# Risk assessment for influenza D in Europe

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## Abstract

Recent studies have identified a new genus of the *Orthomyxoviridae* family, Influenza D virus (IDV). This virus was shown to infect farm animals including swine and cattle, and to efficiently replicate and transmit in ferrets, the animal model of choice for transmission of influenza A virus to humans. This partnering grant on IDV addressed the need for capacity building at EU level to improve the EU's scientific assessment capacity and international competitiveness. We have promoted cross-disciplinary cooperation between the partner institutes representing six Member States (BE, FR, IT, LU, NL and SE). We have shown that the available antigen and genome test systems allow reliable influenza D diagnostics in partners' laboratories, while for a few of the applied antibody testing methods adjustments are recommended. Tools were developed to study virus-host range, with a gain of knowledge on host and tissue tropism of IDV in farm animals but also in wild life and very preliminary data was generated on human tissues. Serological results in European cattle suggest that influenza D virus is enzootic. Virus diversity is still unfolding: new virus introductions were identified, as well as new reassortants whose differential clinical impact or cross-protection levels are still poorly understood. Considering drivers of emergence, IDV was in the top five in comparison with 29 other diseases. The main risk factors of IDV in cattle are related to the animal density, presence of respiratory clinical signs in cattle and contact rates between animals. Simplified quantitative IDV risk assessment exposure model indicated a possible infection of human by IDV through aerosols in cattle farms. Further studies are warranted to fully assess the risk of IDV for both animal and Human health in Europe. 

**Key words:** influenza D virus, diagnostics, tissue binding, surveillance, seroprevalence, genetic diversity, risk assessment

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by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

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# 1. Introduction

**Background and Terms of Reference as provided by the requestor**

This contract/grant was awarded by EFSA to: Institut National pour la Recherche Agronomique

Contractor/Beneficiary: Mariette Ducatez

Contract/Grant title: Risk assessment for influenza D in Europe

Contract/Grant number: GA/EFSA/ AFSCO/2017/01 – GA04

# 2. Methodologies

## 2.1. Methodology for WP1: Evaluation of the diversity, sensitivity and specificity of influenza D detection methods (WP leader: Siamak Zohari, SE-SVA)

To inventory the different assays in place in the partners laboratories to both detect the virus (via molecular tests) and anti-IDV specific antibodies, a questionnaire was developed and allowed for listing all the methods used in the consortium.

Different sera and viruses were then selected in order to set up a proficiency testing scheme (PTS). Two proficiency testing schemes were then carried out with a focus on both molecular and serological diagnostic methods. In 2018, both serology and molecular PTS were organized, with 10 sera and 13 nucleic acid blind samples to be tested, respectively. In 2019 a serology panel alone was organized with 6 antigens to test by hemagglutination test and 10 anonymised coded lyophilised sera for antibodies detection.

## 2.2. Methodology for WP2: Assessment of host range and tissue tropism (WP leader: Hélène Verheije, NL-UU)

Respiratory tract tissues were selected from pig, cow, goat, sheep, horse, roe deer, red deer, reindeer, hog deer, wild boar, river hog, rock goat, springbok and human. Tissues were embedded in formalin and paraffin blocks were sectioned to 3–4 µm. Sections were stained with hematoxylin and eosin (H&E) and assessed microscopically. Human tissue specimens were obtained from bodies through a donation program, where informed consent was given during life that allowed the use of the body for educational and research purposes. The generation of tissue microarray (TMA) blocks has been described before (Wickramasinghe et al., 2015).

Codon-optimized genes encoding HEF of Influenza D strains Oklahoma and Nebraska [GenBank: MG720235 and KM392471 respectively] were obtained from GenScript (USA), and cloned into the pCD5 expression plasmid as described before (Wickramasinghe et al., 2015) . To generate HEF proteins with inactive esterase activity, Q5 side directed mutagenesis (NEB) was performed to change the coding sequence of a serine at position 57 into that of an alanine. In all resulting expression plasmids, the HEF gene was preceded by an N-terminal CD5 signal peptide, and followed by a c- terminal GCN4 trimerization domain (GCN4), superfolder GFP sequence, and Strep-Tag II (ST; WSHPQFEK, IBA GmbH).

Recombinant HEF proteins were produced in a mammalian expression system as described before (Wickramasinghe et al., 2011). Protein histochemistry was performed by applying precomplexed HEF

proteins and Strep-Tactin HRP to TMAs and incubated at 4°C overnight, after which AEC (3-amino-9-ethylcarbazole, Dako, The Netherlands) substrate was used to detect binding of proteins.

## 2.3. Methodology for WP3: Assessment of influenza D virus geographical distribution in Europe (WP leader: Chantal Snoeck, LU-LIH)

A database was developed to gather the extent and the results of serological and virological surveillance activities within the consortium in a harmonized aggregated manner. Surveillance results were shared and discussed during teleconferences calls while the overview table was updated on a regular basis by the partners and shared with the consortium partners.

