



Zoonotic and reverse zoonotic risks of porcine influenza viruses: recommendation to authorities for a monitoring to increase awareness in the swine sector

Influenza viruses are a major public health concern due to their ability to cause seasonal epidemics and occasional pandemics. Europe is currently hit by the worst epidemic of highly pathogenic avian influenza virus (HPAIV) in birds ever recorded. HPAIV became practically endemic in wildlife with substantial losses in the wild bird population as well as regular spill over to the avian production sector. Influenza A viruses (IAV) are found in a wide range of hosts, including birds (avian IAV), humans (human IAV) and pigs (swine IAV). From a One Health perspective, special attention needs to be given to the pig sector during any period of increased circulation of influenza viruses (both avian and/or human IAV).

Importance of monitoring the swine sector, particularly during increased HPAIV or Human IAV activity, from a One Health perspective

Swine influenza virus infection in pigs is not a notifiable disease in Belgium and the EU. Most of the pig industry is localised in the North of Belgium and several veterinary laboratories provide diagnostic capacity to farmers and veterinarians to detect, amongst other pathogens, influenza A viruses in samples (lungs, nasal swabs) of pigs with respiratory symptoms. Furthermore, there are diagnostic tests available to detect antibodies against Influenza A viruses in pigs. Vaccines against swine IAV are available (Van Reeth et al. 2016).

Swine IAV are enzootic in Belgium and swine producing countries worldwide. Swine are susceptible to the same influenza A virus subtypes as humans: H1N1, H3N2 and H1N2. However, swine IAVs are genetically and antigenically distinct from their human counterparts and more diverse (Van Reeth and Vincent 2019). An important difference between humans and swine is the concurrent circulation of multiple antigenically distinct virus lineages and clades within each subtype in swine populations (Anderson et al. 2016). The dominant swine IAVs also differ in different geographic regions. Unlike human IAVs, which has a seasonal peak in activity, swine IAV are circulating year-round.

Swine IAV infections often remain subclinical (Bowman et al. 2012), but may cause economic losses, especially in combination with other respiratory pathogens. Monitoring¹ for swine IAV is patchy (Vincent et al. 2014, Borkenhagen et al., 2019, Henritzi et al. 2020) and dependent on individual national initiatives. Monitoring in Europe is also lagging behind that in the US.

Like other influenza A viruses, swine IAVs are species specific but they may occasionally spill over to humans (Parys et al., 2021, Abdelwhab & Mettenleiter, 2023). These zoonotic swine IAV infections generally cause no or mild symptoms and swine IAV usually fail to transmit further in the human

¹ The systematic (continuous or repeated) measurement, collection, collation, analysis, and interpretation of animal-health and -welfare data in defined populations when these activities are not associated with a pre-defined risk-mitigation plan (although extreme changes are likely to lead to action).



population. The single known exception was the 2009 pandemic H1N1 influenza virus, which was of swine origin (Shinde et al. 2009). This demonstrates that zoonotic transmission of swine IAV may result in a pandemic if (a) human population immunity against the virus is insufficient and (b) the virus is capable of adaptation to humans and onward transmission between humans, as was the case in 2009.

The other way round, human viruses also pose a risk to animal health (reverse zoonotic events). Influenza virus transmission from humans to swine is in fact far more frequent than swine-to-human zoonotic transmission, and is central in the emergence of novel and very diverse viruses in swine populations. The scale of global human-to-swine transmission of the 2009 H1N1 pandemic influenza virus represents the largest 'reverse zoonosis' of a pathogen documented to date. Humans are the drivers of the vast genetic diversity of influenza A viruses in swine. This further highlights the importance of the human-animal interface in influenza virus ecology (Nelson and Vincent, 2015).

