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Influenza Network

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Abstract Book

Organising committee: Enrique María Mateau de Antonio, Gwenaëlle Dauphin, Tomasz Stadejek, Lucia Dieste Pérez, Dinko Novosel - **Contacts:** esflu@swineflu.eu

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Table of content:

16. Lack of seroconversion in pigs infected by influenza A at early ages in endemic farms

Authors: Ivan Domingo Carreño, Gerard Martin, Martí Cortey, Liani Coronado, Hepzibar Clilverd, Enric Mateu

17. Biosecurity Enhanced Through Training Evaluation and Raising Awareness – A new COST Action to improve biosecurity in livestock

AUTHORS: Alberto Allepuz

18. Molecular methods for the surveillance of IAV in the Danish pig population

AUTHORS: Charlotte K Hjulsager, Jesper S Krog, Pia Ryt-Hansen, Lars E Larsen

19. Evaluation of H1N1 Influenza (Swine Flu) in Türkiye

AUTHORS: Handan Hilal Yavuz

20. Viral evolution of SwIAV in Denmark – expansion in novel reassortant viruses

AUTHORS: Pia Ryt-Hansen, Charlotte Kristiane Hjulsager, Ramona Trebbien, Jesper Schak Krog, Sophie George and Lars Erik Larsen

21. Comparison of sampling materials for detection and subtyping of swine Influenza A virus in different age groups in enzootically infected pig herds

AUTHORS: Julia Stadler, S. Gumbert, S. Zwickl, K. Lillie-Jaschniski, V. Skampardonis, M. Ritzmann, A. Graaf, T. Harder

22. The monitoring of swine influenza virus in pig herds in Slovenia

AUTHORS: Jan Plut, Brigita Slavec, Irena Golinar Oven, Marina Štukelj

23. Simulating outbreaks of avian influenza within poultry flocks

AUTHORS: Carsten Kirkeby

24. A short introduction of the project “Swine influenza A virus dynamics at pig farms” (The Netherlands)

AUTHORS: Evelien Germeraad, R. de Swart, T. Tobias, M. Houben, M. Counotte, J.A. Stegeman, W.H.M. van der Poel

25. Presentation of the PhD: “Evaluation of the effectiveness of vaccination strategies at the chain value level: The case of swine influenza virus in France”

Authors: Oumaima Mtaallah, Claire Hautefeuille, Gwenaëlle Dauphin, Andrea Apolloni

TITLE:

1. Occurrence and spread of influenza A(H1N1)pdm09 virus infection in Norwegian pig herds based on active serosurveillance from 2010 to 2014

AUTHORS: Jwee Chiek Er, Eystein Skjerve; Edgar.Brun; Tore Framstad; Bjørn.Lium

ABSTRACT:

Aim: An epidemiological study on occurrence and spread of the first incursion of influenza A virus in the Norwegian pig population.

Context: The incursion of influenza A(H1N1)pdm09 virus was detected by Norway's active serosurveillance of its pig population in 2009.

M&M and results: Active surveillance data from 2010 to 2014 revealed that 54% of 5643 herd tests involving 1567 pig herds and 28% of 23 036 blood samples screened positive for antibodies against influenza A virus. Positive herds were confirmed to have influenza A(H1N1)pdm09 virus infection by haemagglutination inhibition test. In 50% of positive herd tests, ⩾60% of the sampled pigs in each herd had antibodies against influenza A(H1N1)pdm09 virus.

Results & discussion: This within-herd animal seroprevalence did not vary for type of production, herd size or year of test. The overall running mean of national herd seroprevalence, and annual herd incidence risks fluctuated narrowly around the means of 45% and 32%, respectively, with the highest levels recorded in the three densest pig-producing counties. The probability of a herd being seropositive varied in the five production classes, which were sow pools, multiplier herds, conventional sow herds, nucleus herds, and fattening herds in descending order of likelihood. Large herds were more likely to be seropositive. Seropositive herds were highly likely to be seropositive the following year. The study shows that influenza A(H1N1)pdm09 virus is established in the Norwegian pig population with recurrent and new herd infections every year with the national herd seroprevalence in 2014 hovering at around 43% (95% confidence interval 40–46%).

Please see published article at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5080672/

TITLE:

2. Producing personalized recommendations to increase the possibility of seasonal vaccination by using machine learning and XAI methods

AUTHORS: Bekir Çetintav

Burdur Mehmet Akif Ersoy University

ABSTRACT:

Seasonal vaccination is important for people at risk of severe illness from the influenza/flu. Every year, flu vaccination prevents illnesses, medical visits, hospitalizations, and deaths. Flu vaccination also is an important preventive tool for people with chronic health conditions. On the other hand, there is always resistance to vaccination against a particular infectious disease. This is considered an important problem that affects the success of vaccination programs. In the literature, several studies have examined the desire to vaccinate against swine flu in different countries. In these studies, the behavioral data obtained mostly by surveys/questionnaires were analyzed with statistical and machine learning (ML) methods. The results obtained draw a global framework about factors affecting the willingness of vaccination. In the light of these data, more "general" approaches are developed in the field of public health. In our study, the development of "personalized" approaches is discussed. Factors affecting the target variable (in our study, target variable is “get a seasonal vaccination”) in ML models are measured in two ways: feature importance methods for global interpretations, "explainable artificial intelligence (XAI) methods" for local interpretations. In our study, we set up a ML model for estimation whether a person will get a seasonal vaccine. In addition, by applying XAI methods, we can predict the factors that affect each person's condition. In this way, "personalized" recommendations that will increase the likelihood of people to get a seasonal vaccine can be produced, and new preventive medicine applications can be developed.

TITLE:

3. Intra-farm circulation of swine influenza virus in North-east Italy between 2013 and 2020

AUTHORS: Alice Fusaro, Lara Cavicchio, Mery Campalto, Luca Tassoni, Ambra Pastori, Ilaria Belfanti, Marilena Carrino, Monica Mion, Letizia Ceglie, Laura Gagliazzo, Maria Serena Beato

Istituto Zooprofilattico Sperimentale delle Venezie

ABSTRACT:

The high genetic diversity among the swine influenza virus (SIV) characterized in Europe, as a consequence of multiple reassortment events, and the crucial role of swine as a mixing vessel for human and avian influenza viruses make surveillance of SIV crucial both for animal and human health perspectives. The virus endemically circulating in Europe are classified as human-like H1N2 (huH1N2), avian-like H1N1 (avH1N1) and H3N2, with avH1N1 lineage being the dominant subtype. In addition, since the first identification of the human pandemic H1N1 (H1pdmN1pdm) in feral swine in 2009, this viral strain has been repeatedly detected into swine population, in several countries worldwide.

