Quantification of early innate immune responses in chicken blood using microfluidic expression arrays

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French agency for food, environmental and occupational health & safety

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Xth Symposium of the French network for *Domestic Animal Immunology*

Welcome

On behalf of Anses and the Organizing Committee, I am very pleased to welcome all delegates to the Xth Symposium of the French network for Domestic Animal Immunology (i.e. *Réseau Français d'Immunologie des Animaux Domestiques* or IAD) in Ploufragan, France.

As previous IAD symposia, it will gather scientists from INRA (French National Institute for Agricultural Research), Anses (French Agency for Food, Environmental and Occupational Health & Safety), CIRAD (Agricultural Research for Development), French Agricultural and Vet schools and from private firms. We are all interested in immune mechanisms and vaccine developments in livestock, sport or companion animals, and I hope this Xth IAD symposium will be as fruitful as the previous editions promoting national research in this field.

The scientific program will mix keynote lectures given by internationally renowned experts and presentations of short papers selected by the Scientific Committee among abstracts submitted by European researchers from both the academic and industrial sectors. After an exciting opening talk about T lymphocytes in swine, four scientific sessions will focus on "immunomodulation", "vaccination and host immunity", "antiviral innate immunity" and "immunomonitoring". They will permit to give an overview of the current status and ongoing activities on these subjects in different animal species. The Scientific Committee has also selected posters, thus discussion on knowledge and new scientific findings will continue in poster area. A poster prize will award the best poster.

We sincerely thank the members of the Scientific Committee for having dedicated time and energy to make successful this Xth IAD symposium. We are indebted to keynote speakers for having accepted to share their expertise and thank all participants for their productive contribution and scientific input.

I hope you will enjoy the symposium and I wish you a very nice stay in Brittany!

Gaëlle Simon
Chair of the Organizing Committee and Scientific Committee

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AGENDA AND SCIENTIFIC PROGRAMME

Thursday, March 17th

16:00 pm **Shuttle** ***(Saint-Brieuc ⇨ Zoopole Ploufragan)***

16-17:00 pm **Registration**

17:00 pm **Welcome Address**
Gilles SALVAT & Gaëlle SIMON – *Anses Ploufragan*

17:15 pm **Opening Session**
Armin SAALMULLER, *University of Veterinary Medicine, Vienna - Austria*
Porcine CD4+ T lymphocytes and their contribution to an effective immune response after vaccination.

Scientific Session 1 "Immunomodulation"

Chairs: Elodie MERLOT, INRA St-Gilles - Jennifer RICHARDSON, ENV Maisons-Alfort

18:00 pm **Keynote Lecture**
Volker STEFANSKI*, University of Hohenheim, Stuttgart - Germany*
Environmental effects on maternal and offspring immune functions.

18:30 pm ***Stéphanie FERRET-BERNARD****, INRA Saint-Gilles*
Impact of food additive (prebiotic) and food contaminant (mycotoxin) in the maternal diet onto the ontogenesis and responsiveness of mucosal immune system.

18:50 pm ***Elodie BACOU****, ONIRIS Nantes*
Social stress modulates piglet immune system.

19:10 pm ***Marie-Frédérique LE POTIER,*** *Anses Ploufragan*
Effect of *O. porcinus* tick salivary gland extract on the African swine fever virus infection in domestic pig.

19:30 pm ***Philippe HOLZMULLER,*** *CIRAD Montpellier*
Immunomodulating properties of *Amblyomma variegatum* saliva and role on tick-borne disease transmission.

19:50 pm **Reception & Poster Session**

22:00 pm **Shuttle** ***(Zoopole Ploufragan ⇨ Saint-Brieuc)***

Friday, March 18th

7:45 am **Shuttle** ***(Saint-Brieuc ⇨ Zoopole Ploufragan)***

Scientific Session 2 "Vaccination and Host Immunity"

Chairs: Isabelle SCHWARTZ-CORNIL, INRA Jouy en Josas - Daniel DORY, Anses Ploufragan

8:15 am **Keynote Lecture**
Alasdair NISBET, *Moredun Research Institut, Edinburgh -United Kingdom*
Immunobiology in vaccine development against gastrointestinal nematodes of sheep.

8:45 am ***Olivier BOURRY,*** *Anses Ploufragan*
Maternally-derived antibodies (MDA) impair piglet humoral and cellular immune responses to vaccination against porcine reproductive and respiratory syndrome (PRRS).

9:05 am ***Delphine LE ROUX,*** *ENV Maisons-Alfort*
Immune response induced by the Mic1-3 knockout *Toxoplasma gondii* vaccine strain in the parasite definitive host: the cat.

9:25 am ***Ahmed SAMY IBRAHIM,*** *Animal Health Research Institute, Giza- Egypt*
Different avian immune response to immunization and infection with two genetically and antigenically distinct lineages of Egyptian highly pathogenic avian influenza (H5N1).

9:45 am ***Carine CARIOU,*** *MERIAL SAS, Lyon*
The immune fingerprint: a new relevant global immunological tool to assess the compatibility of vaccines.

10:05 am **Coffee Break & Poster Session**

Scientific Session 3 "Antiviral Innate Immunity"

Chairs: François MEURENS, ONIRIS Nantes – Sébastien SOUBIES, Anses Ploufragan

10:45 am **Keynote Lecture**
Pierre BOUDINOT, *INRA Jouy en Josas - France*
TRIM antiviral mechanisms across vertebrates.

11:15 am ***Eugénie BAGDASSARIAN,*** *ENV Maisons-Alfort*
Modulation of the host interferon response by the non-structural polyprotein ORF1 of hepatitis E virus.

11:35 am ***Céline DEBLANC,*** *Anses Ploufragan*
Impact of *Mycoplasma hyopneumoniae* pre-infection on Influenza infection outcome in the porcine model**.**

11:55 am ***Nicolas BERTHO,*** *INRA Jouy en Josas*
The porcine lung dendritic cells/macrophages network and its involvement in influenza and PRRSV pathologies.

12:15 pm **Lunch**

13:20 pm **Best Poster Prize**

Scientific Session 4 "Immunomonitoring"

Chairs: Michel PEPIN, Vet-Agro-Sup Lyon - Mustafa BERRI, INRA Tours

13:30 pm **Keynote Lecture**
Gilles FOUCRAS, *ENV Toulouse - France*
Resistance to mammary infections in ruminants: the unexpected role of SOCS2 on the immune mechanisms and beyond.

14:00 pm ***François MEURENS,*** *ONIRIS Nantes*
Towards the understanding of porcine cellular immune response to *Chlamydia trachomatis* and *suis* infections.

14:20 pm ***Valérie RODRIGUES,*** *CIRAD Montpellier*
Whole blood transcriptome analysis of *Mycoplasma mycoides* subsp. *mycoides*-infected cattle confirms immunosuppression but does not reflect local inflammation.

14:40 pm ***Daniel DORY,*** *Anses Ploufragan*
Development of an avian sub-unit vaccine protocol against *Campylobacter*: assessment of several technical parameters.

15-15:10 pm **Closing Session**

15:35 pm **Shuttle** ***(Zoopole Ploufragan ⇨ Saint-Brieuc)***

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Poster Session

Opening Session

Armin SAALMULLER

University of Veterinary Medicine, Vienna – Austria

Porcine CD4+ T lymphocytes and their contribution to an effective immune response after vaccination

Immune responses have to be guided to guarantee task-directed immune reactions and to avoid uncontrolled over-reactions. T lymphocytes, especially CD4+ T lymphocytes play a central role in the fine tuning of immune responses. After activation, CD4+ T cells differentiate into various effector cell populations to accomplish their diverse functional activities. As a consequence, the CD4+ population represents a functionally heterogeneous population containing a panel of cell subsets with different effector functions: TH1, TH2, TFH, TH17, and regulatory T cells (Tregs). In swine, knowledge about these functionally characterized subsets is still rudimental. Tregs mainly characterized by the expression of the transcription factor Foxp3, play a crucial role in the down-regulation and modulation of immune responses and in swine the majority of them is defined by CD4 expression and co-expression of CD25. Especially CD4+ T cells with high CD25 expression show cell-cell contact dependent regulatory activity. CD25 low positive CD4+ cells seem to be potent producer of IL-10.