“Genetic reassortment” between influenza viruses is very common in swine. Reassortment is a mechanism allowing the exchange of genetic segments between two or more influenza viruses. The last three influenza virus pandemics were caused by reassortant viruses with gene segments of avian (1957, 1968) or swine origin (2009) (Ma et al., 2009; Liu et al., 2012). The 2009 pandemic influenza virus was a reassortant between two long existing swine influenza viruses (Mena et al. 2016). This pandemic virus has now become established in swine populations worldwide. This has led to a multitude of novel reassortant swine IAV, in which part of the gene segments are of pandemic origin and thus potentially better adapted to humans (Sun et al. 2020, Henritzi et al. 2020, Chepkwony et al. 2021, Parys et al. 2023).

Currently, the world is faced with a global pandemic of avian influenza in birds which has led to an increased attention and monitoring of avian influenza viruses. As described above, besides birds, pigs are also a very important animal species regarding the exchange of IAV's between humans and animals. **There is proven zoonotic and reverse zoonotic transmission of influenza viruses between humans and pigs. Therefore, a monitoring in the swine sector is to be advised for One Health reasons, especially during HPAIV or human IAV activity.**

Current monitoring of swine influenza viruses

In Belgium, Sciensano recently started a temporary pilot project to monitor humans at risk for infection with avian or swine IAVs. The monitoring consists of a two-weekly sentinel monitoring by collection of nasopharyngeal swabs (self-swabbing) of people coming in contact with animals in poultry farms, pig farms and wildlife rehabilitation centres. Furthermore, on poultry farms during an outbreak of HPAIV there will be a close monitoring of people in contact with the animals: nasopharyngeal swab every 2 days (self-swabbing) & blood samples. The project has started in the beginning of 2023. Results are not yet available.

The virology lab of the faculty of veterinary medicine of Ghent University (Prof. Kristien Van Reeth) has been monitoring influenza viruses in swine since the late 1990s. The lab is offering diagnosis of influenza in swine, as well as subtyping, genetic and antigenic characterization, and whole genome sequencing of selected samples. The group is performing research on the evolution of swine IAVs, the



role of pigs in the generation of pandemic influenza viruses and immunization against influenza. The results of the swine IAV monitoring are shared with OFFLU and WHO. There is no structural funding for the monitoring, which results in variable numbers of samples for analysis over the years and potential discontinuations (non-sustainable monitoring).

The cell contractual research of the Federal Public Service (FPS) Health, Food Chain Safety and Environment has funded several research projects regarding scientific research on influenza viruses in animals in Belgium in the last 3 years:

- FLUCART: Renewed mapping of sensitive natural areas in Belgium and dynamics of exposure of industrial poultry farms and hobby farms to low and high pathogenic influenza viruses
- mRNAVIFLUE: Development and in vivo evaluation of a self-amplifying mRNA vaccine against influenza A virus in poultry
- PREVENTER: Unravelling the role of influenza D virus in respiratory disease in cattle and humans in Europe
- EMERDIA-H5 II: Development of experimental models to better understand the biology of recent H5Nx highly pathogenic avian influenza (HPAI) viruses and to improve their detection and control

At the international level, the OFFLU network (cooperation between FAO and WOA) has been closely monitoring the evolution of influenza A viruses in animals since 2005 (<https://www.offlu.org>). The network aims at exchanging scientific data and biological materials (including virus strains) within the network, analysing such data, and sharing such information with the wider scientific community. The swine influenza A virus group of OFFLU consists of 20 experts from all over the world. Furthermore, the OFFLU network collaborates with the WHO on issues relating to the animal-human interface, including pandemic preparedness for early preparation of human vaccine. Belgian experts are represented within the OFFLU network.

Furthermore, the GISAID initiative (Global Initiative on Sharing All Influenza Data) promotes the rapid sharing of data from all influenza viruses. This includes genetic sequence and related clinical and epidemiological data associated with human viruses, and geographical as well as species-specific data associated with avian and other animal viruses, to help researchers understand how viruses evolve and spread during epidemics and pandemics.

At the European level, the virology lab of the faculty of veterinary medicine of Ghent University participates in the COST action ESFLU (European swine influenza network, 2022-25). This network includes participants from 26 European countries and aims at becoming the European counterpart of OFFLU.