Between 2013 and 2020, the passive surveillance in pig farms with respiratory signs conducted in Veneto and Friuli Venezia Giulia, in North East Italy, resulted in the collection of 3,522 samples, nasal swabs or lungs, processed at Istituto Zooprofilattico Sperimentale delle Venezie.
Samples were screened by a Real time RT PCR targeting the M gene, and the HA and NA genes of all M gene positive samples were characterized by multiplex Real time RT PCRs. To investigate the virus evolutionary dynamics within a farm, 42 samples - collected from ten swine farms where SIV circulation was reported at least twice over a six months period - were selected for full genome characterization through Next Generation Sequencing (NGS, Illumina MiSeq).

The sequenced SIVs belonged to the H1N1 and H1N2 and H3N2 subtypes and through the complete genome analysis it was possible to identify nine different genotypes circulating in ten pig farms. Six out of ten farms displayed multiple introductions of different genotypes, probably resulting from distinct events. Two of these farms showed the circulation of multiple genotypes in the same year and one of them hosted three different genotypes between 2013 and 2019.
Five farms showed evidence of circulation of the same genotype over a time that ranged from two to seven years, with one farm displaying the circulation of exactly the same virus between 2013 and 2019. In one farm, we identified two H1pdmN1pdm viruses that could represent a recent spill over event from humans.

Our results highlight the importance of monitoring swine farms constantly in order to reveal persistence and/or introductions of different genotypes into pig farms. Such intra-farm longitudinal monitoring approach coupled with proper collection of epidemiological data, may aid in identifying risk factors linked to new virus introductions or persistence of the virus within each farm. Ultimately, data generated may help improving farm management to reduce the risk of multiples introductions and, consequently, reassortment events enabling emergence of new viruses with zoonotic potential.

TITLE:

4. Biomarkers of inflammation, immune system, and antioxidant status in saliva: possible applications to swine influenza

AUTHORS: Lorena Franco Martínez

Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, 30100 Murcia, Spain

ABSTRACT:

This presentation aims to provide with an overview of the potential of using saliva in swine and its potential relevance in the COST ESFLU Project. For this, the research performed in INTERLAB-UMU (University of Murcia, Spain) in the last years using porcine will be briefly presented.

Especially in pigs, where blood collection is a highly stressful, painful, and challenging procedure, saliva collection is an attractive alternative. Saliva sampling is easy, does not require specialized personnel and it is well tolerated by animals. However, despite its employment in the diagnosis and detection of some infectious diseases, it is not still widely used.

Swine saliva can be also used to measure a wide variety of biomarkers of stress, inflammation, immune response, and redox homeostasis. In addition, general rules regarding saliva collection, storage and processing will be addressed, as well as other examples of saliva application that could be extrapolated to the study of porcine influenza. Overall, this presentation illustrates some of the advantages of using saliva samples in swine research and emphasizes the potential of this technique for future studies in the field.

TITLE:

5. Network analysis in animal movements data as an epidemiological tool

AUTHORS: Gavrila Amadea Puspitarani, Amélie Desvars-Larrive

Veterinary Medicine University Vienna

ABSTRACT:

Animal movements represent a major risk for the spread of infectious diseases in the domestic swine population. In this study, we adopted methods from social network analysis to explore pig trades in Austria. We used a dataset of daily records of swine movements covering the period 2015-2021. We analyzed the topology of the network and its structural changes over time, including seasonal and long-term variations in the pig production activities. Finally, we studied the temporal dynamics of the network community structure. Our findings show that the Austrian pig production was dominated by small-sized farms while spatial farm density was heterogeneous. The network exhibited a scale-free topology but was very sparse, suggesting a moderate impact of infectious disease outbreaks. However, two regions (Upper Austria and Styria) may present a higher structural vulnerability. The network also showed very high assortativity between holdings from the same federal state. Dynamic community detection revealed a stable behavior of the clusters. Yet trade communities did not correspond to sub-national administrative divisions and may be an alternative zoning approach to managing infectious diseases. Knowledge about the topology, contact patterns, and temporal dynamics of the pig trade network can support optimized risk-based disease control and surveillance strategies.

TITLE:

6. Adverse effects of Influenza A(H1N1)pdm09 virus infection on growth performance of Norwegian pigs - a longitudinal study at a boar testing station

AUTHORS: Jwee Chiek Er, Bjørn Lium; Saraya Tavornpanich; Peer Ola Hofmo, Hilde Forberg; Anna Germundsson Hauge; Carl Andreas Grøntved; Tore Framstad & Edgar Brun

ABSTRACT:

Background & Aim & Materials & Methods

Influenza A(H1N1)pdm09 virus infection in Norwegian pigs was largely subclinical. This study tested the hypothesis that the infection causes negligible impact on pigs' growth performance in terms of feed conversion efficiency, daily feed intake, daily growth, age on reaching 100 kg bodyweight and overall feed intake. A sample of 1955 pigs originating from 43 breeding herds was classified into five infection status groups; seronegative pigs (n = 887); seropositive pigs (n = 874); pigs positive for virus at bodyweight between 33 kg and 60 kg (n = 123); pigs positive for virus at bodyweight between 61 kg and 80 kg (n = 34) and pigs positive for virus at bodyweight between 81 kg and 100 kg (n = 37). Each pig had daily recordings of feed intake and bodyweight from 33 kg to 100 kg. Marginal effects of the virus infection on the outcomes were estimated by multi-level linear regression, which accounted for known fixed effects (breed, birthdate, average daily feed intake and growth phase) and random effects (cluster effects of pig and herd).

Results & Conclusions

The seropositive and virus positive pigs had decreased (P value<0.05) growth performance compared to seronegative pigs even though feed intake was not decreased. Reduced feed conversion efficiency led to lower average daily growth, additional feed requirement and longer time needed to reach the 100 kg bodyweight. The effects were more marked (P value<0.03) in pigs infected at a younger age and lasted a longer period. Despite increased feed intake observed, their growth rates were lower and they took more time to reach 100 kg bodyweight compared to the seronegative pigs.

