



OFFLU call to discuss Avian Influenza events in mammals 2 March 2023

Agenda for the meeting

1. Update of the situation in mammals including molecular analysis (Europe, North America, South America)
2. Virus dynamic in mammals (pathogenesis, transmission, adaptation, risks)
3. Surveillance approaches in mammals
4. Risk to public health
5. Knowledge gaps

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Update of the situation in mammals including molecular analysis (Europe, North America, South America)

1. Europe

Multiple introductions of avian influenza viruses to mammals were reported from 2020 to 2022. Several cases were recorded in different wildlife species - red fox (*Vulpes vulpes*), Eurasian otter (*Lutra lutra*), lynx (*Lynx lynx*) etc, while fewer events were observed in domestic species such as ferret (*Mustela furo*), cat (*Felis catus*) and American mink (*Neovison vison*). A lot of diversity in terms of species and countries reporting the events. In particular, for the case of H5N1 positive domestic ferrets in Belgium, epidemiological investigations identified feeding of animals with contaminated chicken eggs obtained from the same farm as the most probable exposure to the virus. In the case of the H5N1-positive cat in France, infection originated as a result of proximity with infected farmed ducks, as proven by the genetic

similarity between the cat and duck strains. For the event in intensively farmed minks, a detailed description is available here <https://doi.org/10.2807/1560-7917.ES.2023.28.3.2300001>.

Since 2020, out of the thousands of sequences of HPAI viruses obtained from birds in Europe, only 17 H5Nx viruses present mutation 627K, while for 2 viruses we observed the mutation 701N. However, from the 57 available sequences of HPAI viruses detected in mammals, 56% present mammalian adaptive markers in the PB2 gene (e.g. 627K; 701N; 271A).

An experimental study was conducted in ferrets to characterize the virulence and transmissibility of the H5N1 virus that caused an outbreak in farmed minks in Spain. Results pointed out that this virus causes mortality in 50% of the infected ferrets and transmit to 100% of the contact sentinels. For 75% of the sentinels the infection has a fatal outcome. In moribund ferrets, multiple organs were found positive by RRT-PCR including the olfactory bulb, the brain, lungs, pancreas, spleen, kidney and liver. Interestingly, pancreas and intestine scored high in terms of histological lesions in the majority of the ferrets. In the sentinels, the liver resulted to be one of the most affected tissues, according to both virological and pathological evaluations. Interestingly, nasal shedding lasted for approximately 10 days in both challenged and sentinel ferrets. Progression of the disease was relatively slow with clinical signs such as depression and labored breathing becoming apparent around day 4-6 post infection (p.i.)/exposure (p.e.). Mortality occurred at 12-14 days p.i/p.e. after a severe drop in weight.

2. North America

USA:

<https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/2022-hpai>

In the US, the majority of the viruses characterized from mammals are Eurasian/North American reassortants which are representative of what is circulating in the wild bird population. Since late 2021, thousands of viruses have been characterized from avian species with several spillover detections in mammals. In a small percentage of the mammalian viruses, lysine was detected at position 627 of the PB2 gene. The lysine at this position is associated with mammalian adaptation of avian influenza viruses. At the time of this summary, more than 140 sequences have been characterized from mammals and the 627K change has been identified in foxes (7), racoons (2), skunks (3), a grizzly bear, a black bear and a harbor seal (only one of many characterized), all from different states and multiple genotypes. To date, there is no conclusive evidence of sustained transmission between mammals in the U.S. This mutation has been detected in only 2 wild birds to date, a red-tailed hawk, and a turkey vulture, both of which are likely to predate or scavenge on small mammals. The molecular marker 627K has been detected in a few backyard flocks; while mammals are largely considered dead end hosts, transmission from mammal to bird cannot be ruled out when 627K is present. Other mutations of interest (T271A, D701N) have only been detected in a handful of viruses largely from mammals or raptor species.

There have been no natural detections of these viruses in pigs to date; however, some experimental challenges have been carried out using viruses from both avian and mammalian origin. There was no nasal shedding nor contact from pigs infected with avian viruses, however there was evidence of nasal replication and virus shedding with the 627K mammalian virus. While there were no clinical signs in the experimental pigs, there was some evidence of macroscopic lung lesions.

