Avian influenza in wild canids: an animal and public health threat

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Abstract

The risk of cross-species transmission of infectious diseases and zoonoses has increased due to the adaptive evolution of pathogens and anthropogenic landscape changes. A current example is the avian influenza (AI) virus, which can infect not only avian species but also mammals. In these species, infections are often associated with severe neurological symptoms. AI viruses are primarily not well adapted to mammalian hosts and are not efficiently transmitted among them. To accomplish this, AI viruses must acquire adaptations or mutations that contribute to replication efficiency and virulence in mammals. The exact combination and interaction of mutations that result in optimal adaptation to mammals is still unknown and may vary between host species and virus subtypes. The globally increasing number of infected birds with highly pathogenic AI (HPAI) increases the likelihood of contact between birds and mammals, especially wildlife. One reason for the adaptation of HPAI to mammals is the exposure of wild mammals to infected birds or their carcasses. According to the current data, wild canids, such as red foxes, are among the most infected mammals. However, infections caused by neurotropic viruses, as well as HPAI, have been scarcely studied or not at all in jackals. Since jackals are scavengers that have spread rapidly in Europe and Croatia in recent years, surveillance of AI virus infection in these canids is of the utmost importance. Furthermore, interactions between wild carnivores and domestic animals are of concern, as infections with HPAI pose a public health threat due to the increased risk of mammalian adaptation.

Key words: *influenza; HPAI; wild canids; red fox; golden jackal*

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Introduction

Viruses capable of infecting nervous tissue and causing neurological diseases are among the most dangerous and deadly viruses known. Some have zoonotic potential, meaning that they can infect humans and cause disease, even death. The most studied and best-known neurotropic virus in mammals is the rabies virus, which despite effective preventive measures still leads to the death of around 59,000 people per year (Hampson et al., 2015). As the dominant wild canid in most parts of Europe, the red fox was the main reservoir and vector of the rabies virus until the 1980s, and until 2014 also in Croatia (Lojkić et al., 2021). Mammals, especially canids, can also be infected with other known and frequently studied neurotropic viruses, such as canine distemper virus (CDV), canine herpesvirus-1 (CHV-1) and Aujeszky's disease virus (PRV). CDV has been described in wild canids in numerous studies worldwide, in Europe and in Croatia (Prpić et al., 2023).

Recently, a respiratory virus has stolen all the attention due to its ability to cause neurological symptoms in foxes and other animals. In 2021, a red fox cub was found in the Netherlands with neurological symptoms similar to rabies. The cub's brain tested negative for rabies virus infection but positive for highly pathogenic influenza virus (HPAI) (Rijks et al., 2021). Although HPAI is primarily a respiratory pathogen, it is currently the most emerging neurovirulent virus in mammals, and is therefore described in greater detail in this review.

Avian influenza virus

Avian influenza (AI) is caused by the influenza A virus, a member of the *Orthomyxoviridae* family. All influenza A viruses are classified into subtypes according to differences in the 16 known surface glycoproteins, haemagglutinin (H; H1–H16), and the 9 known neuraminidases (N; N1–N9). All combinations of H and N proteins are apparently possible, from H1N1 to H16N9 (e.g., H5N1), and each combination is considered a separate subtype. Related viruses within a H subtype can be referred to as a lineage.

Natural reservoirs for influenza A viruses are aquatic birds, especially migratory waterfowl, as they often show no signs of the disease (Webster et al., 1992). Avian influenza viruses (AIVs) are classified as low pathogenic (LPAI) or highly pathogenic (HPAI) based on their genetic characteristics and the severity of the disease they cause in terrestrial poultry e.g., chickens and turkeys.