TITLE:

7. Low susceptibility of pigs for experimental infection with recent HPAIV H5N1, clade 2.3.4.4b Objective

AUTHORS: Annika Graaf, Ronja Piesche, Julia Sehl-Ewert, Christian Grund, Anne Pohlmann, Martin Beer, Timm Harder

Friedrich-Loeffler-Institute

ABSTRACT:

Since early 2022, spread of highly pathogenic avian influenza (HPAI) virus H5N1, clade 2.3.4.4b, of the gs/GD-lineage, has exacerbated into a panzootic. Sporadic H5N1 infections of terrestrial carnivores and marine mammals associated with fatal encephalitis were reported. This includes recent HPAI outbreaks among sea lions in South America, and in a mink farm in Spain where even avian-independent transmission was suspected raising public health concerns. Possible adaption of AIV to mammalian livestock and subsequent human exposure is of particular concern, even though the current total of seven human cases globally did not point towards increased zoonotic propensity. The role of pigs as “mixing vessels” for HPAIV is largely unresolved. AIV can potentially be transmitted to pigs and further reassortment with swine influenza A viruses may contribute to the emergence of pandemic strains.

Material & Methods

Pigs were exposed nasally or alimentarily to high doses of a recent (2022) HPAIV H5N1 isolate (genotype Ger-10.21-N1.5) to assess its replication competence in pigs. We selected an HPAIV H5N1 2.3.4.4b isolate that was closely related to a mammal case but did not show any mammalian-adaptive mutations. Two groups of 4 pigs each were inoculated intranasally or by feeding one infected whole embryonated chicken egg per animal; one sentinel pig per group was associated at day 1 post inoculation.

Results

Following exposure, low-titred HPAIV H5N1 shedding, detected by real-time RT-PCR, was limited to dpi 2 (nasal group) and 4 (oral group), respectively. Correspondingly, samples from 4 pigs sacrificed at 4 dpi gave no indication of viral replication in respiratory or gut tissues, regardless of the inoculation route. Only one out of eight pigs, inoculated intranasally with HPAIV H5N1, underwent transient, low-level infection resulting in presence of marginal loads of viral RNA in several tissue specimens and seroconversion at necropsy, 14 dpi. In none of the other seven inoculated animals and two sentinel pigs consistent virus replication was revealed.

Conclusion

We conclude that pigs are unlikely to play a role in transmitting this genotype of HPAIV H5N1 among pigs and across interfaces. However, considering the ongoing massive panzootic, a plethora of new genotypes of the currently circulating strain will emerge, some of which might have higher permissiveness for pigs. Therefore, swine populations must not be a blind spot in HPAIV surveillance programmes, and focusing on periodical reassessment of possible pre-pandemic potentials of dominant circulating HPAIV H5N1 genotypes in the swine model will be indispensable as well.

TITLE:

8. An emerging viral trias of swine influenza A virus (swIAV), porcine respirovirus 1 (PRV1) and swine orthopneumovirus (SOV) in porcine respiratory disease in Germany

AUTHORS: Annika Graaf, Christin Hennig, Kathrin Lillie-Jaschniski, Monika Koechling, Julia Stadler, Jan Böhmer, Ulrike Ripp, Bernd Andreas Schwarz, Martin Beer, Timm Harder

Friedrich-Loeffler-Institute

ABSTRACT:

Objective

Respiratory disease is a common threat in pig production, with a complex etiology involving both viruses and bacteria. SwIAV are widespread in European domestic pig populations and evolve dynamically. The most recent human pandemic in 2009 has highlighted the zoonotic and reverse zoonotic transmissions of swIAV in swine. The availability of rapid, sensitive and specific diagnostic assays to detect and discriminate swIAV in clinical samples is extremely important for monitoring and control of the disease.

Material & Methods

A modified diagnostic algorithm for the reverse transcription real-time PCRs based hemagglutinin and neuraminidase subtype- and lineage-specific detection of swIAV has been developed and validated within this study by using multiplex quantitative RT-qPCR. However, 67.52 % (821/1216) of the samples from 26/123 pig holdings with respiratory signs remained swIAV-negative. As complex and synergistic interactions of swIAV infections with other viral and bacterial infectious agents contribute to an aggravation of respiratory disease in pigs, we further developed an extended simultaneous diagnostic assay for the combined detection of swIAV and the recently described PRV1 andSOV.

Results

Screening for swIAV revealed 30.76 % of the individual samples to be positive and 78.05 % of the farms to be actively infected. Findings included all three main H1-clades (1A-1C) of swIAV as well as several reassortants between them. Subtype H1avN1 was detected most frequently, followed by H1avN2 and then H1pdmN2 and H1pdmN1, respectively. The PRV1 screening revealed the presence of 19.57% positive samples and 61.79 % of the farms to be PRV1 productively infected. For SOV, 14.23 % of the samples tested gave positive results and 25.20 % of the farms were infected. In contrast to swIAV and PRV1, few SOV positive herds were found, but within these herds, a larger number of animals appeared to be SOV positive.

Discussion:

The most recent human influenza pandemic demonstrated the potential impact of swIAV in terms of sparking pandemics, underscoring the necessity for continuous swIAV surveillance. Altogether, our results show that, besides swIAV, PRV1 is widely spread in Germany. All PRV1 infections found were to nearly 30 % associated with swIAV co-infections, while SOV was found to a lesser extent, also in association to any of the other two viruses. The high incidence of PRV1 and SOV infections in pigs with respiratory disease suggest that these viruses could be further players in the porcine respiratory disease syndrome.

TITLE:

9. Low internal biosecurity: factor that favours maintenance of influenza on commercial farrow to wean pig farm in Serbia

AUTHORS: Branislav Kureljušić, Nemanja Jezdimirović, Vesna Milićević, Dimitrije Glišić, Bojan Milovanović, Jelena Maletić, Jelena Maksimović Zorić, Jasna Prodanov Radulović, Božidar Savić

Institute of Veterinary Medicine of Serbia, Belgrade

ABSTRACT:

Swine influenza A is the most common respiratory disease in pig production around the world, as well as an important respiratory pathogen that affects pig health, welfare, productivity, and has zoonotic potential. Internal biosecurity practices during the pre-weaning period are critical in farms with an endemic presence of swine influenza to prevent infection and stop transmission. The current study aimed to determine whether there is a link between internal biosecurity and maintenance of influenza on the farm.

A Biocheck online survey (https://biocheckgent.com) was used to assess biosecurity at one farrow to wean pig farm in Serbia. Each assessed category was scored on a scale of 0 (worst-case scenario) to 100% (the best scenario). In the nursery, 10 udder skin wipes were obtained from lactating sows, whereas in the weaned piglets, 10 samples of oral fluid were collected and analyzed for the presence of swine influenza A RNA using real-time RT-PCR. Throughout a year of monitoring, samples were collected six times every two months.

