives on the New IHR**

P. Aavitsland. Norwegian Institute of Public Health, Oslo, Norway

The new IHR contain 1) a new international system for epidemic intelligence, 2) a procedure for adopting recommendations to guide the response to public health emergencies of international concern, and 3) new international rules on routine measures against international disease spread.

The new IHR represent a shift of paradigm in several ways: Firstly, the objects of surveillance are events, not verified cases of a certain list of diseases. This increases the sensitivity, but lowers the positive predictive value of the surveillance. This is a desired change. Secondly, the member states no longer have monopoly as suppliers of epidemic intelligence to WHO. Now the WHO is allowed to use multiple sources of information, including mass media and non-governmental organisations. Thirdly, the power to define an event that invokes the use of the full powers of the IHR has been moved from member states to the WHO. Even if a member state denies any event is occurring in the country, the WHO may ultimately recommend measures that affects that member state. Fourthly, there are clear obligations on member states to develop capacities for surveillance and response.

From a country perspective, the three main challenges with the new IHR are: a) We must make sure that the national focal points can notify WHO of events independent from political concerns. Deciding to notify WHO of a potential event is a professional decision, not a political one. b) We will need to work hard to build the required capacities for surveillance and response, in our own countries and in less affluent parts of the world. c) We must respect the IHR's underlying ideas of working together to stop disease spread. There should be no withholding of information and no excessive response if we want the IHR to be an instrument to serve global public health.

22.003 **What the New IHR Mean to WHO and GOARN**

G. Rodier. WHO, Copenhagen, Denmark

SESSION 23 (Parallel Session)

Models of Disease Surveillance, Detection and Reporting

Saturday, February 24, 2007
Room: Park Congress
16:30–18:00

23.001 **GPHIN: A Necessary Tool for The International Public Health Community**

A. Mawudeku, R. Lemay, M. Blench, G. Guerrero, Q. Xu, J. Ni, L.F. St. Pierre Dubois, N. Ghanem, E. Rodionova, H. Ghiasbeglou, P. Uhthoff. Public Health Agency of Canada, Ottawa, Canada

Background: The Global Public Health Intelligence Network (GPHIN) is a secure web-based multilingual early warning system that is comprised of an automated function coupled with analysts who have public health expertise and linguistic skills. GPHIN monitors global news media sources in eight languages for potential and emerging public health threats on a 24/7 basis. The scope of public health issues covered is broad and includes animal and human infectious diseases; non-communicable diseases; plant diseases; chemical, radio-nuclear or biological incidents; product safety; food security and safety; and natural and environmental disasters.

Methods: GPHIN monitors global news media sources in Arabic, English, Farsi, French, Russian, Spanish and Simplified and Traditional Chinese. News sources include newswires, newspapers, and television and radio transcripts. Using established search criteria, relevant information is gathered, aggregated, machine-translated and made available to users on a near real-time basis. GPHIN Analysts review the news reports for trends and alerts. Any alerts identified are emailed immediately to subscribers. The alerts notify users about situations that may have serious public health consequences. During an identified public health emergency, Analysts provide status reports throughout the day on the situation. The reports may include data on the estimated magnitude of the incident; geographic distribution of incident; control and prevention measures that are being considered or have been implemented; concerns of the general public; and political implications.

Results: The GPHIN system has been the primordial mechanism for providing to appropriate Canadian and international authorities unverified qualitative and quantitative early warning information and intelligence with regard to disease outbreaks such as Avian Influenza and Severe Acute Respiratory Syndrome (SARS).

Conclusions: Since the development of GPHIN in 1998, this tool has continued to demonstrate its value as a versatile non-traditional early warning surveillance tool for potential global public health threats, particularly during crisis situations.