The HPAI H5 viruses currently circulating in Europe originate from the A/Goose/Guangdong/1/1996 (GD/96) H5N1 HPAI virus, which emerged 28 years ago in the southern Chinese province of Guandong and was first isolated from a goose (Xu et al., 1999). Descendants of this virus are termed GD/96 lineage viruses. After the virus circulated in domestic poultry in Asia for many years, subsequently evolving into many phylogenetic groups, a new clade termed GD/96 virus 2.3.4.4 evolved in 2008, consisting of several subtypes of H5NX viruses that arose by reassortment with gene segments of other AI viruses (De Vries et al., 2015). This clade is further subdivided into eight subclades (2.3.4.4a to 2.3.4.4h): a and b circulate worldwide, while the remaining subclades are present in Southeast Asia, mainly China (Gu et al., 2022). Several subtypes were identified in the outbreaks, but the most common were H5N8 and H5N1. These viruses have spread through migratory wild birds from Southeast Asia to other parts of Asia, Europe, Africa and North America (EFSA, 2023). The adaptation of the HPAI virus to

wild birds has created an additional entry point for the virus in poultry farms and small holdings, expanding the geographic area where the HPAI virus poses a threat to human and animal health (Global Consortium, 2016). The HPAI H5 viruses of subclade 2.3.4.4b have become enzootic in birds and are constantly generating new HPAI virus variants. Since autumn 2020, they have been dominant in Europe and led to the largest global HPAI epidemic to date, with the highest mortality in wild birds and domestic poultry (EFSA, 2023).

Over the last four years (2020-2023), almost 15,000 HPAI virus detections were reported in European countries, with a peak in winter 2021/22. At that time, 2653 HPAI virus detections were reported in as many as 33 European countries, including 1489 in wild birds, 1030 in domestic poultry and 11 in captive birds. In the same period in Croatia, a total of 19 outbreaks of HPAI were detected in wild birds and five in domestic poultry (EFSA, 2024).

HPAI – a threat to wild canid health

The red fox (*Vulpes vulpes*) is one of the most widespread species of wild carnivore in Eurasia. It feeds opportunistically, mainly on birds and small mammals. Red foxes and other wild carnivores usually become infected with avian influenza viruses by eating infected birds or their tissues, although inhalation of excreta from infected birds is probably an important route of exposure. To determine infection susceptibility, a group of foxes were experimentally intratracheally infected by a 2.2 HPAI virus (H5N1) clade, while a second group was fed with infected birds, assuming that this was the natural route of infection (Reperant et al., 2008). It was found that both groups were successfully infected, regardless of the infection route; however, intratracheally infected foxes developed severe pneumonia, myocarditis and encephalitis, while the foxes infected by eating carcasses of infected birds showed only mild or no pneumonia symptoms but could play a role in spreading the virus due to their daily mobility.

Since the beginning of the GD/96 lineage epidemics, many cases of infection with the HPAI virus have been detected in red foxes worldwide. Although the AI virus rarely infects carnivores and causes respiratory symptoms, there is increasing evidence that GD/96 H5 HPAI viruses can cross the blood-brain barrier or the blood-brain-spinal fluid barrier, leading to neurological symptoms (Bauer et al., 2023). Although they are primarily respiratory pathogens, it is a unique characteristic of HPAI viruses that they can cause severe neurological disease in mammals.

Host adaptation factors of the AI virus

Although AI occasionally infects humans and other mammals, AI viruses are primarily not well adapted to mammalian hosts and are not efficiently transmitted among them. To accomplish this, AI viruses must acquire adaptations that contribute to replication efficiency and virulence in mammals. Therefore, one of the major challenges in controlling the HPAI epidemic is to understand which factors, particularly mutations, are critical for HPAI adaptation in mammals.

Several studies have identified and characterised some of these mutations in different HPAI H5NX subtypes. However, the exact combination and interaction of mutations that cause optimal adaptation in mammals is still unknown and may vary between host species and virus subtypes. Therefore, continuous surveillance and risk assessment of HPAI viruses cir-

culating in birds and mammals is crucial to monitor their genetic evolution and potential for adaptation in mammals. Previous studies on neuroinvasive potential, neurotropism and neurovirulence in mammals have revealed a number of components that play an important role. Some of these components are discussed here, although the exact number and combination of components is not yet known.

Influenza viruses use sialic acids (Sia) as receptors to bind to the epithelial cells of the host's mucous membranes via their surface glycoprotein, haemagglutinin. Sialic acids are a type of sugar molecule found on the surface of many cells, including those of the respiratory tract. Avian influenza viruses bind to sialic acids linked to galactose via an α-2,3 link, which is abundant in birds. In mammals, however, the predominant sialic acid binding is α-2,6, which is less well recognized by AIVs. Epithelial cells in the trachea of pigs, for example, contain both the α-2,3 and the α-2,6 linked Sia, which explains the high susceptibility of this animal to human and avian viruses. The CNS also has a high Sia content, with both α-2,3 and α-2,6 linked Sia present in mammalian species including humans (Kim et al., 2013). Therefore, for an AIV to infect and transmit mammals, it must acquire mutations that allow it to bind to α-2,6 linked sialic acids. However, it has also been shown that a single amino acid change in the HA protein can alter receptor binding from α-2,3 to α-2,6 specificity (Tharakaraman, et al., 2013).