Active surveillance activities were carried out in Italy and Sweden; passive activities in France and Luxembourg in various domestic and wild species (including cattle, pig, sheep, goat, horse, wild boar and wild ruminants). The prevalence of influenza D-specific antibodies was assessed by hemagglutination inhibition tests, Mab-based competitive ELISA and indirect ELISA newly developed by members of the consortium.

Real-time RT-PCRs (Faccini et al., 2017; Hause et al., 2013) were used to assess the presence of influenza D virus in upper (nasal swabs, oral fluid) and lower (bronchoalveolar lavage, lung biopsies) respiratory tract samples from animals with respiratory disease or asymptomatic animals.

Partial or complete genome sequencing was carried on original samples or isolated viruses. Viruses were isolated on immortalized cell lines (ST cells, CACO-2 cells, HRT18 cells). Depending on the partner institution and the samples, genome sequencing was performed by Sanger sequencing or Next Generation Sequencing using Illumina MiSeq Instrument. Phylogenetic analyses included all sequences available on public databases as well as sequences obtained within the consortium. Phylogenetic analyses were carried out in MEGA6 software with Maximum Likelihood method after determining the best nucleotide substitution model for each gene segment individually.

## 2.4. Methodology for WP4: Modelling the emergence risk of influenza D virus in Europe (WP leader: Claude Saegerman, BE-ULg)

To study the drivers of IDV emergence, a questionnaire survey was designed using the same template as in Bianchini et al, 2020. An expert opinion was performed to fulfil the questionnaire survey. The risk factors of IDV in cattle were identified using univariate and multivariate logistic regression (joint collaboration between the University of Liège, Belgium, and the Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg).

A retrospective study using archive Human sera was carried out to estimate the exposure of a sub-population at risk (i.e. veterinarians) against IDV using a solid-phase competitive ELISA (SPCE) for antibodies specific to IDV (IZSLER). The sensitivity and the specificity of this ELISA test was previously estimated as 99.78%.The simplified quantitative human exposure risk assessment through airborne transmission was developed in @Risk 7.5.2 software (Palisade Corporation, Ithaca, New York, USA), using a stochastic modelling.

# 3. Assessment/Results

In contrast to IAV, there is only very scarce information on Influenza D and its risks for animal and human health. At the start of our EFSA project, IDV seemed to be circulating at a global level in farm animals, as antibodies against IDV had been detected in cattle in North and central America (USA, Canada and Mexico), Asia (China and Japan), Africa (Benin, Togo, Morocco and Kenya), but also in France and Italy (Chiapponi et al., 2016; Ducatez et al., 2015; Hause et al., 2014; Hause et al., 2013; Horimoto et al., 2016; Jiang et al.,

2014; Mitra et al., 2016; Ng et al., 2015; Salem et al., 2017) . The project generated data showing that the virus circulation is even wider both in terms of geographical localisation and host range: within the project period, IDV antibodies were detected in Swedish cattle, and small ruminants and wild boars were shown weakly seropositive for IDV in France and Italy.

The scope of our project was to generate essential knowledge in order to understand the risk of IDV and perform better risk assessment and to understand the driving force of IDV spread. All parts of the projects including development of diagnostic tools, assessing the virus host range and tissue tropism, its geographical distribution and the modelling the emergence risks of influenza D virus in Europe will act as support for the models for risk assessment. Below is a summary of our results per work package:

## 3.1. Results of WP1: Evaluation of the diversity, sensitivity and specificity of influenza D detection methods (WP leader: Siamak Zohari, SE-SVA)

To the extent possible, the activities of the WP1 had a focus on the partner laboratories diagnostic capacity and performance for the influenza D virus.

The information generated from the questionnaires provided a good overview of the diagnostics used at the partners' laboratories for both molecular and serological diagnostic of influenza D virus. Serological diagnostic tools were developed in order to increase the sensitivity and specificity of the available tests: IZSLER (partner 5) developed a blocking ELISA (Moreno et al., 2019) and SVA (partner 2) developed an indirect ELISA to screen cattle samples.

The two proficiency testing schemes for serological and molecular diagnostics have revealed a few points that require attention and improvement in the participating laboratories such as the type of red blood cells to use (chicken blood seemed to give better results) or the 96 wells plates (V bottom to be preferred to U bottom).

As far as the molecular diagnostics assays were concerned, all participating laboratories were able to successfully detect different IDV strains with their own RTqPCR assays with similar sensitivities.

As far as serology assays were concerned, epidemiological positivity thresholds could be established for HI/ELISA titers and agreements between serology assays were calculated and reached 1:10. While all participating laboratories performed very well in the molecular PTS, discrepancies in results were observed in the serology testing. Comparison of results from the two PTS allowed for recommendations on better protocols. ELISAs showed better sensitivity than HI assays and were more robust (no discrepancy observed between laboratories for the ELISA). For HI assays, chicken red blood cells performed better than horse red blood cells; V bottom plates better than their U bottom counterparts.