Canada:

In Canada there are 85 confirmed cases H5N1 2.3.4.4b in wild mammals, the diversity of species affected have been important, being most of the affected species red fox (*Vulpes vulpes*) and striped skunk (*Mephitis mephitis*) all over the Canadian territory and in Harbor seals (*Phoca vitulina*) in Quebec. Most mammals were affected by reassortant NA/EA H5N1 viruses although few cases in Quebec and Atlantic Canada involved wholly Eurasian H5N1 viruses. Most of the affected mammals had neurological clinical signs such as ataxia, tremors, seizures etc. This was supported by the presence of microscopic lesions and presence of abundant influenza A antigen in the central nervous system by immunohistochemistry. In the lungs, the lesions were extensive but the antigen concentration was minimal.

Some of the H5N1 viruses had a few mammalian adaptive mutations, such mutations involved the PB2 (T271A; K389R; E627K; E627V; D701N; L89V; G309D; T339K; R477G; I495V; A676T); PB1 (D3V, D622G); PA (S37A; N383D, N409S) and NP (N319K).

[Characterization of neurotropic HPAI H5N1 viruses with novel genome constellations and mammalian adaptive mutations in free-living mesocarnivores in Canada \(tandfonline.com\)](https://doi.org/10.1186/s12876-022-02000-0)

Risk assessment studies were conducted in lab animals:

In mice experiments some of the reassortant viruses with 627K, 627V and 701N mutations were virulent. Wholly Eurasian and reassortant H5N1 viruses with 627E were less virulent in mice model. The study in ferrets is still ongoing but early results indicate some of the reassortant viruses with 627K mutation possibly could transmit to ferrets horizontally.

Risk assessment studies were also conducted using primary human airway cells:

All three types of primary human airway cells (HAI cells) were infected with 4 different AI virus – reassortant H5N1 virus isolated from turkeys (627E), Redtailed (RT) hawk (reassortant, 627K), Red fox (wholly Eurasian, 627K) and Red fox (reassortant 627V). In the upper HAI cells, the RT-hawk virus replicated to higher titer starting 48 hrs after infection and reached peak titers 5 to 10 dpi. Red fox (wholly Eurasian, 627K) – reached highest titer by 6 dpi. Turkeys (627E) and Red fox (reassortant 627V) also replicated very poorly. In the trachea-bronchial epithelial cells, the RT-hawk virus replicated very efficiently and reached the highest titer 72 hrs after infection. Red fox (reassortant 627V) replicated to average and reached peak 5 days after infection. Turkeys (627E) and Red fox (wholly Eurasian, 627K) replicated poorly. In the small airway epithelial cells, Turkeys (627E) replicated very poorly, the other 3 H5N1 viruses replicated efficiently and reached peak at 7 dpi.

3. South America

The HPAI outbreak in S. America started at the end of 2022, with major impacts on wildlife. This included a mass die-off of pelicans along the entire coast of Peru, followed by a sea lion mass die off and cormorant and booby species. An opportunistic sampling along the Peruvian coast has confirmed HPAI H5N1 in 3 sea lions, 1 dolphin, 4 pelicans, 1 cormorant and 1 sanderling. From the 10 positive samples, 8 sequences were deposited in Genbank. The most successful samples for viral detection were the brain samples, aligned with the neurological lesions and clinical signs in the animals.

The AI virus found in the Peruvian samples belonged to the same AI lineage as the samples from Chile and Ecuador, while the Venezuelan lineage was phylogenetically more distant and more similar to the North American lineages. This confirms the pathway of the phylogenetic lineages. The Peruvian HPAI fall within

both the Eurasian and American lineage. The mutational analysis revealed no differences between the detected virus in mammals and birds. However, some mutations have been found in the dolphin and sea lions but were not shared between mammal and bird species.

The virus arrived at South America by the Pacific migratory pathways, and it was expected since the first outbreaks in Europe and North America. The virus arrived geographically from the north of the continent starting with Panama, followed by Colombia, Ecuador and Perú. The number of individuals affected were not high in the first countries and it mostly affected poultry, however, when it arrived at Peru it affected thousands of wild birds, >60 000 birds in less than 4 weeks. The virus came down to the Pacific coast of South America, affecting principally sea lions potentially killing at least 3 000 individuals (many of them present clinical signs of being infected by the HPAI virus, but only a few of them have been tested for H5N1 HPAI). The hot-spot of the virus impact on birds and mammals was in Peru and the virus is going south to Argentina and Chile and has already reached Patagonia. The virus will probably extend northward and eastward in the next months during the autumn south-north migration.