In France, a monitoring system for influenza viruses in pigs is running since 2011 (RESAVIP = Réseau national de surveillance des virus influenza A chez le porc). The program is voluntary and targeted on pig farms with symptoms of a possible influenza infection. The goals of the program are to improve virological and epidemiological knowledge and to be prepared for targeted action if necessary.



Recommendations for a monitoring in the pig sector

Recently, EFSA has provided several advices aiming at the guidance of the Member states on monitoring of zoonotic pathogens (EFSA et al., 2023). Swine IAV were regarded in this document as a very important pathogen to be followed by health authorities. Details for a monitoring system in the pig sector were proposed. The RAG-V-EZ is of the opinion that following proposed monitoring constitutes a good basis.

- **Target animal population:**
 - **pigs farms with high incidence of respiratory disease**
 - **pig farms with outdoor access**
 - **mixed poultry/pig holdings** (especially during high avian influenza circulation)
 - **pig farms where the care takers (human) have an influenza infection**
 - **wildlife:** swine influenza viruses may also circulate in wild boar. Furthermore, wild boar might scavenge on birds' carcasses. Therefore, they are at greater risk of being infected with avian influenza. It is advised to also perform a monitoring in wild boar during the next hunting season and to include influenza diagnostics on wild boar which are found dead.
- **Sampling:** the sampling matrix should include nasal secretions and mucosa collected with a swab. In addition, in cases of mortality, tissue samples from the lungs and/or the upper respiratory tract can be used for virus detection. Additionally, alternative sampling matrices such as air sampling and oral fluid collected by ropes could be used (Decorte et al., 2015). Field veterinarians should be incentivized to collect and submit samples of suspected cases.
- **Diagnostic method:** Samples can be screened by PCR. Confirmed influenza positive samples should be further characterized by determining the subtype, and genetic and antigenic characteristics of the surface proteins hemagglutinin (HA) and neuraminidase (NA). This is only possible in specialized laboratories. Whole genome sequencing of a selection of positive samples is recommended, to detect novel reassortants.
- **Data repository:** sequences detected in humans, birds and pigs in Belgium should be uploaded in relevant databases (preferably in already existing international databases (e.g. GISEAD)): this can facilitate phylogenetic/phylogeographic analyses, will allow to monitor changes in the prevailing subtypes and genotypes (De Vleeschauwer and Van den Berg, 2009), and identify genetic changes that may be important for adaptation of animal influenza viruses (including avian strains) to humans. Depositing data in publicly accessible platforms for data analysis and interpretation in conjunction with epidemiological information would also support and accelerate the general advancement of knowledge on the zoonotic and pandemic risk linked to influenza viruses.
- **Implementation:** maximum benefit for all stakeholders (livestock owners-veterinarians-laboratories-risks assessors and managers) should be obtained by applying an integrated approach. This should include the development and implementation of harmonised tools and approaches, exchange of sequence data and enhancing the coordination and dissemination of



the findings from the human, swine and avian networks, as well as from other species, when relevant.

- Human health aspects: It is highly recommended to develop a close collaboration between animal health and human health authorities.
Infection of humans with non-seasonal type A influenza viruses is notifiable. Confirmed infections with influenza viruses in people working in pig or poultry farms should be communicated to allow a monitoring of the animals on the farm.
It is advised to have a regular meeting/workshop to discuss the epidemiology regarding influenza viruses on both the human side as the animal side. It is also advised to install a consultative organ which collects all relevant data and publishes a yearly report.
- Increasing awareness: Communication to physicians and veterinarians regarding the risks of influenza infections and the possibilities/importance for monitoring of influenza should be performed on a regular basis.

Needs for reinforcement of biosecurity and protective measures in the swine sector during increased activity of HPAIV or human IAV

Influenza viruses are **primarily transmitted through aerosols and respiratory droplets**, and close contact with infected animals can increase the risk of zoonotic transmission (World Organization for Animal Health, 2009). In addition, people who work with (living) pigs, such as farmers and veterinarians, may be at increased risk for both zoonotic or reverse zoonotic transmission.