In regard to other CD4+ T-cell subpopulations, swine show the peculiarity of CD8α expression on a major subpopulation of CD4+ T cells. CD4+CD8α- T cells contain in their majority naïve CD4+ T cells. The CD4+CD8+ subset, described as activated and memory T-helper cells contains IFN-γ and IL-17 producing T cells and these cytokine producing T cells seem to represent TH1 and TH17 cells. Moreover CD27 expression discriminates CD4+CD8α+ T cells into CD27+ and CD27- cells with characteristics of central and effector memory T-helper cells, respectively. More detailed analyses of the cell populations involved in the generation of the polarizing milieu showed a contribution of NK cells and TcR γδ T cells. IFN-γ producing NK cells and CD2+ TcR γδ T cells support a TH1 milieu and IL-17 producing TcR γδ T cells contribute to the TH17 response. Together these results demonstrate the high plasticity of porcine $CD4^+$ T cells and their capacity to be involved in a modulation and fine tuning of ongoing immune responses.

Scientific Session 1

Immunomodulation

Chairs:

Elodie MERLOT, INRA St-Gilles

Jennifer RICHARDSON, ENV Maisons-Alfort

Keynote Lecture

Volker STEFANSKI

University of Hohenheim, Stuttgart - Germany

Environmental effects on maternal and offspring immune functions

The immune system is the body's primary defense systems against infection and disease. Its functioning is therefore essential for the survival of an animal in the wild as well as in captivity. Evidence from the field of psychoneuroimmunology amply indicates, however, that the immune system does not only react to immunogenic challenges, but also to environmental influences such as stress. In fact, the immune system is one of the most sensitive to stressor influences among the physiological systems.

In my presentation I will highlight some of the most prominent consequences of (mainly) social stressors on the immune system of adult organisms - with examples provided from wild, laboratory and farm animal species. It will be demonstrated that not all immunological subsystems (cellular vs. humoral) are equally affected and that acute and chronic stressors may elicit quite opposing effects on immunity.

Today, we also know that stressors affecting the maternal organism also affect the intra-uterine development of the offspring. In the second part, I will therefore focus on the impact of early life conditions (mainly the prenatal period) on behavior and immunity of the juvenile and adult organisms, again with emphasis on laboratory and farm animals.

Disease is one of the largest threats that face food animal production today. Farm animals that suffer from illness are less productive and may even pose a human health risk. Furthermore, the welfare of these animals is impaired. Against this background, potential consequences of the above findings for farm animal husbandry will be discussed.

Impact of food additive (prebiotic) and food contaminant (mycotoxin) in the maternal diet onto the ontogenesis and responsiveness of mucosal immune system

Stéphanie Ferret-Bernard(1), Cindy Le Bourgot(1), Laurence Le Normand(1), Julie Seeboth(2), Véronique Romé(1), Gérard Savary(1), Fabrice Laurent(3), Isabelle Le Huërou-Luron(1), Laurence Guzylack(2)

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Keywords: swine, intestinal immunity, dendritic cells, short-chain fructooligosaccharides, mycotoxin

Infant piglets are highly sensitive to infectious enteric diseases notably because of the mucosal immune system immaturity. We evaluated prebiotic maternal supplementation as a possible approach for improving the health of young animals and assessed the impact of food mycotoxin on mucosal immune function. Sows were untreated (CTRL-), received either prebiotic (scFOS) or deoxynivalenol-contaminated food (DON) during gestation and lactation. Half of CTRL- offspring was force-fed with DON for their first week (CTRL+).

First, we analysed dendritic cell (DC) subset frequency among intestinal immune cells by flow cytometry. In DON and CTRL+ groups, CD16+MHCII+ DC decreased ($p<0.05$) respectively at d10 and d21-d49, whereas CD11b+MHCII+ DC decreased at d10 in CTRL+ piglets ($p<0.05$). No effect of maternal scFOS is noticed for any subsets. Then, we assayed cytokine secretion by intestinal mucosal biopsies. scFOS biopsies produced less IL-10 in response to flagellin and CpG, whatever their age, and secreted more TNF (p<0.05) in response to LPS at d49, compared to CTRL- animals. By qRT-PCR, we observed that scFOS biopsies expressed more IL-23 ($p<0.03$), TGF and FoxP3, against flagellin and LPS at d10. A higher expression of IFN and BAFF in d21 unstimulated scFOS biopsies was also revealed. DON biopsies displayed higher expression of IL-23 and those of CTRL+ more IL-1, IL-17 and IL-10 at d49 in response to LPS ($p<0.05$).

Our study demonstrated that maternal prebiotic diet improved mucosal DC secretory functions in neonate piglets whereas mycotoxin significantly compromised their numbers. Therefore, maternal prebiotic supplementation would be a good strategy to reduce the adverse effects of mycotoxin-contaminated food for piglets in order to fight efficiently pathogens.

Social stress modulates piglet immune system

Elodie Bacou (a,b), Karine Haurogné (a,b), Grégoire Mignot (b,a), Jordan Marchand (b,a), Marie Allard (a,b), Yvon Billon (c), Jean-Marie Bach (b,a), Pierre Mormède (d), Julie Hervé (b,a) and Blandine Lieubeau (a,b).

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Keywords: Catecholamines, Cortisol, Immunity, Pig, Stress

Pig husbandry is known as the most intensive breeding system, piglets being submitted to multiple stressful events (early weaning, successive mixing and crowding). Genetic selection carried out for production purpose led in Large White pigs to modifications in the two major stress responsive systems, namely hypothalamo-pituitary-adrenocortical (HPA) and sympatho-adreno-medullar (SAM) axes, whose main mediators are respectively cortisol and catecholamines. The general aim of this project was to describe and analyse consequences of acute social stress on neuroendocrine and immune systems in pig.

For this purpose, we took advantage of two genetically divergent breeds selected on HPA axis activity (SUSoSTRESS program). We submitted 7 week-old piglets of both groups to an one-hour mixing with unfamiliar conspecifics to induce an acute social stress and monitored endocrine and immune parameters variation. Immune traits were recently described as descriptive indicators of pig health; in particular, the whole blood assay (WBA), which measures cytokine release in whole blood in response to LPS stimulation, was proposed to predict individual susceptibility to infectious diseases.

Stressed piglets displayed higher levels of circulating cortisol, epinephrine and norepinephrine. Blood cell counts revealed a stress-induced leukocyte mobilization, which was already described in rodents and men and suggested to enhance the ability of an organism to cope with a contiguous immune challenge. Interestingly, we found out that lymphocyte demargination was specific of some subsets.

We also observed impaired monocyte functions (phagocytosis and cytokine production in WBA) in stressed animals. Altogether, our data associate a stressful event to an altered immune response in pig and pave the way to a better understanding of intensive farming practice consequences on pig robustness.

Effect of O. porcinus tick salivary gland extract on the African swine fever virus infection in domestic pig

Jennifer Bernard (1,3,4), Evelyne Hutet (1), Frédéric Paboeuf (1), Tantely Randriamparany (2), Philippe Holzmuller (3,4), Renaud Lancelot(3,4), Valérie Rodrigues(3,4), Laurence Vial (3,4), **Marie-Frédérique Le Potier** (1)

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Keywords: saliva, Ornithodoros, african swine fever virus, Langerhans cells, Macrophages

African swine fever is a lethal haemorrhagic swine disease with disastrous financial consequences for pig production. Ornithodoros soft ticks are able to transmit the African swine fever virus (ASFV) to pigs in farms, following the natural epidemiologic cycle of the virus. Tick saliva has been shown to modulate the host physiological and immunological responses during feeding on skin, thus affecting viral infection.

To better understand the interaction between soft tick, ASFV and pig at the bite location and the possible influence of tick saliva on pig infection by ASFV, salivary gland extract (SGE) of Ornithodoros porcinus, co-inoculated or not with ASFV, was used for intradermal auricular inoculation.

Our results showed that, after the virus triggered the disease, pigs inoculated with virus and SGE presented greater hyperthermia than pigs inoculated with virus alone. The density of Langerhans cells was modulated at the tick bite or inoculation site, either through recruitment by ASFV or inhibition by SGE. Additionally, SGE and virus induced macrophage recruitment each. This effect was enhanced when they were co-inoculated. Finally, the co-inoculation of SGE and virus delayed the early local spread of virus to the first lymph node on the inoculation side. This study has shown that the effect of SGE was powerful enough to be quantified in pig both on the systemic and local immune response.

We believe this model should be developed with infected tick and could improve knowledge of both tick vector competence and tick saliva immunomodulation.