Comparing the 2 PTS, there was a clear improvement of serology results between year 1 and 2 in terms of number of laboratories performing the tests and in terms of quality of the results obtained (Figure 1 below).

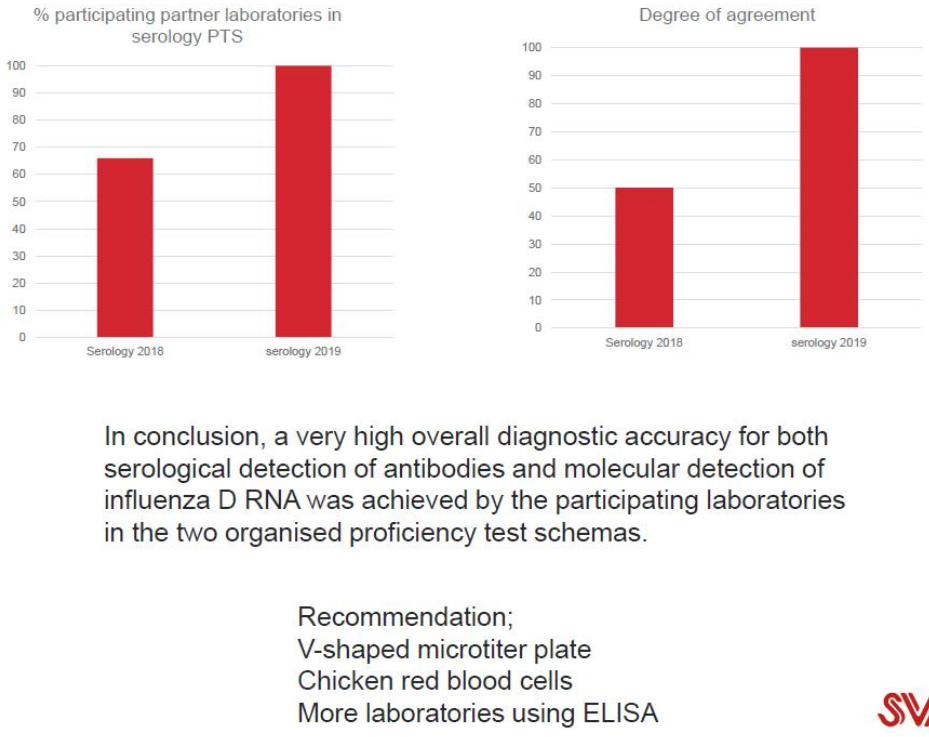


**Figure 1. Improvement of IDV serological diagnostic skills within the project.**

## 3.2. Results of WP2: Assessment of host range and tissue tropism (WP leader: Hélène Verheije, NL-UU)

### Expression and analysis of IDV-HEF

Recombinant HEF proteins of the 2 main IDV clades, D/swine/Oklahoma/2011-like (IDV/OK) and D/bovine/Nebraska/2012-like (IDV/NEB), could be produced from mammalian cell culture cells and detected by Western blot based on the presence of the StrepTag. For all subsequent studies we used HEF proteins from which esterase activity was knocked out to study the presence and localization of the viral receptor on various tissues and hosts.

### Binding of HEF to bovine and porcine TMAs

When applying HEF/OK and HEF/NEB to bovine respiratory tract tissues, both proteins appeared to have equal ability to bind to pharyngeal, as well as nasal epithelial cells, indicating that the host receptor is expressed on these tissues. Interestingly, HEF/NEB showed a stronger staining intensity of epithelial cells in the lung, suggesting that the affinity of this virus strain for lower respiratory tract tissues might be higher compared to that of HEF/OK. Future studies will have to reveal whether this is due to increased avidity for one particular receptor or whether IDV/NEB has the ability to bind to more host factors then IDV/OK.

For porcine tissue, the clearest epithelial cell binding was observed for the nasal epithelium, and not in the other tissues of the respiratory tract. In the pharyngeal epithelium in particular also staining of the subepithelial mucosal glands could be observed. These tissues appear to be rich in sialic acids, but as they are not directly assessible for virus when entering the host, they might not be involved in entry and uptake of the virus.

**Binding of IDV/OK-HEF to goat, sheep, and horse respiratory tissues**

Next we studied the binding of IDV/OK-HEF to respiratory tract tissues of sheep, goat and horse. The results showed that the HEF protein could bind to tissues of all species, being either goblet cells or epithelial cells. It is not yet understood what the impact of the ability of staining the mucus-producing cells is, compared to binding to epithelial cells. Between the three species, slight differences were observed, but tissues of more animals need to be included to confirm these results, and to see whether there are differences between the two IDV strains. The ability of all species to bind the viral attachment protein is in agreement with the reported antibodies against IDV.