The external biosecurity assessment resulted in a score of 88%, compared to the Serbian average of 69% and the global average of 76%. External biosecurity assessments revealed the lowest scores for animal transport, carcass removal, and manure removal (71%). Internal biosecurity revealed a score of 67%, compared to the national average of 44% and the global average of 69%. Internal biosecurity was confirmed to have the lowest percentage score (36%) for the farrowing and suckling period. In this study, the overall biosecurity score for the pig farm was 78%. The national average was 57%, while the global average was 73%.

During 12 months of monitoring, swine influenza A virus positivity rate in investigated farm remained high in both set of samples. For udder skin wipes, the positivity rate ranged from 30% to 60%, and for oral fluid samples, it ranged from 50% to 80%. There was no seasonal variation in the positivity rate.

This study revealed low level of internal biosecurity for the farrowing and suckling period. The following suggestions were made to the farm manager: avoid moving pigs between litters after they are 3 days old; avoid using nurse sows; farm workers should change disposable gloves between litters; not entering in farrowing crates and perform daily disinfection of fomites. Implementing vaccination is another preventive measure that needs to be discussed.

This study helped to identify gaps on biosecurity and risky practices in the pig farm evaluated.

TITLE:

10. Current situation of swine influenza virus in Poland

AUTHORS: Kinga Urbaniak, Katarzyna Podgórska

National Veterinary Research Institute

ABSTRACT:

Introduction
There are no official regulations or surveillance for swine influenza A virus (swIAV) in Poland, but a small-scale 5-year study is currently being funded to provide a crude assessment of the current epidemiological situation.

Materials and methods
Samples were collected in 2020-2022 from pig farms located throughout Poland thanks to voluntary cooperation with local veterinarians and Veterinary Inspection. The main criterion was the farm location in order to obtain a representative number of samples from each region of Poland. Each year a total of 2500 sera and 50 lung tissue or nasal swabs were used for serological and molecular tests, respectively. Sera were tested by HI assay with four swIAV strains representing different lineages: H1avN1, H1pdmN1, H3N2 and H1huN2. Tissue samples and nasal swabs were tested using M1-based rRT-PCR. Positive samples were characterized by multiplex rRT-PCR and/or sequencing. Phylogenetic trees were constructed based on Neighbour-Joining method.

Results
Comparing the results of serological tests over three years, there was an overall decrease in the prevalence of swine influenza. The average percentage of positive results in 2022 did not exceed 10%, while in 2021 and 2020 it was approximately 11% and 15%, respectively. The highest percentage of positive results was recorded mainly in the north-western districts of Poland. One of the highest levels of seroprevalence in three consecutive years was found in the two districts located in northern Poland, despite the continued decline in seroprevalence in these districts. A decrease in seroprevalence was also recorded in three districts located in the south of Poland. Only in the west of Poland an increase in prevalence was detected (Dolnośląskie and Lubuskie districts). As for the occurrence of individual subtypes, the results were mainly positive for H1 with a predominance of H1pdm over H1av. Regarding molecular method, 18.6% of all samples tested over three years were positive. Based on the multiplex rRT-PCR and sequencing results, circulation of reassortant H1avN2 (2020-2022), H1pdmN1 (2020-2021) and H1avN1 (2020, 2022) swIAVs was confirmed.

Conclusions
Both the results of serological and molecular tests have shown that the dominant swIAV in domestic pig herds in Poland are viruses with HA type 1 (H1pdm and H1av). Regarding swIAV subtype, HI assay provide only partial information compared to molecular testing. Therefore, the identification of the currently circulating swIAVs should be based mainly on molecular characterization whereas for an estimation of the SI prevalence serological tests are the method of choice.

TITLE:

11. National Swine influenza surveillance program in The Netherlands, 2022-2023

AUTHORS: Erhard van der Vries, Evelien Germeraad, Annelies Kroneman, Joke van der Giessen, Dirk Eggink, Adam Meijer, Ron Fouchier, Manon Houben

Royal GD

ABSTRACT:

Context
Influenza is highly prevalent in Dutch swine herds, but information about the epidemiology and evolution of circulating influenza viruses, remains fragmented and scarce. To fill this knowledge gap, a national One Health surveillance program was set up by a consortium of the Dutch National Public Health Institute (RIVM), research institutes (EMC, WBVR, Royal GD) and the National Influenza Centre (NIC).

Material and Methods
Swine veterinary practices (n=7) were selected in geographical areas representing three different regions of the Netherlands. Practitioners were requested to collect oral fluid samples and nose swab samples from pigs with influenza clinical signs. All samples were shipped to Royal GD. Here, a RT-PCR was performed to screen for the presence of influenza virus RNA. Nanopore sequencing was then attempted directly on PCR-positive RNA eluates (Ct values < 35), or after a single virus propagation step in MDCK (SIAT-1) cell cultures. Finally, a sequence database was launched at the RIVM to collect and perform metadata analyses using sequence, clinical, epidemiological, and serological data.

Results
From November 2022 to March 2023, 522 respiratory samples were collected from piglets (80%), fatteners (17%) and sows (3%). Of these samples, 45% (235/522) were PCR-positive (Ct < 40) for influenza A virus, the majority were from piglets (80%). Virus-positive samples were from farms (n=37) located in the Northern (n=11; 30%), Middle (n=7; 19%) and Southern (n=19; 51%) part of the Netherlands. Nanopore sequencing was performed on 48 selected samples (Ct values < 35) for virus subtyping and phylogenetic analyses: 27 were of the A/H1N1 and 21 of the A/H1N2 subtype. Phylogenetic analyses of the hemagglutinin gene showed that 13 strains were of the Classical Swine (CS)-lineage (clade 1A.3.3.2-like), 4 of the Human Seasonal (HS)-lineage (clade 1B.1.2.1-like) and 22 of the Eurasian-Avian (EA)-lineage (clades 1C.2.1 and 1C.2.2-like). An influenza A/H1N2 virus from this 1C.2.2 subclade (A/Netherlands/11748/2022) caused a zoonotic infection in a Dutch adult in the fall of 2022. Finally, feedback was provided to the farmers and veterinarians about the virus subtype, lineage (clade) and %-sequence identity to commercially available influenza vaccines.

Discussion
Here, the first data obtained from the Dutch Swine Influenza surveillance program is presented. This ongoing program will allow us to better monitor the circulation of influenza viruses in pigs in the Netherlands. These data will be shared with international public sequence databases (GISAID). These data will increase our understanding of the burden and control of influenza in pigs.

TITLE:

12. Introducing the EU funded project BIOSECURE. Moving towards an enhanced and cost-effective biosecurity in livestock production

AUTHORS: Ilias Chantziaras, Jeroen Dewulf, Evelien Biebaut on behalf of the BIOSECURE consortium

Ghent University

ABSTRACT:

What?