Immunomodulating properties of *Amblyomma variegatum* saliva and role on tick-borne disease transmission

Léo Chamayou(1),Valérie Rodrigues(1),Bernard Fernandez(1),Alexandre Andersen(1),Edith Demettre(2),Martial Seveno(2),Rosalie Aprelon(3),Ken Giraud-Girard(3),Frédéric Stachurski(1),Nathalie Vachiéry(3), **Philippe Holzmuller**(1)

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Keywords: Amblyomma variegatum, tick saliva, immunomodulation, proteomics

The tropical bont tick, Amblyomma variegatum, is a major pest of ruminants, causing direct skin lesions, vectoring the obligate intracellular ricketsiale Ehrlichia ruminantium, the causative agent of heartwater, and being able to reactivate dermatophilosis. In general, tick salivary proteins are the result of host blood feeding adaptation and are known to contain inhibitors of blood clotting, platelet aggregation and angiogenesis, as well as vasodilators and immunomodulators. The general objective of this study was to better understand the role of the saliva in the tick interaction with the host but also in pathogen transmission or diseases activation. We therefore first analysed the immunomodulating properties of semi-fed A. variegatum female saliva on bovine peripheral blood mononuclear cells (PBMCs) in vitro. Flow cytometry and cytokine ELISAs have been used to evaluate the saliva impact on PBMCs. We focused on immunosuppressive properties by analysing both lymphocytes proliferation and monocyte-derived macrophages phenotype characterised by inhibition or induction of stimulatory and co-stimulatory molecules such as MHC-II, CD40, CD80 or CD86, and production of pro- or anti-inflammatory cytokines such as interleukin (IL)-2, IL-4, IL-6, IL-10, IL-12p40, and tumor necrosis factor (TNF)-α. Moreover, a proteomics exhaustive molecular characterisation of A. variegatum saliva was performed to cluster data set according to the evidenced immunomodulatory properties, and allowed refined characterisation of Amblyomma sialome. Bioinformatics functional analysis of tick saliva highlighted molecular determinants that could, at least in part, explain the biological effects observed on bovine PBMCs. Our results bring new insights for a better understanding of tick-ruminant interactions, and open new perspectives to develop integrative strategies to interfere with the infectious pathoimmunological process of such tick-borne infections.

Scientific Session 2

Vaccination and Host Immunity

Chairs:

Isabelle SCHWARTZ-CORNIL, INRA Jouy en Josas

Daniel DORY, Anses Ploufragan

Keynote Lecture

Alasdair NISBET

Moredun Research Institut, Edinburgh - United Kingdom

Immunobiology in vaccine development against gastrointestinal nematodes of sheep

The major cause of parasitic gastroenteritis (PGE) in small ruminants in temperate regions worldwide is infection with the parasitic nematode Teladorsagia circumcincta. We developed a prototype recombinant vaccine to control T. circumcincta which, when administered to 6-7 month old lambs in 2 separate protection trials, reduced nematode egg output by 70% (Trial 1) and 58% (Trial 2). During the period of peak worm egg shedding, vaccinated lambs shed up to 92% fewer eggs than did control (injected with adjuvant only) lambs. Reductions in faecal egg output of this magnitude will have a substantial impact on downstream pasture contamination and could play a central role in sustainable, integrated helminth control programs. However, to ameliorate parasite-induced effects on liveweight gain in young, newly-weaned lambs, the vaccine must be effective in this stage of animal. Initial experiments suggested that the prototype vaccine gave variable results in young lambs in it's current format so we have performed a series of experiments to; 1) determine the underlying immunological causes of variability in vaccine efficacy in young lambs and; 2) determine whether immunisation of pregnant ewes could sustain vaccine-induced protection during the periparturient relaxation in immunity and therefore reduce the associated pasture contamination to which young lambs are exposed. The outcomes of these studies will be described in this presentation.

Maternally-derived antibodies (MDA) impair piglet humoral and cellular immune responses to vaccination against porcine reproductive and respiratory syndrome (PRRS)

C. Fablet (1, 5), P. Renson (2, 3, 5), F. Eono (1, 5), S. Mahé (2, 5), E. Eveno (1, 5), M. Le Dimna (2, 5), V. Normand(4), A. Lebret (4), N. Rose (1, 5), **O. Bourry** (2, 5)

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Keywords: PRRS, vaccination, immune response, maternal antibody

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most costly disease for swine industry worldwide. We recently showed that a modified live vaccine (MLV) can efficiently reduce virus transmission under experimental conditions however the same efficacy seems difficult to achieve in the field. We hypothesized that this discrepancy could be due to the presence of maternally derived antibodies (MDA) which might decrease vaccine efficacy in piglets under field conditions.

We therefore investigated the influence of MDA on piglets' humoral and cellular immune responses to PRRS vaccination under field conditions. Thirty piglets with a low (A-) or high level (A+) of maternally derived neutralizing antibodies (MDNA) were vaccinated (V+) with a MLV at 3 weeks of age. Blood samples were collected before vaccination and at 2, 4 and 8 weeks post-vaccination (WPV) to assess vaccine viremia (RT-PCR), humoral (ELISA and virus neutralization test) and cellular (ELISPOT IFNɣ) immune responses. PRRS vaccine was detected in 60%, 64% and 36% of A-V+ piglets 2, 4 and 8 WPV, respectively. No virus genome was detected in A+V+ piglets during the first 4 WPV but 32% were PCR positive at 8 WPV. 85% of A-V+ piglets and 0% of A+V+ piglets seroconverted (ELISA) between 2 and 4 WPV. Neutralizing antibodies appeared 4 WPV in A-V+ piglets but were not detected at 8 WPV in A+V+ piglets. The number of PRRS-specific IFNɣ secreting cells was significantly higher in A-V+ piglets at 2 and 4 WPV compared to A+V+ piglets. These results show for the first time that MDNA can impair both humoral and cellular immune responses in piglets vaccinated against PRRS. Further studies are required to assess the impact of MDNA on vaccine efficacy after a PRRSV challenge.

Immune response induced by the Mic1-3 Knockout *Toxoplasma gondii* vaccine strain in the parasite definitive host: the cat

Delphine LE ROUX (1,2), Vitomir DJOKIC (2), Solen MORISSE (3), Clément CHAUVIN (1), Vanessa DORE (4), Fanny BOURSIN (3), Aurélie GRASSET-CHEVILLOT (2), Sébastien PERROT (4), Isabelle VALLEE (2), Edouard SECHE (3) and Radu BLAGA (1,2)

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Keywords: Toxoplasma gondii, cat, vaccine, immune response

Toxoplasmosis is a zoonotic parasitic disease caused by the protozoan Toxoplasma gondii. Up to a third of the global human population is estimated to carry a T. gondii infection, which can result in severe complications in immunocompromised individuals and pregnant women [1,2]. The release of the oocyst infective stage by felines, mainly cats, in the environment is at the heart of this public health problem [3].

Immune response to T.gondii has mainly been studied using intermediate hosts (IH) animal models but immune response to the parasite in its definitive host (DH), the cat, has barely been explored. However, studying deeply these parameters in the DH of T. gondii will give a more relevant picture of host-pathogen interactions and will allow identification of new targets for efficient vaccination.

We performed an experimental vaccination of cats using an attenuated live strain of T. gondii [5,6] given either subcutaneously or orally, and could follow their immune response. Both routes gave rise to a high specific antibody titer in cats serum, indicating that the live attenuated strain is highly immunogenic. However, the oral route required more parasites to induce antibody production, compared to sub-cutaneous administration. We could also notice a delay in antibody production when the strain was given orally since 1 out of 4 cats started to produce specific IgG 60 days after the first oral administration. As T.gondii antibodies are believed to be protective in IH, we followed oocysts shedding by vaccinated cats presenting high IgG titers, after oral challenge with a wild-type strain of T. gondii (76K). Surprisingly, high levels of anti-T.gondii IgG do not prevent oocysts shedding by cats, regardless of the vaccination route. This stricking result highlights the particular relationship between T. gondii and its unique DH which we are now currently investigating at the intestinal level.