23.002 The Informal Sector and Emerging Disease Detection

M. Pollack. ProMED-mail, Brooklyn, NY, USA

It is well accepted in the international public health community that no one disease surveillance system will address all needs in detecting newly emerging diseases and that a network of networks is necessary to expand the reach of the current disease surveillance activities. ProMED-mail (Program to Monitor Emerging Infectious Diseases) is a moderated "listserve" that functions as internet based early warning system using a variety of sources, both official and unofficial to provide early notifications of unusual health events around the world. ProMED covers plant, animal and human health issues, and as a non-governmental organization, does not have political constraints to reporting on events it learns about. Sources of information include lay press reports and reports by subscribers. Good examples of how this has functioned to provide early alerts on unusual health events can be seen in an analysis of reports related to the Severe Acute Respiratory Syndrome (SARS) outbreak, avian influenza outbreaks in avians and humans and the spread of chikungunya virus throughout the Indian Ocean region. Early reports obtained through the informal sector resulted in alerts to the formal sector as well as "priming" the proverbial "astute clinician" in geographic areas distant from where disease activity had been seen, and allow for an earlier detection of new geographical spread of disease.

23.003 Market-based Prediction Systems for Infectious Diseases

P.M. Polgreen. University of Iowa, Iowa City, IA, USA

In recent years, specialized markets called prediction markets have been developed solely for their use in forecasting. Prediction markets have accurately forecasted the outcomes of a wide range of future events including sales of computer printers, elections, and the U.S. Federal Reserve's decisions about interest rates. These markets allow trade in artificial financial instruments whose value is determined by the outcome of the event of interest. Participants in these markets are experts with information regarding that event (e.g., an election). Participants buy contracts that are, according to their private information, undervalued by the market and sell contracts that are overvalued. The prices at which these instruments trade reflect a consensus belief about the future value of the instruments and thus can be used as a prediction of the future event.

Prediction markets can also be used for tracking and forecasting emerging infectious diseases such as SARS and avian influenza by aggregating expert opinion quickly, accurately and inexpensively. Data from a pilot study in the state of Iowa suggest that these markets can accurately predict seasonal influenza activity 2-4 weeks in advance by using clinical data volunteered from participating health-care workers. Information revealed by prediction markets may help to inform treatment, prevention and policy decisions. For example, prediction markets can help refine existing surveillance systems by determining who has what information and when. In addition, prediction markets can be used to estimate the success of infection control interventions or to predict the likelihood of new drug or vaccine developments.

23.004 Animal Zoonotic Disease Surveillance Systems: FAO/OIE/WHO Gobal Early Warning System and Response (GLEWS)

K. Ben Jabara. OIE, Paris, France

Early Warning and Early Reaction is based on the concept that dealing with a disease epidemic in its early stages is easier and more economical than having to deal with it once it is widespread. From a public health perspective, early warning of outbreaks with a known zoonotic potential will enable control measures that can prevent human morbidity and mortality. At the international level, the OIE and the WHO mandates include official notification of disease or infection outbreaks to the international community within conditions determined by their Member Countries. The latter have the obligation to report quickly exceptional disease events to the OIE and to WHO respectively for animals and for humans. These conditions have been recently revised and improved.

OIE, FAO and WHO have developed Early Warning and Response Systems that collect, verify, analyse and respond to information from a variety of sources, including official reports, unofficial media reports and informal networks.

The Global Early Warning and Response System (GLEWS) is a joint system that builds on the added value of combining and coordinating the alert and response mechanisms of OIE, FAO and WHO for the international community and stakeholders to assist in prediction, prevention and control of animal disease threats, including zoonoses, through sharing of information, epidemiological analysis and joint field missions to assess and control the outbreak, whenever needed.

The goals and expected outputs of GLEWS are the following:

– Disease alert and early warning messages. They will concentrate on predicting animal disease threats, through epidemiological analysis and the integration of additional factors that could have an impact on the occurrence and spread of such diseases (such as economic factors, civil unrest, climatic changes, etc).

– Development of coordinated responses to animal health emergencies. If in consultation between the three partners there is clear value for onsite assessment of the situation, an urgent joint field mission can be considered engaging the country authorities, in order to obtain a better appreciation of the situation and to offer assistance in the formulation of urgent intervention strategies.

– From a public health perspective, early warning of outbreaks with a known zoonotic potential will enable control measures that can prevent human morbidity and mortality.

SESSION 24 (Parallel Session)

Outbreak Control: What do We Need Besides Good Luck?