Coordinated by Ghent University, BIOSECURE is a forthcoming four-year project supported by the European Union with a budget of 5 million euro and 18 participating partners, from 12 European countries. Biosecurity is critical for management of animal health and disease control however, implementation at farm level remains often insufficient.

Why?

The objective of the BIOSECURE project is to enable decision makers in livestock farming to understand, prioritise and implement evidence-based, cost effective and sustainable biosecurity management systems. This will be carried out through various work packages and tasks that include:

i) Reviewing the current understanding of biosecurity throughout the livestock production chain.
ii)Quantifying the impact of biosecurity practices on the prevention of infection and spread of disease.
iii) Enhancing current biosecurity measures, as well as expanding on these by carrying out field studies and performing experiments.
iv) The socio-economic impact of the BIOSECURE project will be assessed.
v) Stakeholder engagement will be facilitated to support knowledge transfer and implementation of key exploitable results.

Main components

1. Facilitate stakeholder engagement to support interactive knowledge exchange, behavioural change and uptake of key exploitable results.
2. Collect exisiting biosecurity intelligence throughout the livestock production chain.
3. Quantify the impact of biosecurity practices on infection prevention and economics.
4. Enhancement of biosecurity measures through quantitative and qualitative field studies and experiments.
5. Assesss the socio-economic impact of BIOSECURE measures beyond farm level.

How to move forward?

The new or enhanced biosecurity management systems developed in the BIOSECURE project will help farmers of various animal species (pigs, poultry, cattle, small ruminants) and of different production types (indoor – outdoor) to keep animals healthy.

TITLE:

13. An integrative One Health network to monitor and characterize influenza A viruses circulating in the humans and pig populations

AUTHORS: Ronald Dijkman, Jenna Kelly

University of Bern

ABSTRACT:

Background: Influenza viruses can infect many species, including humans and pigs. Pigs play an important role in interspecies transmission since they serve as "mixing vessels" for generation of novel pandemic influenza A viruses (IAV). Despite multiple devastating influenza epidemics and pandemics in the past, our knowledge of the IAV circulating in pigs and humans is limited. Furthermore, our understanding of the zoonotic and/or anthroponotic (reverse zoonotic) potential of these circulating IAV is far from complete.

Aim: Establish an integrative "One Health" network to systematically identify, monitor, and characterize IAV circulating in the human and pig populations.

TITLE:

14. Serological and molecular investigation of influenza in commercial pig farms in Serbia

AUTHORS: Jelena Maksimović Zorić, Ljubiša Veljović, Dimitrije Glišić, Nemanja Jezdimirović, Božidar Savić, Bojan Milovanović, Jelena Maletić, Vladimir Radosavljević, Branislav Kureljušić

Institute of Veterinary Medicine of Serbia

ABSTRACT:

Swine influenza is a worldwide present respiratory disease of swine, in majority of cases caused by H1N1, H1N2, and H3N2 subtypes of swine influenza A virus (swIAV). Exploration of the swIAV in Serbia in recent ten years mainly was oriented to the passive surveillance and examination within PRDC on commercial pig farms. The latest results suggest circulation of the viruses in the majority of the analyzed commercial farms and the presence of two subtypes - H1N1 and H3N2. Even though the problem exists none of the farms apply vaccination as a measure of disease control. The aim of this research is to analyze the results of passive surveillance on the two commercial farms conducted from 2020th to 2022nd.

A total of 94 samples for molecular analyses and 528 samples for serological analyses were submitted to the Institute of Veterinary Medicine of Serbia during this period. These samples were obtained from swine raised in two commercial farms (farm A and farm B) that practice farrow-to-finish production and have implemented satisfactory biosecurity measures. The samples for molecular examination were mummified fetuses, nasal swabs, oral fluids, parenchymatous organs, lungs, and sera. All samples were tested by M gene real-time RT-PCR, according to the accredited WHO protocol (2009) for detection of influenza A viruses. One virus was typed using multiplex real-time RT-PCR protocols described by Henritzi et al. (2016). Serological examination was conducted using ELISA test (Ingezim Influenza A Kit, Ingenasa), and tested sera originated from sows, suckling piglets, and nursing pigs.

During three year period influenza A viruses were detected on both farms, having on average 55,3% of the analyzed samples positive. The highest detection rate was amongst the nasal swab (78.7%) and oral fluid samples (83.3%), while virus was not confirmed in mummified fetuses and sera. Most of the samples analyzed by PCR originated from farm A (80 samples), and the majority of tested were positive (60%). Molecular typing revealed presence of H1N1 subtype in farm A that is in accordance with previous results that confirmed H1N1 as dominating subtype that circulates in commercial farms in Serbia. Serology examination confirmed a high overall seroprevalence of 64,6%. The serum samples from nursing pigs accounted for the highest proportion (95.4%) among those examined, as respiratory issues linked to PRDC typically manifest in this group. Given the endemic presence of swIAVs in the farms under analysis, there is a pressing need to conduct further research on the circulating viruses and enhance the control measures employed on these farms.

TITLE:

15. Presentation of the project proposal Genetic diversity of swine influenza A viruses and their implication on pig production and public health

AUTHORS: Aleksandar Dodovski, Branko Angjelovski (prinicipal investigator), Miroslav Kjosevski

Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine - Skopje

ABSTRACT:

The main goal of this research is to determine the presence, genotype (subtype), phylogenetic origin, and zoonotic mutations of swine influenza A viruses (SIAV) in commercial pig farms in order to protect animal and human health. Norther Macedonian commercial pig farms will be included in the project research, the number of which will be determined based on the equal distribution of farms by region and farm size. Blood samples will be taken from each farm in order to determine the presence of specific antibodies against SIAV which will serve as an indicator of contact with the virus. The number of blood samples from each farm will be statistically determined based on the number of animals present on the farm and the expected seroprevalence and will include pregnant and farrowing sows, pigs in the nursery, and pigs in the finishing period. The blood samples will be tested using the ELISA method. Nasal swabs from three age categories of animals will be taken from serologically positive farms. ELISA-negative farms will only be swabbed in case of the presence of animals showing clinical signs of respiratory infection. Swabs will be tested by RT-PCR for IAV matrix gene detection. The whole genome sequencing will be performed on the positive samples. They will be analyzed using bioinformatics and phylogenetic methods in order to determine the genotype circulating in pig farms in Northern Macedonia, determine their origin and similarity to other SIAVs, and the possible presence of mutations with zoonotic potential. Sequencing of the entire genome of SIAV will be performed in a commercial laboratory. In the project research, a statistical model will be created that will determine the impact of SIAV on the production parameters of the farms. For this purpose, certain annual productive parameters such as fertility, the number of weaned piglets, mortality during production, and the time of finishing period will be collected and analyzed from the selected farms. Next, the degree of biosecurity and introduced biosecurity measures will be assessed and quantified at all farms.