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Different avian immune response to immunization and infection with two genetically and antigenically distinct lineages of Egyptian highly pathogenic avian Influenza (H5N1)

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Keywords: Avian Influenza, H5N1, Chickens, Cytokine, Vaccine, Egypt

Following the introduction of HPAI-H5N1 early at 2006, the Egyptian government implemented a massive poultry vaccination campaign as the cornerstone of policies to control the virus. However, late 2007 two distinct lineages, “classic 2.2.1” and “variant 2.2.1.1” strains have evolved. The underlying host immune responses counteracting infection and/or immunization with these viruses in chickens remain not well understood. In the present study cytokine response to immunization and infection with a classical (C121) and a variant (V1063) strain were compared. Results revealed earlier and higher HI titers and IFNγ levels in sera immunized with C121 whole inactivated virus (WIVs) accompanied by higher expression of IL8, IL10, and IL18 in the spleen compared to chickens immunized with V1063. Furthermore, HD11 cell line stimulated with C121 induced gradual upregulation of pro-inflammatory cytokines, accompanied by upregulation of IFNα. Conversely, V1063 induced very early transient higher expression of pro-inflammatory cytokines accompanied by upregulation of IL10 that subsequently diminished later. In the challenge study, C121 replicated more efficiently than the V1063. Both the C121 and the V1063 increased IFNγ and IL10 expression at 48 hpi in the lung and spleen but with lower expression in case of C121 comparing to V1063. In contrast, in chickens vaccinated with inactivated C121-based vaccine, the C121 replicated less than the V1063. Both challenge with the C121 and that with the V1063 did not increase IFNγ gene expression at 48 hpi; rather, the C121 increased IL-4 gene expression in the lung accompanied with lower viral titer and higher HI titers. In summary, results provide evidence of correlation between adaptive immune responses induced by WIVs and higher expression of IL10 and IL18 in addition IFNα. Furthermore, pathogenicty of HPAI viruses corellated with IFNγ in naïve chicken, whereas vaccine efficacy corellated with IL4 expression in lung.

The immune fingerprint: a new relevant global immunological tool to assess the compatibility of vaccines

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Keywords: Multiparametric analysis, vaccine, humoral, cellular immune responses

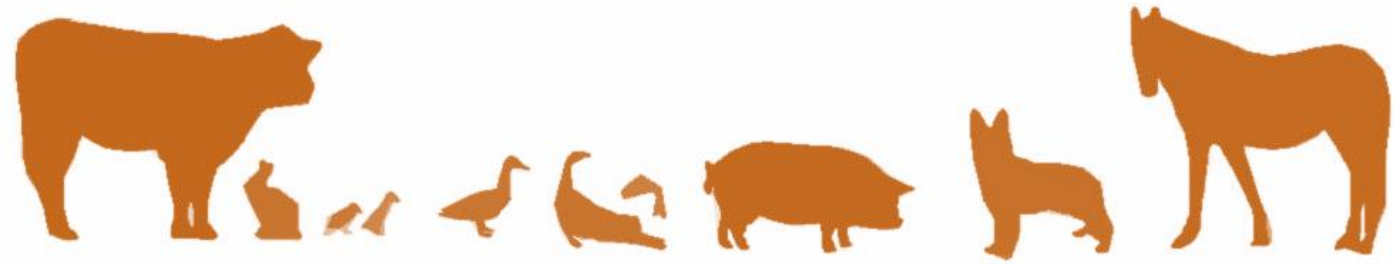
In order to reduce the number of challenges (3R) required for well-established dog combination vaccines, an integrated immunological analysis was performed to evaluate the compatibility between two canine vaccines, Rabisin™ injected alone or with a combined vaccine (EURICAN DAPPi2-Lmulti) at two different sites. This general picture, termed the immune fingerprint, was initially investigated for rabies.

A rabies seroneutralization assay was completed with a panel of humoral and cellular tools that are highly rabies-specific (listed in results).

Humoral parameters such as virus seroneutralization titers, IgG1, IgG3 and avidity indexes were not significantly different between groups ($p \geq 0.29$).

Canine PBMCs were stimulated ex vivo to detect antigen-specific cellular responses. IL10 and IFN-g secreting cells were low and statistically different, whereas IFN-g secretion and the number of circulating plasma and memory B cells showed no impact of the combine association with Rabisin™ vaccine ($p=0.07$, $p=0.94$, $p=0.38$ respectively).

A global analysis focused on these parameters was performed using a principal component analysis (PCA) to summarize the full immune response. The principal axis of variation was determined by the combination of humoral and B cell responses. The second axis was defined by INF-g secretion and IL10. A normal distribution of individual coordinates was found and there was a large overlap between the Rabisin™ vaccine fingerprint alone or with the combination vaccine confirming the immunological compatibility of the vaccines. The immune fingerprint could be therefore a new relevant global tool in view of replacing challenges in well-established products.



Scientific Session 3

Antiviral Innate Immunity

Chairs:

François MEURENS, ONIRIS Nantes

Sébastien SOUBIES, Anses Ploufragan

Keynote Lecture

Pierre BOUDINOT

INRA Jouy en Josas

TRIM antiviral mechanisms across vertebrates

Modulation of the host interferon response by the non-structural polyprotein ORF1 of hepatitis E virus

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Keywords: Hepatitis E virus, interferon, ORF1

Hepatitis E virus (HEV) causes an acute hepatitis in humans. The virus can be zoonotic and has several animal reservoirs, the main one being domestic pigs. Direct contact with infected animals and the consumption of infected meat are risk factors for HEV exposure. As HEV is enzootic in pig farms, it represents an important public-health concern. However, little is known about the biology and pathogenesis of the virus and particularly about is interactions with the host immune response. The interferon (IFN) system is a key component of the host antiviral response, and many viruses have developed different mechanisms to overcome its effects. However, the interactions between HEV and the IFN signalling remain poorly understood. HEV ORF1 encodes a polyprotein with non-structural functions that contains several putative domains such as a papain-like cysteine protease (PCP) and a macro domain that are homologous to other domains found in the “alpha-like” supergroup of viruses. Interestingly, several reports have shown that the PCP and macro domains of different viruses can modulate the host innate antiviral response. This study aims to determine whether the different domains of ORF1 are able to modulate the IFN system. Expression vectors encoding different ORF1 domains were constructed and reporter assays were performed to evaluate the effect of these domains on the IFN system. The results obtained have shown that the HEV PCP domain expressed with the methyltransferase and the Y domains is able to inhibit the activation of both the IFN-β and the ISRE promoters. Thus, it suggests that HEV ORF1 is able to modulate the IFN response. This study provides insights into the interaction between HEV and the host innate immune response.

Impact of *Mycoplasma hyopneumoniae* pre-infection on Influenza infection outcome in the porcine model

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Keywords: inflammation, innate immune response, Mycoplasma hyopneumoniae, swine influenza virus, co-infection

Under experimental conditions, pre-infection of pigs with Mycoplasma hyopneumoniae (Mhp) enhanced the outcomes of H1N1 infection, mainly clinical symptoms’ severity during the first week after virus inoculation and lung injury at 7 days. The present study intended to compare early responses after H1N1 infection, in pigs pre-infected or not with Mhp. Three groups of nine pigs were included: i) H1N1-inoculated at 9 weeks of age (H1N1 group); ii) Mhp-inoculated at 6 weeks of age and H1N1-inoculated 3 weeks later (MH1N1 group); iii) mock-inoculated pigs (Control group). Three pigs from each group were sacrificed at 5, 24 and 48 hours post-H1N1 infection (hpi).

Similar clinical signs were observed in both infected groups and the extent of macroscopic pulmonary lesions increased similarly over time. However, microscopic observation and flow cytometric analyses showed earlier and more severe lung lesions in pigs pre-infected with Mhp, with an important influx of neutrophils. Pre-infection with Mhp had no effect on protein levels of IL12, IFNα, and IFNγ measured in broncho-alveolar lavage fluids, whatever the time of analysis. However, IL6, TNFα and IL1β concentrations were higher at 5hpi in MH1N1 group. Quantification of mRNA related to IFN response and regulation of innate response in lung tissues showed that H1N1 induced the expression of RIGI, IFNβ, Mx1, Mx2, OAS1, PKR and SOCS1 at 24hpi, but Mhp pre-infection did not alter their expression levels. Altogether, these data demonstrated that the Mhp pre-infection induced a higher early inflammatory response following the H1N1 infection and delayed the recovery of the animals.