Saturday, February 24, 2007
Room: Klimt Ballroom 2 & 3
16:30–18:00

24.001 Rapid Response Plan for Pandemic Influenza

D. S. Fedson. Sergy le Haut, France

Most rapid response plans for pandemic influenza focus on non-pharmaceutical interventions (NPI) or community mitigation strategies. Although mathematical models suggest that prompt intervention with conventional inactivated vaccines and antiviral agents could be of some benefit, most experts believe these measures will not be available and/or will be too difficult to implement in most parts of the world. The development of pre-pandemic inactivated H5N1 vaccines has proceeded slowly because of failure to fix the reverse genetics-engineered H5N1 vaccine virus, reluctance to test adjuvanted vaccines at truly low antigen-sparing levels, failure to define vaccine licensing requirements suitable for populations, and the lack of public funding and international coordination for the clinical trials. As a result, today the worlds vaccine companies could produce enough doses of an H5N1 vaccine in six months to vaccinate with two doses only 600 million people. Moreover, anti-neuramindase agents will be in short supply, and evidence from clinical experience with H5N1 infected-patients suggests their efficacy might be limited. Unfortunately, current development efforts fail to address the very real possibility that billions of doses of specific vaccines or pharmaceutical agents could be made available at the onset of or soon after the emergence of a new pandemic virus. Scientific understanding strongly suggests that we could use currently existing industrial capacities to produce within 1–2 months of several billion doses of a live-attenuated H5N1 vaccine and/or a

recombinant hemagglutinin (rHA) vaccine. In addition, laboratory, clinical and epidemiological findings suggest that generic statins might be useful for pandemic treatment and/or prophylaxis because of their anti-inflammatory and immunomodulatory effects. Given the potential enormity of an H5N1-like pandemic, rapid response plans must include consideration of the potential utility of these specific interventions.

24.002 Disease Surveillance After the Outbreak

K. Mandl. Health Science Technology, Harvard Medical School, Boston, MA, USA

Modern biosurveillance approaches rely on the behaviors, symptoms, signs, or laboratory findings that ill persons exhibit and which can be tracked through a variety of data sources.

The main goal of outbreak detection is to generate an alert whenever observed data depart sufficiently from an expected baseline. Four basic methodologic stages are required to process data for outbreak detection: 1) the disease grouping stage, in which data are used to assign each patient to a particular case definition or syndrome group; 2) the modeling stage, in which historic data, including data for patients during the past year(s), are analyzed to establish a model from observed temporal and spatial patient distributions; 3) the detection stage, in which the expected values are compared with observed values to determine whether abnormal activity is occurring, and; 4) the alert stage, in which thresholds are set to evaluate whether an unusual pattern warrants notifying public health authorities.

I will discuss AEGIS—an open source, advanced, modular biosurveillance system which is used by the Massachusetts DPH. AEGIS fully automates data collection, integration, processing, visualization, interpreting and alerting, using emergency department (ED) data from twelve hospitals.

Work on AEGIS has lead to important methodological advances as well as to scientific discoveryfor example our findings about the force of influenza transmission by preschool aged children, recently lead to a national change in vaccine policy. The latest module of AEGIS is AEGIS-Flu, shifts the emphasis from an outbreak detection model to a disease tracking model and prediction model.

I will also address the privacy concerns raised by biosurveillance and the architectural approaches health data exchange that attempt to optimize privacy preservation while conserving detection capabilities.

24.003 Immune Survivors in the Management of Influenza

D. Lucey. Georgetown University Department of Microbiology and Immunology, Washington, DC, USA

Background: Influenza virus infection is generally considered to confer immunity in survivors against repeat infection with the identical virus in the near term, although not against drift variants of the same subtype or different subtypes. The large majority of persons infected during the influenza pandemics of the 20th century survived. A vaccine is unlikely to be available during the first wave of the next flu pandemic.

Methods: Review of influenza literature, including international (WHO) and national pandemic influenza plans, and personal communication with planners.

Results: A partial list of considerations includes: (1) Validation of the concept that survival after infection with the next human pandemic influenza virus confers solid immunity against reinfection; (2) The need for a rapid lab assay to document immunity to the circulating pandemic flu virus; (3) Establish guidelines for how documented pandemic flu immune survivors could best be used locally, nationally, and internationally to maintain critical infrastructures such as public health, home care, security, transportation, energy, and food-water supplies; (4) If and how blood products from immune survivors could be used as immunotherapy (Ann Intern Med 2006;145:599-609); (5) How best to address the complex risk communication, ethical, and legal issues; (6) Whether the immune responses of survivors can help identify the optimal immune responses to be elicited by pandemic flu vaccines; (7) Guidelines on whether immune survivors would need masks and other PPE, vaccination, or antivirals.