Influenza A viruses primarily infect the cells of the respiratory system and can spread from there to the CNS. The respiratory and olfactory mucosa (OM) lines the nasal cavity, making the olfactory and respiratory tracts the primary routes of neuroinvasion. The olfactory bulb serves as the connection between the OM and the brain and thus represents the entry point of the virus into the CNS (Van Riel et al., 2015). In mice, for example, influenza viruses can enter the CNS from the nasal cavity via the olfactory and trigeminal nerves (Tanaka et al., 2003; Yamada et al., 2012), but also via the *nervus vagus* and sympathetic nerves from the lungs (Matsuda et al., 2004). The surface area ratio of the respiratory and olfactory mucosa differs between mammalian species. Canids are up to 100 times more sensitive to odours than humans and have on average ≈100 cm^2 of olfactory epithelium, as opposed to only 2-10 cm^2 in humans, while marine mammals such as dolphins have a reduced olfactory apparatus (Issel-Tarver and Rine, 1997). So how does all this contribute to neuroinvasion in humans with small olfactory mucosa or even in marine mammals with a reduced olfactory apparatus? It appears that in this epidemic, the key element of infection is the consumption of infected prey, so that the size of the olfactory mucosa contributes but is not critical for the virus to invade the CNS. The combination with other factors discussed here is necessary.

An important feature of HPAI is the presence of multi-basic cleavage sites (MBCS). MBCS is a sequence of amino acids in the HA protein that enables cleavage and activation of HA by ubiquitously expressed proteases. In contrast, AIVs containing a monobasic cleavage site can be cleaved by trypsin-like serine proteases (Garten et al., 2015). AI viruses with MBCS have a higher virulence and can infect a wider range of hosts than those with a monobasic cleavage site. In ferrets, MBCS is essential for the neuroinvasiveness of HPAI H5N1 through the olfactory nerve (Schrauwen et al., 2012). However, this feature is not solely important for neuroinvasion, as it has been shown that the insertion of MBCS into AI viruses other than HPAI H5NX does not increase their neuroinvasive potential (Schrauwen et al., 2011).

The polymerase genes encode three subunits (PA, PB1 and PB2) that are essential for viral replication and transcription. Mutations in polymerase genes, particularly in different domains of PB2, can enhance polymerase activity and thus modulate viral fitness and virulence and increase affinity for mammalian receptors (Matrosovich et al., 2009). The mechanisms by which these mutations cause adaptation to humans are not yet fully understood, but they could alter the binding affinity of PB2 to host factors, modulate the stability of the polymerase complex or influence the nuclear import of PB (Mehle et al., 2008; Rameix-Welti et al., 2009; Zhou et al., 2023). Several mutations in this gene have been shown to enhance viral replication and transmission in mammals. The best-studied mutation in PB2 is the substitution of glutamate by lysine at position 627 (E627K) (Subbarao et al., 1993). It enhances viral replication and pathogenicity in mammals by favouring viral replication at low temperatures (33°C) similar to those of the human upper respiratory tract (Massin et al., 2001; Bordes et al., 2023; Vreman et al., 2023), and is considered a hallmark of influenza virulence and transmission to mammals, including humans. In mice and ferrets, the E627K substitution enhances the interaction of PB2 with other viral proteins or with the host factor, leading to increased polymerase activity in mammalian cells (Resa-Infante et al., 2008) and increased neuroinvasive potential (Gabriel et al., 2011). Another mutation, aspartic acid to asparagine at position 701 (D701N), enhances nuclear entry of PB2 and polymerase activity in mammalian cells (Gabriel et al., 2008) and enables replication and virulence of human GD/96 lineage H5N1 virus in mice (Gabriel et al., 2011). Remarkably, all mammalian viruses have at least one of these two mutations, suggesting that they are essential for efficient virus replication in mammals. These mutations likely occur during transmission to mammals, as they were rarely detected in clade 2.3.4.4b HPAI viruses isolated from birds prior to the outbreak. The exception is the D701N substitution in the PB2 gene, which occurs naturally in GD/96 lineage H5N1 viruses in ducks (Li et al., 2005), which could facilitate the transmission of these viruses to mammals. Adaptation to mammals poses a threat to public health as it contributes to the increased virulence of the virus and thus the risk of transmission to other mammals and humans. There are other mutations in the PB2 gene that have been found in mammals (e.g., T271A or K526R). However, more than half of the mammalian sequences do not contain any of these mutations (Suttie et al., 2019), and as such do not represent reliable evidence of mammalian adaptation.