The porcine lung dendritic cells/macrophages network and its involvement in influenza and PRRSV pathologies

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Keywords: Lung, Dendritic cells, Macrophages, Influenza, PRRSV

Respiratory diseases in pigs are the most important health concerns for swine producers today, as illustrated by a prevalence of pneumonia at slaughter higher than 50%. Respiratory diseases are aggregated in the so call 'porcine respiratory disease complex' (PRDC) which can include, depending of the farm, PRRSV, Influenza virus, M. hyopneumoniae, and several opportunistic bacteria. Despite these burden, there is a lack of knowledge about the immunity of the porcine respiratory tract. Here, we segregated and studied six populations of pig lung dendritic cells (DCs)/macrophages (Mθs): conventional dendritic cells (cDC) 1 and cDC2, inflammatory monocyte-derived DCs (moDCs), monocyte-derived Mθs (moMθs) and interstitial and alveolar Mθs. The three DCs subsets present migratory and naïve T cells stimulation capacities. As observed in human and mice, porcine cDC1 and cDC2 were able to induce Th1 and Th2 responses, respectively. Interestingly, porcine moDCs increased in the lung upon influenza infection, as observed in the mouse model. Pig cDC2 shared some human characteristics that are not observed in mice, such as the expression of FCεRIα and Langerin and an intra-epithelial localization. Thanks to this seminal work (Maisonnasse, Mucosal Immunol 2015) we then explored, for two of the main component of the PRC : the PRRSV and the influenza virus, two virus known for their ability to target DC and macrophages, the specific roles of these different populations in virus production and inflammation.

Scientific Session 4

Immunomonitoring

Chairs:

Michel PEPIN, Vet-Agro-Sup Lyon

Mustafa BERRI, INRA Tours

Keynote Lecture

Gilles FOUCRAS

ENV Toulouse

Resistance to mammary infections in ruminants: the unexpected role of SOCS2 on the immune mechanisms and beyond

Mastitis is an infectious disease mainly caused by bacteria invading the mammary gland. Genetic control of susceptibility to mastitis has been widely evidenced in dairy ruminants, but the genetic basis and underlying mechanisms are still largely unknown. Through a genome-wide association study, a major QTL associated with elevated milk leukocyte count or SCC as a proxy for mastitis, was identified on ovine chromosome OAR3. Full genome sequencing helped identification of one mutation in the coding sequence of a highly-conserved gene in mammals, *suppressor of cytokine signalling 2* (*Socs2)*. The frequency of the variant was 21.7% and the *Socs2* genotype explained 12% of the variance of the SCC trait. The mutation induces a R96C substitution in the SH2 functional domain, which is the binding site of SOCS-2 to various ligands, like the growth hormone receptor (GHR). Using surface plasmon resonance, we showed that the p.R96C point mutation completely abrogates SOCS-2 binding affinity for the phospho-peptide of GHR. Additionally, size and weight of homozygote sheep carrying the mutated alleles were significantly increased by 24% and 18% respectively, when compared to wild type sheep, supporting the view that the point mutation causes a loss of SOCS-2 functional activity. Whole blood gene expression was measured by RNA-sequencing in order to identify underlying mechanisms related to immunity. A significant alteration of the type I interferon signalling was revealed. Furthermore cytokine gene expression upon stimulation with heat-killed Staphylococcus aureus, a frequent mastitis pathogen in sheep, indicated that IL-17 production might be altered in relation with the mutation. Altogether these results provide strong evidence that besides milk production, SOCS2 controls the host's inflammatory response to mammary infections.

Towards the understanding of porcine cellular immune response to Chlamydia trachomatis and suis infections

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Keywords: Pig, genital mucosa, T cell, Chlamydia, pre-clinical model

Chlamydia trachomatis (CT) infections cause serious diseases including infertility and trachoma. A vaccine against CT is not available but urgently needed. A recent study shows that pigs could serve as an affordable and relevant pre-clinical animal model but the porcine cellular immune response to the disease is poorly understood. Therefore, our aim was to establish a comprehensive analysis of porcine chlamydia-specific T-cell subsets. Pigs were synchronized and infected in standing estrus with 10^8 IFUs C. suis (CS) or CT intra-vaginally and intra-uterine. Then they were clinically monitored, and serum, swabs and blood were taken to analyse the humoral immune response, detect chlamydia, analyse immune cell counts and for PBMC isolation. Chlamydia-specific CD4+ T cells, CTLs, and gamma-delta-T cells were detected after in vitro restimulation of CFSE-labelled PBMC via flow cytometry while cytokine production was analysed via multiplex. Clinical scores, qPCR and serology confirm CS and CT infection with gross pathological changes in 3/4 CS-infected and 2/4 CT-infected animals. Proliferation analyses showed a chlamydia-specific CD4+ T-cell response while CTLs and gamma-delta-T cells responded less effectively. Multiplex analyses revealed IFNg and IL17 indicating a strong TH1 and TH17 responses. We incorporated recent advances regarding porcine toolbox to analyse the chlamydia-specific cellular immune response demonstrating the important role of a TH1 response upon in vivo CT infection of pigs. With the ability to comprehensively analyse not only the humoral but also the cellular responses, pigs can now serve as an important animal-model in chlamydia vaccine development bridging the gap between mice and primates.

Whole blood transcriptome analysis of Mycoplasma mycoides subsp. mycoides-infected cattle confirms immunosuppression but does not reflect local inflammation

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Keywords: cattle; contagious bovine pleuropneumonia; mycoplasma mycoides mycoides; whole blood transcriptome; immunosuppression.

Contagious bovine pleuropneumonia (CBPP), caused by Mycoplasma mycoides subsp. mycoides (Mmm), is a severe respiratory disease of cattle responsible for major economic losses in sub-Saharan Africa. Disease control relies mainly on the use of empirically attenuated vaccines that provide limited protection. Thus, understanding the virulence mechanisms used by Mmm as well as the role of the host immune system in disease development, persistence, and control is a prerequisite for the development of new, rationally designed control strategies. The aim of this study was to assess the use of whole blood transcriptome analysis to study cattle-Mmm interactions, starting by the characterization of the bovine response to Mmm infection during the acute form of the disease. For that purpose, we compared the transcriptome profile of whole blood from six cattle, before challenge by contact with Mmm-infected animals and at the appearance of first clinical signs, using a bovine microarray. Functional analysis revealed that 680 annotated genes were differentially expressed, with an overwhelming majority of down-regulated genes characterizing an immunosuppression. The main bio-functions affected were "organismal survival", "cellular development, morphology and functions" and "cell-to cell signaling and interactions". These affected functions were consistent with the results of previous in vitro immunological studies. However, microarray and qPCR validation results did not highlight pro-inflammatory molecules (such as TNFα, TLR2, IL-12B and IL-6), whereas inflammation is one of the most characteristic traits of acute CBPP. This global gene expression pattern may be considered as the result, in blood, of the local pulmonary response and the systemic events occurring during acute CBPP. Nevertheless, to understand the immune events occurring during disease, detailed analyses on the different immune cell subpopulations, either in vivo, at the local site, or in vitro, will be required. Whole blood transcriptome analysis remains an interesting approach for the identification of bio-signatures correlating to recovery and protection, which should facilitate the evaluation and validation of novel vaccine formulations.

Development of an avian sub-unit vaccine protocol against *Campylobacter*: assessment of several technical parameters

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Keywords: Campylobacter, Vaccination, Poultry

Camylobacteriosis is a major public health concern with nine million cases each year in Europe. Poultry constitutes the main reservoir of Campylobacter and poultry meat the main source of human contamination. Poultry vaccination could be a potential way to reduce Campylobacter intestinal loads and therefore impact human disease incidence. However, despite many studies, no vaccine is available yet. The goal of the present study was to develop an avian sub-unit vaccine protocol against Campylobacter. Therefore, three sequential in vivo trials aimed to assess different parameters in term of immunogenicity and protection. Each trial consisted in two immunizations using the flagellin as antigen, followed by Campylobacter infection. Concerning the immune response, ELISA tests showed that DNA/DNA vaccination by the subcutaneous or intramuscular route was ineffective in inducing an immune response whereas DNA/protein and protein/protein vaccinations by the intramuscular route were effective. The time of vaccine administration did not impact antibodies levels. Concerning the protection, none of the tested parameters allowed the decrease of Campylobacter intestinal loads for conventional Ross broiler chickens. However, we were able to totally remove Campylobacter from SPF Leghorn chickens using DNA/protein vaccination. Considering these results, new antigens, in silico selected will be assessed in vivo to improve the avian vaccination and induce both immune and protective responses

Posters

Pathways to Antigen Expression after Oral Delivery of Adenovirus-Based Vaccines

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Keywords: vectored vaccines - vectored vaccines, oral delivery

P 01

Recombinant adenoviruses (rAd) represent a promising platform for the development of orally delivered vectored vaccines. Nevertheless, the humoral and cell-mediated immune responses elicited against transgene-encoded antigen after oral delivery of rAd are currently unsatisfactory. In order to conceive strategies to optimize their administration by the oral route, a better understanding of the fate of rAd in the digestive tract is a prerequisite.