Conclusions: Consideration of how immune survivors during each successive wave of the next flu pandemic could help provide continuity of public health and other critical societal infrastructures is deserving of enhanced international dialogue and interim guidelines (e.g., under WHO leadership). The recent report (Lancet 2006; 368:2211-18) that most deaths during the flu pandemic of 1918-20 occurred in nations with scarce health resources should be considered in planning for use of any national or international pandemic flu immune survivor teams.

SESSION 25 (Plenary Session)

Drivers of Disease: Human–Wildlife Linkages

Sunday, February 25, 2007
Room: Park Congress
08:30–09:15

25.001 Drivers of Disease: Human–Wildlife Linkages

W. Karesh. Wildlife Conservation Scoiety, Bronx, NY, USA

The threat of emerging infectious diseases spreading between people and animals continues to rise. The vast majority of humans on the planet still live in a world like our great-grandparents, buying their food fresh, salted or smoked in open-air markets, or gathering it themselves. For much of the world, there are no systems of inspections for these markets and few people have access to good health care, education on hygiene, common vaccinations or antibiotics. The global transport of animals and animal products, which includes hundreds of species of wildlife, also provides safe passage for commensal and pathogenic microbes alike. The inadvertent movement of infectious agents due to wildlife handling and trade, and domestic animal movement, impacts not only human and livestock health, but also includes agents that can devastate native wildlife which serve as biological linchpins for environmental integrity, as well as providing a range of cultural and quantifiable economic values. Meanwhile, no government agency is responsible for, or currently capable of, the surveillance and prevention of the myriad of diseases residing around the world. None are given the responsibility for robustly pursuing the simplest of concepts the health of people, animals, and the environment in which we all live, are inextricably linked.

SESSION 26 (Parallel Session)

Emerging Vectoborne Diseases in Humans and Animals

Sunday, February 25, 2007
Room: Park Congress
09:30–11:00

26.001 Chikungunya: An Emerging Outbreak from East-Africa to Indian Ocean, 2004–2007

A. Flahault [1], G. Aumont [2], V. Boisson[3], X. de Lamballerie[4], F. Favier[5], D. Fontenille[6], B.A. Gaüzère[7], S. Journeaux[8], V. Lotteau[9], C. Paupy[6], M.A. Sanquer[10], M. Setbon[11]. [1]Inserm-UPMC UMR-S707, Paris, France; [2]Inra, Tours, France; [3]GHSR, Saint-Pierre de la Réunion, France; [4]Université Aix-Marseille, Marseille, France; [5]Inserm-GHSR-CHD-URML, La Réunion, France; [6]IRD, Montpellier, France; [7]CHD, Saint-Denis de la Réunion, France; [8]General Practice, Sainte Marie de la Réunion, France; [9]Inserm, Lyon, France; [10]DASS, Mayotte, France; [11]CNRS, Aix-en-Provence, France

Many triggering factors for onset of emerging infectious diseases are now recognised: globalisation, demographic increase, population movements, international trade, urbanisation, and forest destruction, climate changes, loss in biodiversity, and extreme life styles such as poverty, lack of food and war. Epidemic burden is often leading to disasters, in terms of human losses, as well as economic, political or social consequences. These outbreaks may jeopardize in a few weeks or months industry, trade, or tourism in countries not prepared for that. When dengue, and dengue hemorrhagic fever is spreading all over the tropical world, another arbovirosis, chikungunya which dramatically spread in Indian Ocean islands where 30 to 75% of population catch the viral infections between 2005 and 2006, now extends its progression towards India, with more than a million people infected. Patients experience sequelae with disability, work loss, and rarely severe outcome recently identified in La Réunion and Mayotte, France. No country, no part of the world may consider as protected against such events. However, consequences of emerging or re-emerging diseases are more inacceptable when they impact the poorest countries in the world. Viruses, bacterias, as well as wild animals, birds, or arthropods do not know borders. It is time now to promote barriers against infectious diseases, including prevention, anticipation, disease surveillance and research. This is not only for humanitarian reasons, but also for contributing to a sustainable development with equity for worldwide population.