However, none of these components alone is responsible for the neuroinvasive and neurovirulent potential of the virus. The neurovirulence of HPAI H5 virus infection is related to the virus' entry into the CNS and its spread, as well as the resulting inflammation and tissue damage. The GD/96 lineage HPAI H5NX viruses are highly neurovirulent in contrast to seasonal or pandemic influenza A viruses in many mammalian species. The neurovirulence caused by the high neuroinvasive and neurotropic potential of the HPAI H5 virus, together with the induction of systemic cytokines, can contribute to the development of severe neurological symptoms, including behavioural changes such as fearlessness of humans or aggression. For these reasons, rabies or CDV is most suspected in wild and domestic canids with neurological symptoms. At the same time, canids and other mammals infected with HPAI show no symptoms of the respiratory system, so influenza is not

suspected. Nevertheless, many mammals, particularly foxes, ferrets and martens, infected with the HPAI H5 virus of clade 2.3.4.4b often show no neurological symptoms or mortality.

This demonstrates the need for increased surveillance of all wildlife to monitor infections and mutations, regardless of neurological signs. Increased surveillance of the emergence and development of new AI strains should also include other wild and domestic species, especially rapidly spreading wild scavengers such as the golden jackal.

Spillover events, transmission from mammal to mammal

Spillover events of HPAI H5N8 and H5N1 in mammals were first observed in seals and foxes in the United Kingdom in late 2020 (Floyd et al., 2021) and in animals from a wildlife rescue centre in the Netherlands in spring 2021 (Rijks et al., 2021).

According to EFSA (2023), the HPAI subtype H5 clade 2.3.4.4b has been detected in 50 mammalian species from the families *Canidae, Felidae, Mephitidae, Mustelidae, Otariidae, Phocidae, Procyonidae, Ursidae, Delphinidae* and *Suidae*.

There is no definitive evidence of transmission among wild mammals. However, a recent outbreak of HPAI H5N1 virus in American minks (*Neovison vison*) on a farm in Spain may indicate that mammal-to-mammal transmission is possible (Aguero et al., 2023). In addition, carnivores showing no neurological signs and not found dead have also tested serologically positive, suggesting that HPAI infection in mammals can be asymptomatic (Chestakova et al., 2023). Similar to wild carnivores, domestic cats, which are good hunters, can also come into contact with AI viruses. As they are in close contact with humans, they can pose a risk for the transmission of adapted HPAI viruses from animals to humans.

The jackal problem

Anthropogenic changes can also affect the change in the dominance of a particular population of animals in a given area. The golden jackal (*Canis aureus*), as a rapidly spreading wild canid, is increasingly conquering new habitats, especially urban areas, and the incidence of viruses, especially neurotropic viruses, which have been studied mainly in red foxes, has been studied little or not at all in jackals. According to the Croatian NRL for rabies, the number of jackals shot as part of a monitoring programme for the effectiveness of oral vaccination of foxes increased from 3.5% to 18.3% in the last six years, indicating an increase in the population. Jackals are opportunistic omnivores that can adapt to different environments and prey on small mammals, birds, reptiles, insects, fruit and carcasses. They can also compete with or prey on other carnivores such as foxes, wolves, lynx and wildcats. In the last 15–20 years, the golden jackal has begun to spread over a larger area in Croatia (the first shooting took place in Slavonia in 1997), and a particularly strong increase in jackal populations has been recorded in eastern and western Croatia and Istria. Given the increasing spread of jackal populations and the takeover of fox habitats, as well as the increasing number of cases of jackal attacks on livestock, the study of these viruses would provide new insights into the detectability and prevalence of emerging neurotropic viruses, especially those previously more commonly found in red foxes. One reason for the adaptation of HPAI to mammals is the exposure of wild mammals to infected birds or their carcasses. Since jackals are carrion eaters and their spread across Croatia and Eu-

rope has been rapid in recent years, surveillance of AIV infection in these canids is of the utmost importance.