To provide a global vision of transgene expression in the intestine, whole body bioluminescent imaging was performed after intragastric administration of a rAd encoding firefly luciferase in C57Bl/6 and BALB/c mice. In complementary experiments, quantitative RT-PCR was performed in the intestine and in peripheral tissues after intragastric delivery of vector in the two mouse strains. These studies revealed that both bioluminescence and antigen-encoding transcripts were confined to the intestine, and were restricted to delimited anatomical zones. Moreover, the distribution of bioluminescence and antigen-encoding transcripts was dissimilar in C57Bl/6 and BALB/c mice. Immunohistochemical staining of tissue sections from the zones of interest revealed that at least some of the transduced cells were enterocytes.

Finally, in order to characterize the pathways by which the vector crosses the intestinal epithelium and is captured by sentinel cells, a fluorescent-labelled vector has been administered to C57Bl/6 and BALB/c mice by the intragastric route. Cells that handle vector at an early time point are currently being characterized by fluorescence microscopy of tissue sections sampled along the intestine.

Identification of the bottlenecks in expression of transgene-encoded antigen should instruct optimisation of rAd-based vaccines for oral delivery.

Genetic selection on feed efficiency impacts vaccine immune responses in the chicken

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Keywords: Chicken; Genetic selection; Feed efficiency; Humoral immunity

P 02

According to the resource allocation trade-off hypothesis, excessive selection on production traits may jeopardize animal robustness, including the capacity to respond to immune challenges. Residual feed intake (RFI) measures feed efficiency and is defined as the difference between observed and expected feed intake (FI) based on metabolic body weight, growth and egg production. We set out to compare the immune response to a classical viral vaccine program in the divergently selected low efficient (R+, high FI) and high efficient (R-, low FI) chickens from a layer breed, displaying the same body weight at any given age (Bordas et al, 1992). We observed a significant higher humoral immune response to IBV, NDV, IBDV and CIAV live vaccines between 8 and 12 weeks of age after nasal and/or oral delivery in R+ chickens. However R- chickens developed a better cellular response considering numbers of circulating helper and cytotoxic T cell. Strikingly R+ chickens displayed much higher numbers of circulating heterophils and macrophages compared to R- chickens. However further studies are needed to assess putative differences in the innate immune responses of the divergent chicken lines to pathogen infections. In conclusion we demonstrated that the genetic selection on a production parameter such as RFI impacts strongly specific immunity in the chicken, which most likely reflects a difference between high and low efficient birds in the allocation of metabolizable energy.

Detection of antibodies specific to Koi Herpes Virus (KHV) in carps: an illustration of the interest of serological tests in the improvement of fish diseases surveillance

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Keywords: Koi Herpesvirus (KHV); Serum neutralisation test; Antibody response; Common carps; Koi carps

P 03

Serological investigations are common and useful in mammals and poultry especially for long term control of infectious diseases. In fish, such indirect methods were developed these past years but are still relatively little used for routine surveillance, despite the fact that diseases survivors often become latent carriers with significant antibody responses. The main explanations of this under-utilization are the lack of validation data and of knowledge on the kinetics of the antibody response at various water temperatures. In this study, the analytical and diagnostic performances of an indirect and non-lethal serum neutralization (SN) test developed in our laboratory for the detection of Koï Herpes Virus (KHV) specific antibodies was assessed using 151 sera or plasma from healthy, naturally or experimentally infected carp or koi. The French KHV isolate 07/108b was used efficiently to be neutralized by sera from carps infected with European, American and Taiwanese KHV isolates but no neutralization was observed using sera specific to other aquatic herpesviruses (Chanel Catfish Herpesvirus, Herpesvirus Anguillae, Cyprinid Herpesvirus type 1). Diagnostic sensitivity, diagnostic specificity and repeatability respectively of 95.9%, 98.8% and 99.3% were obtained as well as a compliance rate of 89.9% among six laboratories involved in an inter-laboratory proficiency test of the assay. Neutralizing antibodies were detected in latently infected carps more than 25 months post-infection, with various titres as a function of water temperature. The results suggest that SN test could be used in a close future to improve the epidemiological surveillance and control of KHV disease in Europe.

In ovo vaccines based on recombinant NetB toxin and Montanide™ IMS adjuvants induced protective immunity against Necrotic Enteritis in chickens

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Keywords: in ovo vaccine, necrotic enteritis, adjuvant

P 04

The current study was conducted to investigate the effects of in ovo injection of recombinant clostridium NetB toxin plus Eimeria profilin proteins in combination with Montanide adjuvants in modulating immune system in chickens infected for experimental necrotic enteritis (NE) disease. First, the safety of adjuvants was tested for in ovo injection in broiler eggs at 18 days of incubation. In a second step, broiler eggs were injected with 100µl of PBS, profilin, profilin plus NetB, profilin plus NetB/Montanide IMS OVO adjuvant (OVO). Intestinal lesion scores, body weight gains, Net-B toxin antibody levels, and proinflammatory cytokine and chemokine levels were measured as outcomes of protection following oral co-infection with C. perfringens and Eimeria maxima. Birds in ovo vaccinated with recombinant profilin plus NetB proteins/OVO showed significantly increased body weight gains and reduced gut lesions compared with the profilin-only group, respectively. Greater NetB toxin antibody titers were observed in the profilin plus NetB/OVO group compared with the other three vaccine groups. Finally, decreased levels of gene transcripts encoding interleukin-8, tumor necrosis factor superfamily 15, and TNF-α factor were observed in the intestinal lymphocytes of chickens in ovo injected with profilin plus NetB toxin in combination with OVO compared with profilin protein alone bird. These results suggest that in ovo injection with recombinant profilin plus NetB proteins in combination with Montanide IMS adjuvants enhances protective immunity against experimental NE disease.

Quantification of early innate immune responses in chicken blood using microfluidic expression arrays

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Keywords: Gallus gallus, innate immunity, microfluidic arrays, total blood, TLR agonists

P 05

Developing high throughput applications for high precision analyses of individual variations of innate immune abilities is essential to develop preventive strategies such as genetic selection to improve chicken health and robustness. We followed the kinetics of expression levels of 13 genes involved in the early innate immune response in the blood of 6-weeks-old laying hens stimulated by intravenous injection of the TLR3/4 agonists, poly(I:C) and LPS. The 40 animals being tested belonged to two divergent lines selected for their residual feed intake (RFI), which presumably possess distinct immune response abilities (cf Quéré et al., IAD2016). Gene expression was assayed using a Biomark microfluidic dynamic array. Our results show a strong difference of expression according to time (prior to and 4/8 h after stimulation) and to the agonist injected. Some of the genes analyzed, e.g. TLR4 or IL-8, also vary according to the genetic line, indicating that RFI and immune response are two physiologically related traits (cf Quéré et al., IAD2016). Hence, we successfully employed the microfluidic array analysis for the determination of individual gene expression variations in animals with different immune abilities and stimulated with different molecules. The development of more elaborated microfluidic arrays could be a useful means for testing individual immune response abilities in a high throughput setting using samples obtained by minimally invasive blood collection.

Innate and adaptive immunity synergize to trigger inflammation in the mammary gland

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Keywords: Mastitis; hypersensitivity; IL-17; IFN-gamma

P 06

The mammary gland is able to detect and react to bacterial intrusion through innate immunity mechanisms, but mammary inflammation can also result from antigen-specific adaptive immunity. We postulated that innate and adaptive immune responses could synergize to trigger inflammation in the mammary gland. To test this hypothesis, we immunized cows with the model antigen ovalbumin and challenged the sensitized animals with either Escherichia coli lipopolysaccharide (LPS) as innate immunity agonist, ovalbumin as adaptive immunity agonist, or both agonists in three different udder quarters of lactating cow. There was a significant amplification of the initial milk leukocytosis in the quarters challenged with the two agonists compared to leukocytosis in quarters challenged with LPS or ovalbumin alone. This synergistic response occurred only with the cows that developed the ovalbumin-specific inflammatory response, and there were significant correlations between milk leukocytosis and production of IL-17A and IFN-γ in a whole-blood ovalbumin stimulation assay. The antigen-specific response induced substantial concentrations of IL-17A and IFN-γ in milk contrary to the response to LPS. Such a synergy at the onset of the reaction of the mammary gland suggests that induction of antigen-specific immune response with bacterial antigens could amplify the initial immune response to infection, hence reducing the bacterial load and contributing to protection.