**Preliminary data on IDV/OK-HEF binding to wildlife and human tissues**

Initial studies on binding of OK-HEF to respiratory tract tissues of various wild life species demonstrated that the nose of some, but not all, express receptors for attachment of IDV. Conclusions on the receptor expression in wild life is currently limited by the fact that for the mentioned species tissues of only one animal are available, while also sometimes the quality of the tissues was not ideal due to long time between death of the animal and collection of the tissues.

On respiratory human tissues, initial results showed binding of HEF to submucosal glands, and not to the epithelial cells. However, we cannot exclude that the receptors on the tissues were affected by the long term storage and preservation of these tissues in formalin. Again, more research will be needed to confirm these results.

## 3.3. Results of WP3: Assessment of influenza D virus geographical distribution in Europe (WP leader: Chantal Snoeck, LU-LIH)

Based on continuous data exchange between consortium partners, the geographical distribution of influenza D in partner countries could be assessed. Exchanges of sequences also allowed to assess virus diversity.

**Serological surveillance of influenza D virus**

Seroprevalence in cattle ranged from 36.3% in France to 81.8-84.2% in Luxembourg and Italy. Seroprevalence in pigs ranged from 3.1% in Luxembourg to 9.3% Italy (Foni et al., 2017; Oliva et al., 2019; Snoeck et al., 2018). Seroprevalence in sheep ranged from 0.8% in France (Oliva et al., 2019) to 5.5% in Italy. Seroprevalence in other species was very low (below 1%).

These results suggest species and regional differences in exposure to influenza D virus. Very high seroprevalence levels in cattle is consistent with the hypothesis that cattle are likely the main host of influenza D virus. High exposure rates in Luxembourg and Italy are also in line with high seroprevalence reported in Ireland (O'Donovan et al., 2019) , and suggest that the virus likely circulates in cattle herds in other European countries given cattle trade. Cattle may also potentially be the source of virus circulation in other species, as reflected by the consistent higher seroprevalence in Italy in all species investigated, compared to other countries.

**Molecular surveillance of influenza D virus**

Similar trends were observed, as compared to serological surveillance. A significant ten-fold difference in virus detection in samples from cattle compared to pigs was observed ($p<0.001$). In Italy, virus prevalence in cattle varied between 6.3% to 9.5% but did not vary significantly according to surveillance year ($p=0.087$), while prevalence in pigs ranged between 0.3 and 2.8% (Foni et al., 2017; Snoeck et al., 2018). Despite differences in surveillance schemes between Italy (mainly passive surveillance) and Luxembourg (active surveillance), virus prevalence in pigs did not significantly differ between both countries ($p=0.256$).

The type of samples used for molecular surveillance had significant influence on the virus detection rates observed. For both cattle and pigs, virus detection in nasal swabs was significantly higher than compared to lung biopsies or oral fluid.

**Genetic diversity of influenza D viruses**

Although the majority of viruses were isolated from cattle, some influenza D viruses of swine origin were obtained. Phylogenetic analyses revealed that influenza D strains from cattle and swine are interspersed and no separate clusters according to species appeared, suggesting multiple cases of interspecies transmission.

Geographic clustering was however observed. Until March 2018, all EU strains belonged to a single cluster (D/OK-like), with two exceptions. One strain from France and one from Ireland (partial sequence) clustered in a separate group (Ducatez et al., 2015; Flynn et al., 2018). As of March 2018, a new cluster of strains appeared in Italy (D/660; (Chiapponi et al., 2019). These results suggest a limited number of virus introductions in Europe that later spread within Europe. Parallel introductions of viruses of similar genetic background cannot be ruled out, although less likely. Within the first EU cluster (D/OK-like), no clear country specific clustering was visible, suggesting virus exchanges between countries.

At the farm level, some within farm variability was observed. In Italy for instance, the presence of D/OK and D/660-like strains in a single farm was observed (Chiapponi et al., 2019) within a 2 week interval of sampling, highlighting the need to deepen the sequencing effort to properly understand true genetic variability and virus exchanges.

Phylogenetic analyses of all genes also highlighted an increasing virus diversity in Europe. Indeed, genetic diversity within each cluster is increasing due to virus evolution over time but also improved surveillance. New genotypes and new reassortants are also emerging divergence, justifying the need for maintaining molecular surveillance. Those analyses also revealed that the phylogenetic nomenclature initiated based on the two variants initially described in the USA (i.e. D/OK and D/660) may no longer be suitable to reflect virus diversity. As the diversity increases, the need for a more robust classification and reassortant gene mapping is raising.

## 3.4. Results of WP4: Modelling the emergence risk of influenza D virus in Europe (WP leader: Claude Saegerman, BE-ULg)

**Questionnaires (linked with samples collected in WP3) to evaluate putative risk factors/indicators for cattle (probable reservoir)**

There were difficulties to obtain data set(s) available with relevant information on cattle sera due to: (i) limited reliable data available on risk factors in Europe (e.g. results of serological test available frequently without epidemiological data at animal and herd level), (ii) no information on incidence of IDV cases (no longitudinal study available) and (iii) access to TRACES data was only possible for own country but not for other Member States.