26.002 Vectorborne Bacterial Zoonoses: Rickettsia, Ehrlichia, Anaplasma

P. Parola. WHO Collaborative Centre for Rickettsial Reference and Research, IFR 48, CNRS UMR6020, Faculté de Médecine, Marseille, France

During most of the 20th century, it was thought that a single pathogenic tick-borne spotted fever group (SFG) rickettsia occurred on each continent and that the many other rickettsias isolated from ticks were not pathogenic to humans. This concept has been ruled out with the identification from 1984 through 2006 of at least 13 additional rickettsial species or subspecies that cause tick-borne rickettsioses around the world. Of these agents, 9 were initially isolated from ticks, often years or decades before a definitive association with human disease was established, including in developed countries, such as Rickettsia parkeri in the USA, or R. massiliae in Europe. Rickettsia felis, an emerging agent belonging to the spotted fever group of Rickettsia but transmitted by fleas has been detected throughout the world. Also, outbreaks of the louse-borne epidemic typhus have re-emerged in Africa and might threaten homeless people parasitized by lice in industrialized countries if they have close proximity with bacteremic imported cases. On the other hand, although bacteria of the genus Anaplasma ssp and Ehrlichia ssp have been thought for a long time to be of veterinary importance only, three species are now known human pathogens, including Ehrlichia chaffeensis, the agent of human monocytic ehrlichiosis, Anaplasma phagocytophilum, the agent of human anaplasmosis, and Ehrlichia ewingii, the agent of a granulocytic ehrlichiosis. We present here recent data on the epidemiology of these diseases. Finally, although molecular tools have increased the panel of assays available for the diagnosis of rickettioses, they should be carefully used to avoid any misdiagnosis caused by contaminations. Doxycycline remains the drug of choice for treating empirically, as soon as the disease is clinically suspected, all rickettsioses, most of them having a high rate of mortality if inappropriately treated.

26.003 Emerging Parasitic Infections. Reports from the ProMED Surveillance

E. Petersen. Department of Infectious Diseases, Aarhus University Hospital-Skejby,, AArhus, Denmark

The review of emerging parasitic diseases used reports from ProMED surveillance reports to give an overview of present problems of changing disease distribution and emergence of control failure of major parasitic diseases.

Parasitic diseases include infections with protozoans and helminths and are distributed worldwide. The protozoans include malaria, leishmaniasis and trypanosomiasis, which are endemic in many tropical countries. The distribution of these infections have often not changed for decades, but appearance in new geographical areas or major outbreaks signalling changing epidemiology or development of resistance and failure of control programmes need to be reported. ProMED has over the years reported on outbreaks of malaria in India and emergence of imported and autochthonous in Jamaica and Bahamas and even Corsica (France). The emergence of malaria in the former Soviet Union counties has been followed closely. African trypanosomiasis has increased in central Africa and outbreaks of food-borne Chagas disease in Brazil have highlighted this previously unnoticed mode of transmission.

Helminths include nematodes, trematodes and cestodes. ProMED has reported several outbreaks of Schistosomiasis and in particular reported on the increase of Schistosoma japonicum in China associated with the hydro electrical projects. Reports of smaller outbreaks of infection with Trichinella from Russia and other eastern European countries, show that Trichinella is widespread and the control measures not working properly. The reports also demonstrate that food is send over borders for private consumption giving rise to infection in emigrant populations. Reports from ProMED also indicate that infections with Opistorchis are widespread in humans in Russia perhaps spreading into eastern China.

SESSION 27 (Parallel Session)

Detection, Surveillance and Control of Emerging Diseases in Animals: The Case of Avian Influenza

Sunday, February 25, 2007
Room: Klimt Ballroom 2 & 3
09:30–11:00

27.001 Avian Influenza: The Moving Target

I. Capua[1], D.J. Alexander[2]. [1]OIE/FAO Referenvce Laboratory for Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy; [2]VLA, Weybridge, Addlestone, United Kingdom

Avian Influenza (AI) is a complex infection of birds, of which the ecology and epidemiology has undergone substantial changes over the last decade. AI viruses infecting poultry can be divided into two groups. The very virulent viruses causing highly pathogenic avian influenza (HPAI), with flock mortality as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all H5 and H7 viruses cause HPAI. All other viruses cause a milder, primarily respiratory, disease (LPAI), unless exacerbated by other infections or environmental conditions.