TITLE:

16. Lack of seroconversion in pigs infected by influenza A at early ages in endemic farms

Authors: Ivan Domingo Carreño, Gerard Martin, Martí Cortey, Liani Coronado, Hepzibar Clilverd, Enric Mateu

Universitat Autònoma de Barcelona

ABSTRACT:

Swine influenza has become endemic in most indoor pig farms of Europe, causing economic losses to the affected farms and being a potential threat to public health. Drivers of the endemic situations are not well known. Previous reports indicated that, in the endemic farms, infection may happen very early in live when animals still have maternally derived antibodies (MDA). The aim of the present study was to assess whether the presence of MDA correlated with the lack of seroconversion after infection or with prolonged shedding or reinfection.

We conducted two longitudinal studies of pigs from birth to the end of the nursery phase in two farms assessing nasal shedding of the virus and the presence of antibodies (ELISA and haemagglutination inhibition assay, IHA). The results showed that at the first week of age, most animals had detectable antibodies by either ELISA or IHA, likely indicating a colostral origin. Despite the strong evidence for viral circulation in both herds, most of the infected animals did not show any clear seroconversion and, actually, most of them ended the observation period being seronegative or with lower titres than when they were infected. No differences were observed in the IHA titres of animals at the moment when they were first detected infected and the titres of the virus-negative pen-mates. Additionally, no clear relationship could be established between the antibody titres and the prolonged shedding.

Overall, our study provides some insight into the dynamics of influenza-specific antibodies in endemic farms and highlights the complexity of these situations.

TITLE:

17. Biosecurity Enhanced Through Training Evaluation and Raising Awareness – A new COST Action to improve biosecurity in livestock

AUTHORS: Alberto Allepuz

Universitat Autònoma de Barcelona

ABSTRACT:

The BETTER COST Action began to run in October 2021 with the aim to reduce the risk of infectious disease introduction and spread by improving the implementation of biosecurity measures in animal production systems. At the moment around 250 people from 40 different countries have joined the Action to i) evaluate how biosecurity is currently used and to understand motivators and barriers for biosecurity implementation; ii) perform a comparison of existing methods used to evaluate biosecurity and to promote the development of tailored options in farms; iii) to identify training needs through the evaluation of existing training materials and to iv) recommend priority research areas for future biosecurity improvement in animal production systems.

TITLE:

18. Molecular methods for the surveillance of IAV in the Danish pig population

AUTHORS: Charlotte K Hjulsager, Jesper S Krog, Pia Ryt-Hansen, Lars E Larsen

Statens Serum Institut (SSI)

ABSTRACT:

Influenza disease is widespread in the Danish pig population. Several influenza A virus (IAV) subtypes are enzootic in Danish pigs. The distribution of subtypes has changed over the years due to the emergence of new IAV variants. The most prevalent subtypes are now H1avN2sw and IAVs with H1pdm09 in combination with different NA subtypes, mainly N2sw, N1av and N1pdm. IAVs with gene segments of human seasonal IAV origin is regularly detected in Danish pigs.
Systematic passive surveillance of circulating IAVs in pigs has been conducted in Denmark since 2011. The aim of the surveillance is to monitor the IAV subtypes and genotypes present in Danish pig population, focusing both on veterinary and zoonotic aspects.

IAVs for the investigations are obtained from samples submitted for diagnostic investigations with a request for IAV diagnostics due to respiratory disease. IAVs are subtyped and genotyped by real-time PCR and whole genome sequencing. The diversity of IAVs has increased over the years and accordingly, a constant optimization of the detection and subtyping palette has been necessary for adequate diagnostics and monitoring of the IAVs circulating in the Danish pig population.
The presentation will focus on the design of the laboratory testing scheme for the Danish surveillance of IAV in pigs, including recent adaptations of methods for detection, subtyping and genotyping of IAVs.

TITLE:

19. Evaluation of H1N1 Influenza (Swine Flu) in Türkiye

AUTHORS: Handan Hilal Yavuz

Cukurova University, Faculty of Veterinary Medicine

ABSTRACT:

Swine influenza is a highly contagious respiratory disease infection caused by influenza A field conditions have variable virulence and secondary infections may be involved. Pigs are able to replicate influenza viruses of avian and human origin and play an important role in the epidemiology of influenza. In addition pigs have a possible effect in the emergence of zoonotic strains with pandemic potential.

People can be infected by pigs, but swine strains of IAV do not spread easily in humans. However, it could be lethal in immunocompromised people. In contrast, human seasonal influenza viruses become established in pigs rarely, contributing to the overall genetic diversity of IAVs found in pigs. In 2009, an H1N1 IAV strain of swine origin spread globally; it infected humans, swine, poultry and fewer dogs, cats, and other animals.

On June 2009 the World Health Organization (WHO) declared that the influenza A/H1N1 disease is a pandemic. The first confirmed case of influenza A/H1N1 was reported in Mexico on 23 April 2009. The first laboratory-confirmed case of influenza A/H1N1 was reported on 16 May 2009 from Türkiye, and the number of laboratory-confirmed cases of influenza A (H1N1) totalled 128 of whom 38 were indigenous cases on 17 July 2009. Turkish Ministry of Health confirmed the first death on 25 October.

In the pandemic period, findings showed that swine influenza (H1N1) entered Türkiye through visitors most of coming from the United Kingdom and the United States. The big parts of confirmed cases in Türkiye had a travel history from highly contaminated areas. However, confirmed cases from the Turkish Cypriot community were mostly indigenous cases with no history of travel. The majority of the confirmed cases consisted of young adults as reported from other countries. This could be related to the frequency of travel among the young population. In the other hand, NATO İncirlik air base maj in Adana, Türkiye where primarly used by American and Turkish air forces declerated that there was no swine flu case during the pandemic.

Prevention of swine flu infection is essential. Turkish Ministry of Health gives advice on swine flu vaccination to all risk groups such as health workers, elderly and immunosuppressived people. These people are periodically called and invited to take their swine flu vaccination at the first step health care units by personal phone call. Also, vaccination is suggested for other populations. However, a resistance to vaccination is indicated. In a survey study showed that only 23% of 718 were vaccinated. In another survey, only 9.6% of medical students were vaccinated. The most frequent reasons for refusing pandemic vaccine were fear of side effects and doubts about vaccine efficacy.