To overcome these difficulties, we combined three strategies to identify putative drivers / risk factors of IDV. The first strategy consisted in an expert elicitation on drivers of emergence for IDV. The information generated from the questionnaire survey (28 experts included) provided a good overview of the drivers of emergence of IDV in a country (50 drivers grouped in 8 different domains) and allowed the ranking of this disease with a data set of 29 other diseases affecting livestock (Bianchini et al., 2020). IDV was ranked in the top five.

In the second strategy, a cross-sectional survey in cattle farms from Luxembourg (WP3) with additional completion of information on several putative risk factors was performed under a joint collaboration with the Luxembourg Institute of Health (laboratory tests), IZSLER (providing ELISA kit) and ULiège (statistical analysis). Several risk factors were thus identified.

In addition, the third strategy consisted in selecting risk factors from a recent study carried out in Togo, Africa (Fusade-Boyer et al., 2020) .

Finally, taking into account the results of the three above strategies, putative risk factors of interest in the European context were proposed and classified in three groups (i.e. animal density, presence of respiratory clinical signs in cattle (especially in young animals) and contact rates between animals).

**Evaluation of dynamics of infection considering factors captured during the previous task and population structure and contacts**

The development of a quantitative import risk analysis was not possible due to a lack of data. Consequently, an infectious index was built taking Belgium into account as a case study. This index integrated three main factors contributing to the infection: (i) density of hosts (mainly cattle, the proposed reservoir), (ii) prevalence of bovine respiratory disorders, and (iii) number of contacts between cattle. For the first parameter, data was easily available and mapped at province level. For the second parameter, no accurate information was available for Belgium and was identified as a knowledge gap. For the third parameter, only a surrogate data was found as the number of cattle movements. A probability of animal movements at province level was thus estimated based on SANITEL data (i.e. Belgian registration of cattle farms and animal movements).

**Risk-based surveillance plan according to results obtained in tasks 4.1. and 4.2.**

The zoonotic potential of IDV is still not completely clear, but serological and virological studies suggested that the virus might infect human (Hause et al., 2013) , especially exposed to cattle, which was considered as the most probable reservoir (White et al., 2016) .

In Italy, a serological survey on historical human sera (Trombetta et al., 2019) indicated a progressive exposition of the human population in time against the IDV. Additional results of a joint collaboration between LIH (tests in the lab), IZSLER (providing ELISA kits) and ULiège (sampling and statistical analysis) indicated IDV exposure of a target sub-population in contact with cattle (veterinarians). Consequently, a simplified quantitative IDV risk assessment exposure model was developed for human through airborne transmission route and using results from all the previous WPs. This model indicates a possible infection of human by IDV.

## 3.5. Results of WP5: project management

To coordinate the present project, we had 3 consortium meetings: kick-off and final meeting in Parma, Italy (February 2018 and February 2020), as well as a mid-term meeting in Utrecht, the Netherlands (February 2019). We also shared teleconference calls every other month. Below is a summary of the dissemination of our results.

**Communications**

| Authors | Presentation title | Location, dates | Target audience |
|---|---|---|---|
| CJ Snoeck | Surveillance and characterization of important livestock and zoonotic viruses in Luxembourg: Annual Report 2017 | Administration des Services vétérinaires de l'Etat, Strassen, Luxembourg, March 12th, 2018 | National animal health authorities |
| CJ Snoeck, A Sausy, J Oliva, M Pauly, M Bourg, S Losch, F Wildschutz, CP Muller, JM Hübschen, MF Ducatez. | Widespread circulation of Influenza D virus in Luxembourg. | ESVV-Epizone 2018, Vienna, Austria, 27-30 August 2018 | Scientists, research community |
| CJ Snoeck | Surveillance and characterization of important livestock and zoonotic viruses in Luxembourg: Annual Report 2018 | Administration des Services vétérinaires de l'Etat, Strassen, Luxembourg, June 3rd, 2019 | National animal health authorities |
| Ducatez M.F., Salem E., | Virus influenza D : un | Journées d'animation | Scientists, research |