Conclusions

Viruses generally pose a threat to wildlife and human health, particularly when they cross the species barrier and adapt to new hosts. A recent example of such a virus is HPAI, which can infect not only avian species but also mammals (including humans), such as wild canids, martens and marine mammals. HPAI viruses can cause respiratory, systemic and neurological symptoms and have a high mortality rate in infected animals. Cases of AI in mammals have been spatially and temporally associated with mass mortality in birds. Transmission of the HPAI virus from birds to mammals is favoured by environmental factors, especially habitat overlap, which is facilitated by feeding on infected, sick birds or their carcasses. A large amount of the virus is usually required, and after shedding, the virus must be able to bind to the receptor on the cell of the new host and consequently replicate in the infected cell (Driskell et al., 2014). The main route of infection for mammals with HPAI viruses is the consumption of infected, sick or dead wild birds. In many mammalian species, infections are often associated with severe neurological symptoms, which is a unique feature of the GD/96 HPAI H5 viruses compared to other influenza A viruses. Frequent shedding and spillover infections, including some evidence of mammal-to-mammal transmission, have raised concerns about the possibility of further adaptation to mammals. Minks are susceptible and permissive to both avian and human influenza A viruses, leading to the theory that mustelids could serve as a potential transmission route between birds, mammals and humans, as is already known for pigs. Interactions between wild carnivores and domestic animals are of concern as HPAI H5 virus infections in domestic animals can lead to severe disease, high mortality and costs, as well as public health risks due to the increased risk of mammalian adaptation. Awareness of the potential transmission of HPAI viruses from wild birds to domestic animals should also be raised. Therefore, increased surveillance of wild animals, especially wild canids, is needed to monitor infections and mutations independent of neurological signs. The control and prevention of neurotropic viral infections in wild canids remains an important public health issue.

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Ptičja influenca u divljih kanida – prijetnja javnom zdravlju i zdravlju životinja

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Tijekom godina, rizik od međuvrsnog prijenosa zaraznih bolesti i zoonoza se zbog zbog adaptivne evolucije patogena i antropogenih promjena u okolišu povećao. Trenutačni primjer je virus influence ptica (IP), a koji osim ptica može zaraziti i sisavce u kojih su infekcije često povezane s teškim neurološkim simptomima. Virusi IP ponajprije nisu dobro prilagođeni sisavcima kao domaćinima i ne prenose se među njima učinkovito. Da bi to postigli, moraju se prilagoditi ili mutirati što pridonosi učink-

ovitijoj replikaciji i povećanju virulencije u sisavaca. Točna kombinacija i međudjelovanje mutacija koje rezultiraju optimalnom prilagodbom virusa IP na sisavce još uvijek je nepoznata i može varirati između domaćina i podtipova virusa. Vjerojatnost kontakta između ptica i sisavaca (posebice divljih) povećava se sa sve većim brojem zaraženih ptica visokopatogenim virusom IP (VPVIP) diljem svijeta. Jedan je od razloga prilagodbe VPVIP na sisavce i izlaganje divljih sisavaca zaraženim pticama ili njihovim lešinama. Među najugroženijim sisavcima su divlji kanidi (lisice i čagljevi). Neurotropni virusi, kao i VPVIP, su vrlo malo ili nikako istraženi u čagljeva. Uzevši u obzir povećanje broja i širenje staništa čagljeva u Europi, ali i u Hrvatskoj i činjenicu da se hrane lešinama, od iznimne je važnosti pratiti infekciju IP u ovih kanida. Infekcija VPVIP zbog povećanog rizika prilagodbe na sisavce predstavlja prijetnju javnom zdravlju što je posljedica sve češćeg kontakta ljudi, domaćih životinja i divljih zvijeri.

Ključne riječi: *influenca, VPVIP, divlji kanidi, lisica, čagalj*