Canigen® DHPPi/L vaccine protects dogs against clinical signs of parvovirosis and mortality due to CPV-2c variant

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All authors are employed by VIRBAC

Keywords: Parvovirosis, vaccine, CPV-2c, Canigen

Four types of canine parvovirus (CPV) are responsible for parvovirosis: the original CPV-2 and three antigenic variants (2a, 2b, 2c). The protection provided by dog vaccines against all variants, especially CPV-2c which is the most recent, is controversial *(1)*.

The study objective was to demonstrate the efficacy of Canigen® DHPPi-L (Virbac, Carros, France) against a CPV-2c induced parvovirosis.

Twelve healthy conventional beagle puppies were randomly assigned to three groups. In Group 1, five puppies were vaccinated with Canigen® DHPPi/L (CPV780916 strain, 104.9 CCID50/mL). In Group 2, five puppies received an experimental multivalent vaccine against canine parvovirosis. In both groups, vaccination was performed at 9 and 12 weeks of age. In Group 3, two puppies remained unvaccinated. At 15 weeks of age, all puppies were exposed via oro-nasal route to 106.5 CCID50 of a virulent CPV-2c strain (D0) and observed daily for signs of parvovirosis during 2 weeks. Blood samples for leucocyte counts were taken on D-7, -3, 0, 3, 5, 7 and on the last day.

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In Group 1, 0/5 puppies developed any clinical signs of parvovirosis at any time. Conversely, all puppies of Groups 2 and 3 developped mild to severe parvovirosis (diarrhoea to haemorrhagic diarrhoea, anorexia, apathy, dehydration, >30% decrease in leucocytes count) starting four days after exposure leading to the death of 4/5 puppies of Group 2 at D6.

Canigen® DHPPi/L vaccine protects dogs against clinical signs of parvovirosis and mortality due to CPV-2c variant.

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Relationship between general and pathogen specific passive immune transfer in puppies on example of canine parvovirus antibodies

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Keywords: passive immune transfer, maternally derived antibodies, canine parvovirus, puppy

Passive immunity in dogs relies nearly entirely on colostrum intake. Deficit in passive immune transfer is nonspecifically characterized by a low blood immunoglobulin G concentration (IgG). This study aimed to evaluate the relationship between non specific immunity (IgG) and specific immunity against a ubiquitous and aggressive pathogen, canine parvovirus (CPV2). Evaluation will be conducted at two days of age (D2) and at 28 days of age (time of immunological gap).

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Puppies (n=151) were included within a multi-breed kennel with circulating CPV2. At 2 and 28 days of age, blood was collected for IgG assay (Dog IgG Quantitation Kit, Bethyl Lab, Montgomery, USA) and CPV2-specific antibodies assay (haemagglutination inhibition test; Decaro 2005). Linear regression was used to evaluate relationship between IgG and CPV2 MDA (maternally derived antibodies).

At D2, median IgG was 6.0 g/L [interquartile range: 3.6; 9.9 g/L] and CPV2 MDA was 1:320 [1:80; 1:640], with moderate correlation between both parameters ($r=0.63$; $p<0.001$). Among all puppies with MDA at D2 of 1:320 (n=44), IgG at D2 varied from 2.3g/L to 14.5g/L, with coefficient of variation of 40.4%. At D28, IgG was 1.7 g/L [1.4; 2.0 g/L] and CPV2 MDA was 1:40 [1:20; 1:80], with no correlation observed ($r=0.01$; $p=0.91$).

The absence of a strong correlation between serum IgG and specific immunity in puppies suggests that assaying IgG only provides an inaccurate evaluation of the protection level against specific pathogen. Although some puppies achieve adequate IgG transfer, it does not guarantee sufficient antibody level to prevent them from CPV2 infection.

CIRCOVAC® vaccination in piglets triggers a high and complex cell-mediated immunity

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Keywords: PCV2, vaccine, IFNg response, monocytes, T cells

Porcine Circovirus type 2 (PCV2) is the causative agent of several pig diseases. CIRCOVAC is an inactivated whole PCV2 vaccine in an oily adjuvant registered in piglets and sows. The aim of this work was to explore the cellular mediated immune (CMI) response in piglets following CIRCOVAC vaccination.

SPF piglets were monitored for their CMI response after vaccination at 3 weeks of age (D0) with CIRCOVAC or with an experimental vaccine formulated with the same PCV2 inactivated antigen, compared to control animals.

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The number of PCV2-specific IFNg producing cells was significantly higher in vaccinated piglets compared to controls at D8 for CIRCOVAC ($p<0.05$) and at D41 for experimental vaccine ($p<0.05$) and was sustained up to D132 in each group with no significant difference between vaccination groups.

Surprisingly, following irrelevant restimulation or even in the absence of ex vivo restimulation, an increase in IFNg secretion was observed only in cells collected from CIRCOVAC-vaccinated piglets at D8 ($p<0.05$) and it decreased over time up to D132. An additional experiment demonstrated that pigs injected with CIRCOVAC adjuvant only did not display this response either.

Furthermore, CD5+ T cells and SWC3+ monocytes were isolated from blood of vaccinated and control piglets. We demonstrated that only the co-culture of CD5+ T cells and SWC3+ monocytes from CIRCOVAC-vaccinated animals could lead to IFNg production without any ex vivo antigenic stimulation suggesting a long-term presentation of the antigen, the mechanism of which has to be explored.

Those studies demonstrated the efficacy of CIRCOVAC to induce a quick, high and prolonged CMI towards PCV2. Moreover, an additional activation of PBMCs derived from CIRCOVAC-vaccinated pigs was evidenced and could be linked to the particular combination of CIRCOVAC antigen and its adjuvant.

The cellular protein gC1qR of the complement differently interacts with the capsid proteins of the porcine circoviruses

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Keywords: capsid, complement, porcine circovirus

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Porcine circoviruses (PCV) are small, non-enveloped single-stranded DNA-viruses. Porcine circovirus type 2 (PCV-2) is associated to several diseases named porcine circovirus associated disease (PCVD) including post-weaning multisystemic wasting syndrome (PMWS) whereas porcine circovirus of type 1 (PCV-1) is non- pathogenic. gC1qR is a membrane-located receptor of the complement protein subunit C1q and interacts with PCV capsid proteins. The mechanisms associated with the triggering of PMWS are not well known and gC1qR may have a role in the viral life cycle and eventually in the pathogenicity of PCV. The objective of this study was to determine the region of PCV-2 capsid protein (Cap) required for the interaction with gC1qR and to evaluate the interaction of gC1qR with several PCV Cap proteins using double yeast hybrid assay. It has been demonstrated that the 59 N-terminal amino acids of PCV-2 Cap, an arginine-rich region non-exposed to the surface of the virus, are involved in the interaction with gC1qR. Porcine gC1qR interacts with Cap proteins of two pathogenic viral strains, PCV-2a and PCV-2b, while interaction has been observed with only one Cap protein of two investigated strains of PCV-1. This result suggests that the different interaction of gC1qR with PCV Cap protein may have an impact on the pathogenicity of the PCV.

Marine Sulfated Polysaccharide extract stimulates in vitro intestinal immune mediators, and enhances colostrum IgG and milk IgA in lactating sow

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Keywords: Seaweed, immunity, pig model

P 14

Marine algae contain in their cell wall water-soluble sulfated polysaccharides with potential biological activities such as antiviral, antibacterial and immunomodulating activities that are being explored to be used as an effective alternative to antibiotics. A Marine Sulfated Polysaccharide extract (MSP) was prepared from the green algae Ulva armoricana harvested in Brittany region (France). The ability of this MSP to stimulate the expression of the immune response mediators was evaluated using an in vitro system of porcine differentiated intestinal epithelial cells IPEC-1. Analysis by RT-qPCR showed a significant increase of mRNA expression of several cytokines such as IL1_, IL1_, L6, IL8, TNF_, TGF _and CCL20 chemokine as well. This stimulation of immune response mediators by the MSP was shown to involve the activation of TLR2 and TLR4 receptors.
In this study, we also assessed whether feed supplementation of sow with 2g (EA1), 8 (EA2) and 16 (EA3) g/day of MSP could increase anti-Bordetella bronchiseptica IgG in colostrum and total IgA in milk. The kinetics between the serum taken before the farrow and the colostrum showed an increase in the anti-Bordetella bronchiseptica IgG titer in the EA3 group ($P<0.05$). Regarding the IgA level in the colostrum and the milk, the E2 group showed a higher level of IgA than the control ($P<0.05$) whereas the EA1 and EA3 groups denoted an inhibition compared with the EA2 group.
All these results suggested that the MSP supplementation could be used as a new prophylactic strategy to stimulate the specific gut immune response in sows and the subsequent transfer of lactogenic immunity in suckling piglets.