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|---|---|---|---|
| Oliva J., Meyer G. | nouvel Orthomyxovirus avec un large spectre d'hôtes et une circulation à l'échelle planétaire | scientifique du département Santé animale INRA, Nantes, France, October 2018 ; and XXèmes Journées francophones de virologie, *Paris*, France, March 2018 | community and INRA Animal Health Department (France) |
| Oliva J., Salem E., Snoeck C., O'Donovan T., Ryan E., Czirjak G., Greenwood A., Pereradra M., Peiris M., Linden A., Volpe R., Clavel S., Ortiz K., Le Loc'h G., Meyer G., Ducatez M.F. | Influenza C and D viruses: a wide host tropism and a global geographic distribution | 8th Orthomyxovirus research conference, Hanoi, Viet Nam, September 2018 | Scientists, research community |
| M. Ducatez | Surveillance and pathogenesis of the recently identified influenza D virus | Symposium: Managing Zoonotic Infections in the era of One Health, Guangzhou Medical University, Guangzhou, China, October 2019 | Scientists, research community |
| M. Ducatez | Surveillance and pathogenesis of influenza D virus | Makerere University, Kampala, Uganda, August 2019 | Scientists, research community |
| M. Ducatez | Surveillance and pathogenesis of the recently identified influenza D virus | 4th International NIV Symposium on neglected influenza viruses, Brighton, United Kingdom, April 2018 | Scientists, research community |
| J. Oliva | Eco-épidémiologie du virus Influenza D : évaluation du spectre d'hôtes et du risque d'émergence | PhD defence, Toulouse, France, October 2019 | PhD defense audience |
| S.Zohari | Influenza D in cattle | National veterinary Institute-2019 | The Veterinarian working with ruminants diseases at the private sector for animal health organization |
| S.Zohari | Retrospective serological and virological survey of influenza D virus among cattle in Sweden | National veterinary Institute-2019 | The Veterinarian working with ruminants' diseases at the governmental agency |
| Ana Moreno, Davide Lelli, Antonio Lavazza, Enrica Sozzi, Irene Zanni, Chiara Chiapponi, Emiliana Brocchi, Emanuela Foni | MAb-based competitive ELISA for the detection of antibodies against influenza D virus | 4th International Symposium on Neglected Influenza Viruses, Brighton, UK, 18-20 April 2018 | Scientists, research community |
| Silvia Faccini, Ana | Detection of a new | Epizone 2019 Berlin | Scientists, research |

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|---|---|---|---|
| Moreno, Alice Prosperi, Andrea Luppi, Carlo Rosignoli, Marianna Merenda, Giovanni Loris Alborali, Laura Baioni, Chiara Chiapponi | genetic cluster of Influenza D virus in Italian Cattle. | | community |
| Nika Nemanichvili | IDV: assessment of host range and tissue tropism | Departmental meetings, Faculty of Veterinary Medicine, University of Utrecht | Scientists, research community |

## Material exchange

| Line to column transfer | France | Netherlands | Sweden | Luxembourg | Belgium | Italy |
|---|---|---|---|---|---|---|
| France | | D/bov/France/5920/2014; Plasmid for RTqPCR standard curve; Hedgehog fixed tissues for TMA | D/bov/France/5920/2014; Plasmid for RTqPCR standard curve; reference animal sera to use in PTS | D/bov/France/5920/2014; Plasmid for RTqPCR standard curve; | | Wild fauna sera; D/bov/Nebraska/9-555/2012 |
| Netherlands | | | | | | |
| Sweden | Profi ciency Test Scheme | | | PTS | | PTS |
| Luxembourg | | | Reference animal sera to use in PTS | | | |
| Belgium | | | | Human sera | | |
| Italy | Anti-IDV MAbs | Formaline fixed tissues of various wild life species for TMA<br><br>Anti-IDV Mabs | Anti-IDV MAbs | In house ELISA kits | | |

## Data exchange

- Regular exchange of serological and molecular screening data (all partners involved)

- Between LU and BE and between FR and BE for tests comparisons and setup of epidemiological serology assays threshold

- Between LU and BE for risk factor analyses

- Protocols:

    - HI: initially, FR and IT shared their protocols with the consortium; then 2nd PTS: protocol from SE shared to all PTS participants
    - ELISA: IT and SE shared their protocols
    - PCRs: protocols exchanges

- Sequencing: protocols exchanges between FR and LU

**Visits and discussions**

- LU & BE for risk factor analyses on serological data, by phone (several times)
- C Saegerman in LU on 31/01/2020

**Extra consortium collaborations**

- Participation of Ireland to all the consortiums activities (TC every other month, PTS)
- LU with Lao PDR: kits from Italian partner sent to local partners, sample cohort to be screen soon
- Start of a parallel project between LU and BE
- Pathogenesis project of IDV in cattle (FR and SE)
- Surveillance project of IDV in Ireland (molecular epidemiology and Seroprevalence studies): IR and FR

# 4. Conclusions

The present project enabled gains of knowledge on IDV, capacity building in Europe, and the development of working hypotheses for future projects. The main conclusions of this project are:

- The proficiency testing scheme demonstrated that the available antigen and genome test systems allow reliable influenza D diagnostics in partners' laboratories, while for serology methods adjustments are recommended;
- In our evaluation, the commonly used haemagglutination inhibition test performed poorly when compared with two in-house ELISAs from two partners' laboratory. with significant better sensitivity and specificity;
- The project allowed for the development of tools to study virus-host range (tissue binding microarrays);
- We have gained knowledge on host and tissue tropism of IDV in farm animals (clear binding to respiratory tract tissues) but also in wild life and very preliminary data was generated on human tissues;
- The comparison of 2 distinct IDV strains was carried out: we are able to look for binding specificity of different strains of.
- Serological results in cattle suggest that influenza D virus is enzootic in this species and is likely present at a larger geographical scale that the consortium countries.
- Other species are permissive to influenza D infection (small ruminant, swine) but do not yet appear to be major virus hosts. Increased seroprevalence in countries with higher virus circulation in cattle may suggest that interactions between cattle and other hosts may drive infections.
- Considering drivers of emergence, IDV was in the top five in comparison with 29 other diseases.
- Main risk factors of IDV in cattle are related to the animal density, presence of respiratory clinical signs in cattle and contacts rate between animals. The epidemiology and transmission of IDV infection needs to consider the three above main risk factors.
- A preliminary exposure model studying airborne transmission suggest that farmers and veterinarians are more at risk of IDV infection compared to the general population.

Taken together, the project allowed for sharing knowledge, skills, competencies and expertise in the field: it has enabled capacity building within Europe. The output of the project has enhanced European cooperation and generated a sustainable network necessary for detecting, preventing and responding to an emerging animal disease that could constitute a threat to animals, the feed and food chain. Influenza D virus has now clearly been shown to play a role in the bovine respiratory complex.

# 5. Recommendations

## 5.1. IDV detection: recommended protocols

- Molecular detection: assays published by Hause et al in 2013 and Faccini et al in 2017 are both as sensitive and as specific and can be used as described in the literature;
- ELISA tests should be preferred to HI tests whenever possible to detect anti-IDV antibodies
- A cut off of 1:10 is recommended to classify serum samples being negative/positive using the HI test. Chicken blood should be preferred to horse blood for HI tests, V bottom to U bottom 96 wells plates.

## 5.2. Risk assessment of IDV

- Main risk factors for IDV occurrence (according to experts): farm size, trade of living animals, age of cattle, respiratory signs.
- More studies are needed on the true prevalence of respiratory clinical signs in cattle (longitudinal studies, serology studies combined with observational studies).
- More investigations are needed to estimate more deeply the human exposure to IDV, especially in cattle farms.
- Virus diversity is still unfolding: new virus introductions were identified, as well as new reassortants whose differential clinical impact or cross-protection levels are still poorly understood, justifying the need for maintaining surveillance. In this context of increasing virus diversity, monitoring fitness-for-purpose of diagnostic tools is also warranted. Efforts on a standardized nomenclature system for IDV genotypes should also be made.

## References

Bianchini, J., Humblet, M.F., Cargnel, M., Van der Stede, Y., Koenen, F., de Clercq, K., Saegerman, C., 2020. Prioritization of livestock transboundary diseases in Belgium using a multicriteria decision analysis tool based on drivers of emergence. Transboundary and emerging diseases 67, 344-376.

Chiapponi, C., Faccini, S., De Mattia, A., Baioni, L., Barbieri, I., Rosignoli, C., Nigrelli, A., Foni, E., 2016. Detection of Influenza D Virus among Swine and Cattle, Italy. Emerging infectious diseases 22, 352-354.

Chiapponi, C., Faccini, S., Fusaro, A., Moreno, A., Prosperi, A., Merenda, M., Baioni, L., Gabbi, V., Rosignoli, C., Alborali, G.L., Cavicchio, L., Monne, I., Torreggiani, C., Luppi, A., Foni, E., 2019. Detection of a New Genetic Cluster of Influenza D Virus in Italian Cattle. Viruses 11.

Ducatez, M.F., Pelletier, C., Meyer, G., 2015. Influenza D virus in cattle, France, 2011-2014. Emerging infectious diseases 21, 368-371.

Faccini, S., De Mattia, A., Chiapponi, C., Barbieri, I., Boniotti, M.B., Rosignoli, C., Franzini, G., Moreno, A., Foni, E., Nigrelli, A.D., 2017. Development and evaluation of a new Real-Time RT-PCR assay for detection of proposed influenza D virus. J Virol Methods 243, 31-34.

Flynn, O., Gallagher, C., Mooney, J., Irvine, C., Ducatez, M., Hause, B., McGrath, G., Ryan, E., 2018. Influenza D Virus in Cattle, Ireland. Emerging infectious diseases 24, 389-391.

Foni, E., Chiapponi, C., Baioni, L., Zanni, I., Merenda, M., Rosignoli, C., Kyriakis, C.S., Luini, M.V., Mandola, M.L., Bolzoni, L., Nigrelli, A.D., Faccini, S., 2017. Influenza D in Italy: towards a better understanding of an emerging viral infection in swine. Sci Rep 7, 11660.