Until recently HPAI viruses were rarely isolated from wild birds, but for LPAI viruses extremely high isolation rates have been recorded in surveillance studies, particularly in feral waterfowl.

In recent years there have been costly outbreaks of HPAI in poultry in Italy, The Netherlands and Canada and in each millions of birds were slaughtered to bring the outbreaks under control. However, these outbreaks have tended to have been overshadowed by the H5N1 HPAI virus, initially isolated in China, that has now spread in poultry and/or wild birds throughout Asia and into Europe and Africa, resulting in the death or culling of hundreds of millions of poultry and posing a significant zoonosis threat.

Since the 1990s AI infections due to two subtypes, LPAI H9N2 and HPAI H5N1, have been widespread in poultry across large areas of the World, resulting in a modified eco-epidemiology and a zoonotic potential. An extraordinary effort in a transparent environment is required to manage these epidemics both from the human and animal health perspectives.

27.002 **Role of Poultry Vaccination in Helping to Prevent a Pandemic in Man**

P. Jones. International Federation for Animal Health (IFAH), Brussels (Belgium)

The disease of Avian Influenza is caused by virus strains that are divided into two groups based upon their pathogenicity and their ability of the virus to produce disease. These groups are called low pathogenic (LP) and highly pathogenic (HP). Low Pathogenic or "low path" Avian Influenza (LPAI) occurs naturally in wild birds and can spread to domestic birds. In most cases it causes either no signs of infection or only minor symptoms in birds. These strains of the disease pose little significant threat to human health, and are common around the world. Highly Pathogenic or "high path" Avian Influenza (HPAI) is often fatal in chickens and turkeys and has been the course of the serious outbreaks reported in Asia and Africa which has also resulted in serious illness and mortality in in-contact humans. HPAI spreads rapidly and has a higher death rate in most bird species than LPAI. LPAI viruses can mutate to HPAI. The virus is particularly adept at mutating and changing its genetic structure, for example by different strains exchanging genes, and this property helps the virus to survive and remain circulating in the population, whether that population is birds, mammals or humans. The large number of subtypes coupled with the virus's ability to mutate into different strains within a subtype makes influenza a particularly difficult vaccine target.

The paper will describe that the use of vaccination to control Avian Influenza stressing that vaccination alone cannot be used to replace other control measures. Vaccination is an additional tool in a comprehensive control strategy, combining biosecurity, surveillance, quarantine and eradication of infected birds as the traditional measures used to prevent or control an outbreak of Avian Influenza before it has the opportunity to spread to a large geographic area. The advantages and benefits of vaccination against AI will be reviewed especially the reduction of environmental contamination and the risk of infection both to other birds and people, and the importance of the latter in avoiding mutation/reassortment in man so helping to preventing the emergence of a human pandemic.

27.003 **Avian Influenza and Wild Birds: Victims or Vectors**

D. Pfeiffer. Royal Veterinary College, United Kingdom

The importance of wild birds as a source and particularly in the large distance spread of H5N1 HPAI virus infection is still being debated. It now seems likely that migratory wild birds have had a role in the spread of the virus from Eastern Asia across Asia to Europe. Their relevance in the still comparatively limited spread into the African continent remains unclear. Any attempt at controlling the infection needs to take account of the interaction between the various factors contributing to spread and maintenance of the virus, and wild birds are only one of those, and this issue will be discussed in this presentation.

SESSION 28 (Parallel Session)

Balancing Science, Surveillance and Society: Who Needs to Know What and When

Sunday, February 25, 2007
Room: Park Congress
11:30–13:00

28.001 **Outbreaks: When and How to Inform the Public**

J. Giesecke. ECDC, Stockhom, Sweden

The issue of when to inform the public about outbreaks is becoming more and more academic: with modern world-wide, round-the-clock communications the public is usually informed at the same time or before the public health community. In fact, a lot of modern outbreak surveillance tools rely heavily on media reports (GPHIN, MedISys). However, even when the surveillance professionals learn about a possible outbreak first, the rule should be to be as transparent as possible. It is almost always better to report what (little) we know, and to try to convey our insecurity plus a clear account of what is being done to gather more knowledge than to withhold the information for fear of public reaction.