In the university student population who are Faculty of Arts and Sciences, Health Services Vocational High School, Faculty of Economics and Administrative Sciences, Faculty of Education), the average score of knowledge level about swine flu obtained from the students was slightly above the middle level. The major sources of their information of most respondents reported television/radio was about swine flu (82.8%). The proportion of those who had knowledge about the fact that the most important and effective means for the prevention of swine flu is vaccination was only 32.0% in a Turkish university.

There is no study data about swine flu infection or virus detection in pigs in Türkiye.

TITLE:

20. Viral evolution of SwIAV in Denmark – expansion in novel reassortant viruses

AUTHORS: Pia Ryt-Hansen, Charlotte Kristiane Hjulsager, Ramona Trebbien, Jesper Schak Krog, Sophie George and Lars Erik Larsen

University of Copenhagen

ABSTRACT:

Since 2011, Denmark has had a national passive prospective swine influenza A virus (swIAV) surveillance program making it possible to track the changes in prevalence of HA and NA lineages over time. In addition, sequencing of selected submissions have been performed continuously and in 2022 the HA and NA lineages were based mainly on whole genome sequencing using Illumina MiSeq, in summary creating a great collection of swIAV sequences for studying the viral evolution. In 2018, a novel reassortant appeared being an "H1pdm09N1av" reassortant carrying all genes except for the NA gene of H1N1pdm09 origin. The NA gene was of Eurasian avian H1N1 origin. This reassortant spread rapidly within the Danish herds and by 2022 constituted 27 % of all submissions in which the HA and NA lineage was determined. In 2020, a similar H1pdm09N1av virus was recorded, the only difference being that this reassortant carried a NS gene of Eurasian avian H1N1 origin. By 2022, 68 % of the H1pdm09N1av viruses carried an avian NS gene. A similar pattern was observed for the swine adapted H1N1pdm09 viruses and for other of the common Danish HA and NA lineages. Interestingly, two zoonotic cases of swIAV was recorded in Denmark in 2021, one being a swine adapted H1N1pdm09 virus and one being a H1pdm09N1av virus, both with a NS gene of Eurasian avian origin. These results emphasize the importance of having continuous swIAV surveillance and illustrates how genetic data can aid in explaining patterns observed in the swine herds. Moreover, the surveillance provides a unique dataset making the basis for future research areas, where different viral properties can be studied.

TITLE:

21. Comparison of sampling materials for detection and subtyping of swine Influenza A virus in different age groups in enzootically infected pig herds

AUTHORS: Julia Stadler, S. Gumbert, S. Zwickl, K. Lillie-Jaschniski, V. Skampardonis, M. Ritzmann, A. Graaf, T. Harder

Clinic for Swine at the Centre for Clinical Veterinary Medicine, LMU Munich, Oberschleißheim, Germany

ABSTRACT:

Background and Objectives

Influenza A virus (IAV) is an important porcine respiratory pathogen worldwide. Varying infection dynamics in farms with unspecific clinical signs underline the need for adequate sampling strategies for virus surveillance. The present cross-sectional field study aimed at comparing individual and group sampling strategies to assess the most suitable sampling material for IAV detection and identification of subtypes in enzootically infected farms.

Material and Methods

20 IAV-positive German pig breeding farms with attached nurseries were sampled with different materials (nasal swabs, tracheobronchial swabs, udder skin wipes, surface wipes, dust samples, oral fluids) in different age groups (sows, suckling piglets, beginning/mid/end of nursery). Samples were analyzed for IAV genome presence and subtype by multiplex RT-qPCR. Furthermore, clinical signs (coughing, sneezing) were assessed in suckling piglets and nursery pigs.

Results

In general, the IAV detection rate was higher in group-samples compared to nasal swabs. It was shown that suckling piglets and nursery pigs were more likely to have a positive result compared to sows. Interestingly, piglets beginning and mid of nursery were more likely to have a positive PCR result compared to the piglets end of nursery. In addition, the analyses revealed differences in the percentage of subtypeable samples between the investigated age groups and sampling materials correlating with viral loads. Individual nasal swabs were significantly more often subtypeable then other sampling materials. Nasal swabs of suckling piglets showed the highest percentage of subtypeable samples (88.2%, n=15/17) compared to nursery 61.8% (n=21/34) and sows 0% (n=0/9). Subtypes detected were H1avN1, H1avN2, H1pdmN1pdm, H1pdmN2, H1huN1, H1huN2. In 25% of the farms, more than one subtype was found, in 4 of these farms they were found in different age groups. Clinical signs were significantly more often observed in nursery piglets compared to suckling piglets ($p<0.001$). However, the mixed effect logistic regression model revealed that clinical signs did not necessarily correlate with IAV-detection.

Discussion and Conclusion

Our results highlight group sampling methods facilitate screening of farms for the presence of IAV. However, nasal swabs remain the preferable sampling material for IAV subtyping (PCR). Furthermore, exclusive sampling of single age groups does often not cover all circulating subtypes on farm. As IAV detection does often not correlate with the appearance of clinical manifestations it is advisable, particularly for suckling piglets, to sample also animals without obvious IAV-related clinical signs.

TITLE:

22. The monitoring of swine influenza virus in pig herds in Slovenia

AUTHORS: Jan Plut, Brigita Slavec, Irena Golinar Oven, Marina Štukelj

Veterinary faculty, University in Ljubljana

ABSTRACT:

Although Swine influenza Virus (SIV) generally causes low mortality and mild clinical signs in pigs, disease is important as it causes growth retardation of the whole herd due to high morbidity. Viral strains present in pigs can also be the source of infection for humans and potentially cause outbreaks with pandemic potential like the case was in 2009. The national Targeted research program (V4-1803, Detection of Influenza A viruses in environmental samples, feed, and litter, and setting of an algorithm for the diagnosis of influenza in pigs) started in 2018 aiming to estimate the SIV prevalence in Slovenian pig herds, setting the protocol and algorithm for monitoring of SIV in samples of live animals and environment, and determine the SIV subtypes present in Slovenian pig herds. The situation about the presence of SIV and it's characteristics in the pig population has not been thoroughly analyzed in Slovenia since 2011. The aim of the study was to determine the prevalence of influenza viruses and the most common subtypes in Slovenian pig herds.