Molecular, enzymatic and cellular responses of rainbow trout exposed to herbicide and challenged with infectious hematopoietic necrosis virus (IHNV)

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Keywords: rainbow trout, herbicide, infectious hematopoietic necrosis virus (INHV), qPCR

Streams and ground water all around the world are contaminated by pesticide and several studies have shown that contaminants can modulate fish immune system and reduce host resistance to pathogens.
Main objective of this study was to evaluate the susceptibility of rainbow trout, Oncorhynchus mykiss to an experimental challenge with infectious hematopoietic necrosis virus (INHV) after a chronic exposure to pendimethalin, an herbicide frequently used in agriculture and measured at high level in river of several countries.
After 28 days exposure to chemical, fish have been challenged by immersion in water containing 10^4 TCID50 mL−1 of IHNV. Four conditions were tested: 1) control, 2) contaminated by pendimethalin, 3) challenged with IHNV and 4) exposed to pendimethalin and IHNV. Mortalities were recorded during the 44 days post-infection (dpi) and organs were collected from dead fish for virological examination. After the chemical contamination and 24h, 96h and 6 weeks after the bath immersion, survivor fish were sampled to analyze several specific and non-specific immune markers. Lysozyme concentration, complement activity and the detection and quantification of trout anti-IHNV antibodies were assessed in trout plasma. Moreover, expression of 8 genes implicated in immune system (complement C3-1 and C3-4, interferon γ, interleukin 1β, tumor necrosis factor α 1 and 2, toll-like receptor 3 and β-defensin 3) were followed in spleen.
Pendimethalin exposure seems to have no direct impact on fish immunity but the chemical contamination modulates the susceptibility and the immune response of rainbow trout in presence of IHNV. Few significant differences were observed at the cellular level whereas more fish were positive to virus with lower viral particles concentration in the group exposed to herbicide and virus. Moreover, β-defensin expression was down-regulated and TNF expression seems early and up-regulated too in this group than those of the group only challenged with virus. In conclusion, even if no direct effect of pollutant could be demonstrated, the fish immune response in presence of virus could be modulated by contamination.

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In vitro polarization of bovine macrophages

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Keywords: bovine, M1 macrophage, M2 macrophage

Macrophages are major cells of the innate immunity. Macrophages derived from monocyte precursors undergo specific differentiation depending on the local tissue environment. Similar to the T helper type 1 and T helper type 2 polarization, two distinct states of polarized activation for macrophages have been defined in mouse and humans: the classically activated (M1) macrophage and the alternatively activated (M2) macrophage phenotypes. On the other hand, these different patterns of macrophage differentiation drive adaptive responses during the stages of infection, hence restraining inflammation and favoring tissue repair. In vitro generation and characterization of these subpopulations are essential to perform relevant studies understanding the host-pathogen interactions. Currently, several in vitro differentiation and polarization protocols are used to induce M1 or M2 mouse and human macrophages but none have been developed for the bovine species.

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We developed a method for in vitro differentiation and polarization of bovine macrophages using GM-CSF and IFNg to induce M1, and IL-4 to induce M2 phenotype. We characterized M1/M2 macrophages by specific morphology, production of cytokines (IL-10, IL-12, TNF-a), NO production, and phenotypic markers such as CD206 and TLR2.

Towards a better definition of the immuno-proteome in the frame of contagious or vector-borne animal diseases

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Keywords: proteomics, immuno-capture, antibodies, biomarkers

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Defining the repertoire of antigenic targets is central to better understanding the immune responses against wether contagious pathogens and those transmitted by arthropod vectors. Traditional molecular approaches of antigen discovery have identified many immunodominant antigens, but they afford limited proteome coverage. Advances in proteomic technologies that are based on peptide library and the increase in genome sequencing that enriched molecular databases, allowed the definition of new analytical strategies with interrogation of the entire proteome for antigens. At the same time, improved technologies for antibodies purification for serum as well as antigens immunocapture lead scientists to revisiting the characterisation of immuno-proteomes, particularly in the frame of contagious or vector-borne animal diseases. Here, we propose an analytical workflow to illustrate how to deepen the definition of the immuno-proteomes, and illustrate the proof of concept targeting Mycoplasma mycoïdes, the causative agent of contagious bovine pleuropneumonia (CBPP).

Bovine innate immunity against *Mycobacterium bovis*

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Keywords: Lung, innate immunity, bovine tuberculosis, mycobacteria, cattle

P 18

Bovine tuberculosis (bTB) is a chronic disease of farmed cattle and wildlife, which may also be transmitted to human, presenting a zoonosis risk. The cost of the active abattoir surveillance (13 millions €/year) so far allows the maintaining of a "bTB" status in France since 2001. Nevertheless, this status is fragilized by the increasing incidence of bTB in some regions (Dordogne, Côte d'Or, Camargue). Even thought the circulation of Mycobacterium bovis (Mb) the main agent of bTB is not precisely characterized, the transmission through the respiratory route as been associated with manifestation in the lungs and the associated lymph nodes. The analysis of the cattle alveolar environment and the associated lymphoid tissues will allow us to better understand the critical primary steps of the mycobacteria infection.

Our laboratory "Infection mycobactérienne animale" has a deep expertise on paratuberculosis and the host response to Mycobacterium tuberculosis (Human tuberculosis) and BCG (attenuated Mb used for Human vaccination) and has just starting to study bTB one year ago. Our primary goal is to characterize the cattle lung environment at the basal level and following Mb interaction to evaluate the initial immune response and more globally the tissue signature following Mb infection.

From different sources, we are currently recovering bovine samples: blood, broncho-alveolar lavages (BAL), lung and draining lymph nodes. We have set up a protocol for alveolar macrophages culture from BAL, and now aim to study their response after a co-culture with Mb. We will also use ex vivo approach, with precision cut lung slices (PCLS), to decipher the cross-talk between Mb and the host.

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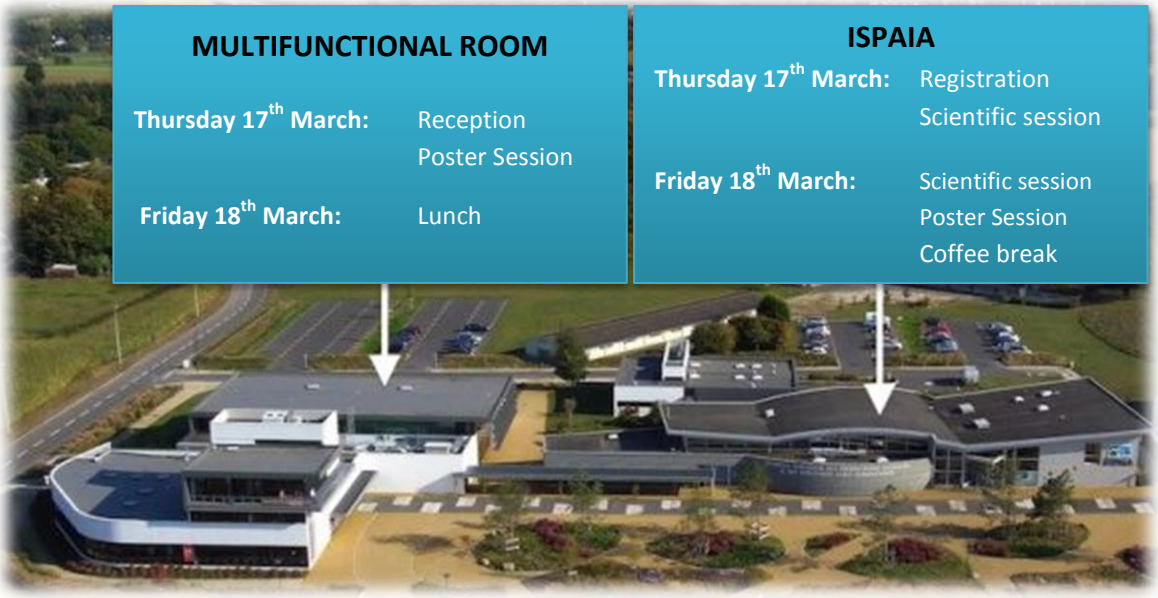
ZOOPOLE
développement



MULTIFUNCTIONAL ROOM

Thursday 17th March:	Reception
	Poster Session
Friday 18th March:	Lunch

ISPAIA

Thursday 17th March:	Registration
	Scientific session
Friday 18th March:	Scientific session
	Poster Session
	Coffee break