As for 'how', the main imperative is to be co-ordinated among different national and international public health authorities. A rapid common risk assessment has to be performed among the parties involved, and after that an agreement on what should be communicated and how it should be phrased. There is little room for 'solo' play here: conflicting messages from different authorities probably creates the most public anxiety in any outbreak situation.

28.002 **The Use of Internet-based Resources in Emerging Disease Surveillance**

J. Brownstein. Harvard Medical School, Boston, MA, USA

Internet-based resources such as discussion sites and online news sources, accessible through free and unrestricted subscription, have proven to be valuable sources of information. Technologies such as GPHIN and ProMED have effectively leveraged these types of data for disease surveillance, reporting and early notification of outbreaks (e.g.: SARS). These data also exemplify unprecedented potential for increasing public awareness on public health issues and early warning of disease prior to their widespread recognition. However, despite an abundance of disparate electronic resources, none is comprehensive. Each has geographic, population and expertise gaps. In this talk, we first evaluate the utility of web-based information for surveillance. We find that data from news sources tend to have greater value for monitoring more common endemic illnesses and seasonal infections. Given their high volume, new sources are especially valuable for monitoring spatial and temporal patterns of larger scale epidemics. Surveillance that relies on individual reporting through mailing lists such as ProMED may provide richer and timelier data, especially for rare infections. However, we find that different data sources provide complementary information given the differences in geographic coverage and disease focus. In an attempt to address the value of data integration, we describe Healthmap.org, a free and open web resources. Healthmap is a multi-stream real-time surveillance system, which aggregates alerts across data sources (e.g.: news wire, RRS feeds, mailing lists, WHO alerts). Information is automatically acquired through screen scraping, natural language interpretation, text mining and parsing to obtain disease name and geocode the location of the outbreak. Through this approach, we achieve unified and comprehensive view of current global infectious disease outbreaks in space and time. Overall, we find that internet-based alert mapping offers a promising tool for surveillance and that there is value in the integration of distributed electronic resources for public health communication and intervention.

28.003 The Media in the Detection of Emerging Diseases: How to Inform the Public

R. Knox. National Public Radio, Washington, DC, USA

Beginning with the SARS episode in 2003, infectious disease outbreaks and even sporadic cases are reported on almost a daily basis by news media throughout the world. Due to the 24/7 media culture and the Internet, there is no longer any line separating reports within the public health community and information broadcast to a global public. This is a generally a good thing. It heightens public awareness of the risks of both emerging and established diseases at a time when pathogens can be quickly transmitted around the world in the space of an incubation period. It sometimes helps WHO get early warning of outbreaks before official channels transmit them. But the 24/7 news culture also requires continuous assessments of the size and nature of these risks so that quick judgments can be made about the appropriate journalistic and public health response. This is especially critical for outbreaks or isolated cases of H5N1 influenza. What should the threshold be for reporting new cases of H5N1 in animals or humans? Since H5N1 may or may not transform into the next pandemic strain, how should health officials and journalists keep appropriate focus on the need for pandemic planning when the advertised pandemic fails to appear? Finally, what can authorities and journalists do now to ensure that the public gets the information it will need when the next flu pandemic strikes? This presentation will provide some guide-points on how health officials and ID specialists can address these questions in an era of near-instantaneous communications.

SESSION 29 (Parallel Session)