Firstly, 700 sera samples from fattening pigs were collected on 140 pig farms between 2017 and 2019 and checked for presence of antibodies against SIV with two commercial ELISA test kits. Of 140 farms sampled, 45% (63 of 140) were positive with both commercial ELISA kits; 48% with the ID Screen® Influenza A Ab Competition Multi-species Kit and 57% with the IDEXX Influenza A Virus Ab Test Kit. Half of the sera that tested positive with the ELISA test (143 total) were also retested with the IHA (inhibition of hemagglutination) test. Thirty-five percent of the samples (50 of 143) tested positive for one of the selected antigens; 78% of the samples (39 of 50) tested positive for H1N1 subtype and 22% of the samples (11 of 50) tested positive for H3N2 subtype, whereas none of the samples tested positive for H1N2 in the IHA test.

Second, 114 nasal swabs and 35 environmental swabs (pool samples from feeders, drinkers, enrichment material) were collected for virologic testing from 6 farms with reported respiratory problems. Two real-time methods RT-PCR were used to detect the influenza type A M gene. The method for detection of avian strains and the modified method which is more sensitive for detection of swine strains were used. Different real time RT-PCR assays were used for subtyping (H1N1, H1N2, and H3N2). Viral RNA was detected in four out of six farms with average Ct values of 26.6 for nasal swabs and Ct 31.5 for environmental swabs, both using the modified real-time RT-PCR method. In all positive cases, the H1N1 subtype was confirmed. In one farm, both types of swabs were collected 14 days apart. All nasal and environmental swabs collected were positive at the first examination with an average Ct value of 24 and 27, respectively. After 14 days, only 67% of nasal swabs (10 of 15) were positive with a Ct value of 33, whereas all environmental swabs (2 of 2) were positive with an average Ct value of 36. Whole genome sequencing was successfully performed on positive nasal swabs from three positive farms. Genome comparison showed that the pathogen belongs to a group of avian strain-like H1N1 swine influenza viruses.

The results show that SIV infections are widespread in the Slovenian pig population, but the results may be influenced by the detection method used.

TITLE:

23. Simulating outbreaks of avian influenza within poultry flocks

AUTHORS: Carsten Kirkeby

University of Copenhagen

ABSTRACT:

In this ongoing research project, we are utilizing literature estimates combined with a SEIR simulation model to investigate the possible course of outbreaks of avian influenza in poultry flocks. The results show that the outbreaks can be of different duration and magnitude, depending on the poultry species and the type of avian influenza involved.

TITLE:

24. A short introduction of the project “Swine influenza A virus dynamics at pig farms” (The Netherlands)

AUTHORS: Evelien Germeraad, R. de Swart, T. Tobias, M. Houben, M. Counotte, J.A. Stegeman, W.H.M. van der Poel

Wageningen Bioveterinary Research (WBVR)

ABSTRACT:

The project focuses on influenza A virus (IAV) transmission between pigs, both within and between pig farms in the Netherlands. Over the past decades, the infection dynamics of IAV in pigs have changed from incidental outbreaks to virus detection throughout the year. In case of endemic circulation within a farm, the theoretical risk of genetic shift (resulting from combination of gene segments of different viruses) is increased. In addition, endemic circulation of strains with human IAV gene segments may result in transmission back to humans.

This project will be carried out in parallel with a study of the Dutch National Institute of Public Health (in collaboration with Erasmus MC, Royal GD and WBVR) that focuses on preparing an inventory of the genetic characteristics of IAV strains that are present in Dutch pig farms, including an analysis of the origin of the gene segments of these viruses.

The overall aim of the current project is to increase our understanding of porcine IAV transmission for human and animal health and provide practical tools for intervention options. This will be achieved by pursuing the following specific objectives:

- How and at what rate are IAV strains transmitted within and between pig farms
- Which strains are associated with morbidity, in which animals or age categories
- What are the immunological correlates of protection from IAV infection

Based on this information mathematical transmission models will be developed, in which different intervention strategies can be tested in silico. Using these models and the data obtained, we aim to develop and improve control strategies and reduce the risk of introduction, transmission and development of new IAV strains.

TITLE:

25. Presentation of the PhD: “Evaluation of the effectiveness of vaccination strategies at the chain value level: The case of swine influenza virus in France”

Authors: Oumaima Mtaallah, Claire Hautefeuille, Gwenaëlle Dauphin, Andrea Apolloni

CIRAD, UMR ASTRE, F-34398, Montpellier, France and ASTRE, Univ Montpellier, CIRAD, INRAE, Montpellier, France

ABSTRACT:

Context:
Swine influenza A (SwIA) viruses cause significant economic losses in pig industry and represent a potential risk of zoonotic emergence. The control of SwIA viruses is crucial for both the pig industry and public health. Vaccination, coupled with good biosecurity measures, is the main tool to prevent and control swine flu. In France, no conventional vaccination strategy has yet been implemented. But the choice of vaccination relies on the willingness of farmers/owners. At its turn, this choice is often linked to the farm’s production type and its specific epidemiological status.

Global Objective of the Ph.D. work:
An evaluation of the impact of different vaccination strategies will be performed based on their impact on the spread of viruses and their cost-benefit in order to compare and identify the most efficient vaccine strategies. The case study will focus on France.

Methods:
The first part of the thesis work will be a literature review on the efficacy of swine influenza vaccines in Europe. The aim of this activity is to review the characteristics of existing vaccines and requirements for vaccines to be developed taking in account the conditions of application and the circulating virus strains. This activity will be conducted within the European Swine Influenza Network Cost action framework.
The second part of this thesis work will be a description of the French pig value chain. Data will be collected from literature reviews and expert’s opinions, taking into account farm types, farm numbers and size, localization, number of animals in the farms, and the flow of animals between different steps of the production chain.
The third part of the work will be to develop two models to compare vaccination strategies. The first one is a matrix model to follow the evolution of immunity dynamics by age groups. The objective of this model is to provide information on the level of immunity in the absence of circulating virus as a function of demographic dynamic. This model will provide insights on how viruses are maintained and identify period of higher and lower protection. The second will be an epidemiological model, coupled with the matrix model, to study the SwIA transmission dynamics. It will take into account the demographic dynamics, epidemiological parameters and the structure of the value chain. This model will aim to evaluate the impact of different vaccination strategies on the spread of SwIA in the value chain. The fourth part of this work will be a cost-benefit analysis using the result of the epidemiological model to compare vaccination strategies looking at their direct impact on the reduction of spread of the virus and at the financial benefits they can bring. This PhD started in December 2022 and will end in November 2025. It is part of the BioFluARN project which is funded by Bpifrance and Ceva-animal health. The project is carried out by Cirad and Ceva-animal health. The aim of this project is to develop self-replicative RNA vaccines against animal influenza viruses and to provide tools to optimise the use of these new vaccines.