Nearly all countries in South America, excluding Paraguay, Brazil, Suriname and the Guyanas, have reported HPAI in birds (wild and poultry), and for mammals the only countries reporting were Peru and Chile.

4. Asia

In Japan, a case of a fox and a tanuki was detected without any mutation.

Surveillance approaches in mammals and what questions to we want to address collectively:

- Opportunistic surveillance in mammals is needed, especially if there are events of mass mortality.
- Serology is also necessary as relying solely on the mortality rate in mammals may not capture milder infections.
- Defining what samples should be taken from mammals when diagnosing HPAI and also discuss best practice when having opportunistic access to carcasses to facilitate maintenance of cold chain for enhanced chances of virus isolation.
- Need to investigate and define risk pathways: Ecological studies are required to understand host interactions in order to target surveillance. There are cases of animals in zoos being fed raw meat coming from poultry farms where animals were infected and is a risk for the dissemination of the virus.
- Additionally genomic data is needed in order to understand transmission pathways.
- Surveillance in swine populations could be considered, however such would need to address difficulties in obtaining quality samples.

Risk to public health

So far only avian viruses have been used in transmission models with a limited number of genotype viruses. It is a priority to try this with mammalian viruses and try and reproduce some effects which have been described above. A new antigen to be prepared as a WHO pre-pandemic candidate vaccine virus has been recommended given some antigenic diversity of viruses in North America (although there is no difference between mammalian and avian virus antigenically and genetically at the level of the hemagglutinin currently circulating viruses). A WHO formalised risk assessment TIPRA will be carried out soon.

Polymerase changes of Avian influenza viruses in humans have been similar to what has been already discussed in mammalian infections, indicating intra host adaptation of an avian virus in infected humans who have had close contact with birds. In China there have been human infections that have resulted in severe and fatal infections which is in contrast with the asymptomatic infections in the US, Spain and UK. However, the data must be carefully analysed because some cases may not have been true infections making it difficult to quantify and generalise the Public Health risk, which is still considered low in the US and there have not yet been important widespread mutations in the HA indicating changes in receptor binding.

In the US, individuals participating in depopulation or disinfection activities of H5N1 affected farms are monitored for 10 days. The asymptomatic individuals are tested and out of all the 6000 people that have been monitored, only about 100 had any symptoms and all of those were negative, with exception of the case in Colorado. Another point to note is to recommend that depopulating workers should be recommended not to have contact with live poultry afterwards for a time period to reduce the risk of carriage and spread of infection by depopulation workers.

ECDC also assessed the risk for general population as low and higher for those directly exposed. Reports of neurological symptoms in mammalian species also forced public health authorities to update and adapt guidance for testing and included patients with neurological symptoms without known aetiology.

Using the example of Peru there is direct contact between potentially infected mammals and members of the public. Currently people are going to the beach, and there is no public knowledge about the risks associated with these animals. It's not uncommon also to see pets directly in contact with these animals, especially when these animals are disoriented. There have been cases of people taking the sick animal to their homes to try to rehabilitate them. For the moment there is no sampling in humans because the monitoring has been led by veterinarians. It is important to suggest the advocate for a public health awareness campaign to try to limit the interaction and contact between humans and potentially infected animals.

Particularly in instances where avian influenza occurs near bodies of water used for recreational activities it could be helpful to propose clear recommendations in terms of Public Health risks and activities limitations.

The best way to monitor people working with infected poultry and to minimise the chances of contamination (to avoid false positives from environmental contaminants), public health protocols should clearly specify the right conditions and timing for swabbing. As a result of the event described (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9951258/>) it is stated that human cases will only be considered confirmed if samples are taken under adequate hygienic conditions, i.e. wearing clean clothes and sampling at a healthcare center rather than on location, and by swabbing 5 and 7 days after the last exposure and avoiding the sampling after the workday.

Summary knowledge gaps

- Surveillance in mammals to understand the baseline level of infection
 - Public health awareness of infected wild animals and associated risk
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