Vaccines Against Emerging Diseases

Sunday, February 25, 2007
Room: Klimt Ballroom 2 & 3
11:30–13:00

29.001 New Tools in the Control of Rabies

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From public health, veterinary, and economic standpoints rabies is one of the most serious viral zoonosis that is presently encountered worldwide. The disease however, is not a candidate for global eradication, largely as a result of wildlife involvement on all major continents and in particular the evolutionary and ecological role of bats as reservoirs. Global elimination of rabies from the principal reservoir, the domestic dog, should however be a major objective for international organizations involved with disease control strategies. For example, in Western Europe, cases of rabies have substantially decreased, due to the success of oral wildlife vaccination programmes. Over the next few years, terrestrial rabies will only occur east of a line from the Baltic Sea to the Adriatic Sea, with an overall aim to eliminate terrestrial rabies from the whole European Union. Elimination of rabies from the less rich countries of Eastern Europe and in the longer term the protection of Europe requires the maintenance of a cordon sanitaire along the eastern boundary of the EU. In contrast, throughout parts of Africa and Asia rabies continues to be a significant public health concern. There is little doubt that the lack of effective surveillance systems and diagnostic capabilities in parts of Africa and Asia confounds our lack of knowledge of the viruses in these regions. New tools used in controlling rabies, underpinned by basic rabies research, are in development and include the use of more effective vaccines, anti-viral strategies and human prophylactic regimens. Ultimately, major financial commitment and serious long term partnering with interested member countries and organizations are pre-requisites for appreciable recognition of global rabies eradication goals.

29.002 Cell Culture Technology for Development of Pandemic Influenza and other Emerging Disease Vaccines

N. Barrett. Baxter AG, Orth/Donau, Austria

The rapid spread of avian influenza (H5N1) and the transmission to humans has induced world-wide fears of a new pandemic. Vaccines are considered the most effective means to control influenza outbreaks. The favoured strategy for pandemic influenza vaccine production involves use of genetically attenuated reassortants to manufacture vaccine in embryonated eggs. A disadvantage of this strategy is that the time required for generation and safety testing of such reassortants may be upto three months. In addition, egg supplies could be endangered by H5N1 infections of chicken flocks. Also clinical trials to date with H5N1 split vaccine formulations have demonstrated that very high antigen doses are required to induce seroconversion in immunized subjects. We report here on an alternative strategy which involves use of wild-type virus grown in a continuous cell culture (Vero) system to derive an inactivated whole virus vaccine. Candidate vaccines based on clade 1 (Vietnam/1203/2004/H5N1) and clade 2 (Indonesia/05/2005/H5N1) strains have been developed and demonstrated to be highly immunogenic in animal models. The vaccines induce both cross-neutralising antibodies and highly cross-reactive T-cell responses and induces protection in a mouse challenge model not only against the homologous virus but against other H5N1 strains including those from another clade. These data indicate that this strategy allows the high yield production of a pandemic vaccine and the whole virus vaccine based on the wild-type virus has the potential to induce broadly protective immune responses. This strategy is also currently being utilized to develop vaccines against other emerging viral diseases such as SARS coronavirus and West Nile virus.

29.003 Safety and Immunogenicity of a Cell Culture (Vero) Derived Whole Virus H5N1 Vaccine

M. Müller[1], H. Oh[2], P. A. Tambyah[3], S. Fritsch[4], A. Loew-Baselli[4], N. Vartyan-Boehm[4], R. Bobrovsky[4], B. G. Pavlova[4], O. Kistner[4], H. J. Ehrlich[4], N. Barrett[4]. [1]University Hospital, Vienna, Austria; [2]Changi General Hospital, Singapore, Singapore; [3]National University Hospital, Singapore, Singapore; [4]Baxter BioScience, Global R&D, Vienna, Austria

It is widely believed that the emergence of a new influenza pandemic caused by an avian virus strain is highly likely if not inevitable. The outbreaks of highly pathogenic H5N1 influenza in multiple avian species and humans are unprecedented and the wide distribution of this virus has raised considerable concern that H5N1 viruses may have the potential to cause a devastating pandemic. Vaccination against H5N1 influenza is considered to be the most effective option to limit its spread. However the conventional methodologies to manufacture and formulate influenza vaccines have a number of disadvantages and raise concerns whether sufficient quantities of an effective vaccine can be made available early enough after the onset of a pandemic to have an impact on public health. In addition published clinical studies to date with conventional split vaccine formulations have demonstrated poor immunogenicity. We report here on the first clinical study with a candidate H5N1 vaccine, which is based on a cell culture derived whole virus vaccine derived from the wild type virus, strain A/Vietnam/1203/2004. Preliminary data demonstrate (i) the vaccine is safe and has an excellent tolerability profile (ii) vaccine doses as low as 3.75 or 7.5 μg are highly immunogenic against the homologous H5N1 strain (iii) the non-adjuvanted formulation is more immunogenic than an alum adjuvanted formulation (iv) the vaccine induces antibodies which are capable of neutralizing not only clade 1 strains but also a widely divergent clade 2 strain (A/Indonesia/05/2005).