Fusade-Boyer, M., Pato, P.S., Komlan, M., Dogno, K., Batawui, K., Go-Maro, E., McKenzie, P., Guinat, C., Secula, A., Paul, M., Webby, R.J., Tran, A., Waret-Szkuta, A., Ducatez, M.F., 2020. Risk Mapping of Influenza D Virus Occurrence in Ruminants and Swine in Togo Using a Spatial Multicriteria Decision Analysis Approach. Viruses 12.

Hause, B.M., Collin, E.A., Liu, R., Huang, B., Sheng, Z., Lu, W., Wang, D., Nelson, E.A., Li, F., 2014. Characterization of a novel influenza virus in cattle and Swine: proposal for a new genus in the Orthomyxoviridae family. mBio 5, e00031-00014.

Hause, B.M., Ducatez, M., Collin, E.A., Ran, Z., Liu, R., Sheng, Z., Armien, A., Kaplan, B., Chakravarty, S., Hoppe, A.D., Webby, R.J., Simonson, R.R., Li, F., 2013. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. PLoS pathogens 9, e1003176.

Horimoto, T., Hiono, T., Mekata, H., Odagiri, T., Lei, Z., Kobayashi, T., Norimine, J., Inoshima, Y., Hikono, H., Murakami, K., Sato, R., Murakami, H., Sakaguchi, M., Ishii, K., Ando, T., Otomaru, K., Ozawa, M., Sakoda, Y., Murakami, S., 2016. Nationwide Distribution of Bovine Influenza D Virus Infection in Japan. PloS one 11, e0163828.

Jiang, W.M., Wang, S.C., Peng, C., Yu, J.M., Zhuang, Q.Y., Hou, G.Y., Liu, S., Li, J.P., Chen, J.M., 2014. Identification of a potential novel type of influenza virus in Bovine in China. Virus genes 49, 493-496.

Mitra, N., Cernicchiaro, N., Torres, S., Li, F., Hause, B.M., 2016. Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus. The Journal of general virology 97, 1771-1784.

Moreno, A., Lelli, D., Lavazza, A., Sozzi, E., Zanni, I., Chiapponi, C., Foni, E., Capucci, L., Brocchi, E., 2019. MAb-based competitive ELISA for the detection of antibodies against influenza D virus. Transboundary and emerging diseases 66, 268-276.

Ng, T.F., Kondov, N.O., Deng, X., Van Eenennaam, A., Neibergs, H.L., Delwart, E., 2015. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. Journal of virology.

O'Donovan, T., Donohoe, L., Ducatez, M.F., Meyer, G., Ryan, E., 2019. Seroprevalence of influenza D virus in selected sample groups of Irish cattle, sheep and pigs. Ir Vet J 72, 11.

Oliva, J., Eichenbaum, A., Belin, J., Gaudino, M., Guillotin, J., Alzieu, J.P., Nicollet, P., Brugidou, R., Gueneau, E., Michel, E., Meyer, G., Ducatez, M.F., 2019. Serological Evidence of Influenza D Virus Circulation Among Cattle and Small Ruminants in France. Viruses 11.

Salem, E., Cook, E.A.J., Lbacha, H.A., Oliva, J., Awoume, F., Aplogan, G.L., Hymann, E.C., Muloi, D., Deem, S.L., Alali, S., Zouagui, Z., Fevre, E.M., Meyer, G., Ducatez, M.F., 2017. Serologic Evidence for Influenza C and D Virus among Ruminants and Camelids, Africa, 1991-2015. Emerging infectious diseases 23, 1556-1559.

Snoeck, C.J., Oliva, J., Pauly, M., Losch, S., Wildschutz, F., Muller, C.P., Hubschen, J.M., Ducatez, M.F., 2018. Influenza D Virus Circulation in Cattle and Swine, Luxembourg, 2012-2016. Emerging infectious diseases 24, 1388-1389.

Trombetta, C.M., Marchi, S., Manini, I., Kistner, O., Li, F., Piu, P., Manenti, A., Biuso, F., Sreenivasan, C., Druce, J., Montomoli, E., 2019. Influenza D Virus: Serological Evidence in the Italian Population from 2005 to 2017. Viruses 12.

White, S.K., Ma, W., McDaniel, C.J., Gray, G.C., Lednicky, J.A., 2016. Serologic evidence of exposure to influenza D virus among persons with occupational contact with cattle. J Clin Virol 81, 31-33.

Wickramasinghe, I.N., de Vries, R.P., Eggert, A.M., Wandee, N., de Haan, C.A., Grone, A., Verheije, M.H., 2015. Host tissue and glycan binding specificities of avian viral attachment proteins using novel avian tissue microarrays. PloS one 10, e0128893.

Wickramasinghe, I.N., de Vries, R.P., Grone, A., de Haan, C.A., Verheije, M.H., 2011. Binding of avian coronavirus spike proteins to host factors reflects virus tropism and pathogenicity. Journal of virology 85, 8903-8912.