Measures to reduce the risk of zoonotic transmission (from animal to human):

- Avoid close contacts with pigs.
- Wear a mask when handling or taking care of pigs with respiratory clinical signs
- Respect basic hygienic rules when handling animals: regular hand washing with soap, separate clothes and footwear only used during contact with animals, ...
- Avoid any mucosal contacts (eye, nose) with unwashed hands during contact with pigs.
- Call a veterinarian when pigs are developing any respiratory clinical signs.
- Hunters who come in contact with wild boar should practice good hygiene to prevent any potential infection. Evisceration in the field is also not recommended.

Measures to reduce the risk of reverse zoonotic transmission (from human to animal):

- Any human working with pigs and developing respiratory clinical signs should avoid contact with pigs or wear a mask during activities with pigs; only FFP2 masks are recognised to sufficiently reduce the risks of transmission through droplets during close contacts.
- Inclusion of swine and poultry workers in category A of the yearly recommendation of the Superior Health Council for vaccination against seasonal flu (see further).

Measures to reduce the risk of HPAIV infections in pigs:



- Prevent any contact with wild birds, poultry or other animals that may carry HPAIv; from this point of view particular attention should be paid to the management of outdoor pigs or pigs with a temporary outdoor access.
- More information regarding the risks of infection of other species with HPAI and recommended protective measures can be found in the following document of the RAG-V-EZ: https://www.favy-afsca.be/professionnels/productionanimale/ragvez/ documents/Zoonotic-risk-of-avian-influenza_V9.pdf

Vaccination

Human seasonal influenza vaccination is not intended to prevent infection with animal influenza viruses. However, it can reduce the risk of virus transmission from humans to swine. For this reason, it is recommended to include swine and poultry workers in category A in the yearly recommendation of the Superior Health Council for vaccination against seasonal flu.

Data originating from the monitoring and antigenic/genetic characterisation of swine IAV is needed to monitor the evolution of these viruses and to aid in the selection of swine influenza vaccine strains.

Conclusion and final recommendations

Circulation of influenza viruses at the human/animal interface can potentially lead to the emergence of novel strains with pandemic potential. In the One Health One Medicine framework, animal and public health authorities need to tackle the risks of animal influenza viruses at their sources. Therefore, it is recommended to install a monitoring program of influenza virus in pigs. Monitoring programs can help to identify influenza viruses circulating in pig populations. Monitoring can also help to identify potential risk factors for zoonotic transmission and give input to the development of interventions to prevent or control the spread of influenza viruses in pig populations. Monitoring data can provide early warning of the emergence of new strains of influenza viruses in pigs and can help to inform public health authorities in order to develop appropriate measures to prevent or control the spread of the virus (Smith et al., 2009). **The RAG-V-EZ recommends the establishment of such a monitoring system in the pig sector, targeted to all pig farms where a high incidence of respiratory disease is observed, to pig farms with outdoor access, to mixed poultry/pig holdings (especially during high avian influenza circulation), to pig farms where the care takers (human) have an influenza infection as well as to wild boars.** For that purpose, already existing structures and channels can be expanded rather than starting up new initiatives. It is however very important to have a centralised, sustainable and long-term monitoring system. Such a monitoring system would allow a greater understanding of the evolution of swine IAVs at the national and global level and could establish a baseline to support alerts for the emergence of new reassortants. To reach a high participation of pig farms with acute respiratory disease, this monitoring component could be accompanied by a **disease awareness campaign and incentives for farmers and veterinarians to submit samples.**



Furthermore, the RAG-V-EZ recommends to **increase protection and biosecurity measures at the animal/pig interface and to increase seasonal influenza vaccine coverage** of people who may have exposure to infected animals in order to reduce the risk of recombination events.

Lastly, it is highly recommended to develop a **close collaboration between animal health and human health authorities**. This should include the development of an influenza network and implementation of harmonised tools and approaches, exchange of sequence data and enhancing the coordination and dissemination of the findings from the human, swine and avian networks, as well as from other species, when relevant. The ultimate goal is to detect zoonotic and reverse zoonotic infection in an early stage.

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