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Review paper

Cats infected with H5N1 avian influenza – a new infectious disease in Poland

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Abstract

Avian influenza virus (AIV) infections in cats are a new and not fully understood problem in Poland. These infections have drawn the attention of both veterinarians and human medical practitioners, mainly because of their zoonotic potential, i.e. possible spreading to humans and other mammals. In wild felids as well as in domestic cats, AIV can cause severe infections, often ending in death. Highly pathogenic avian influenza virus (HPAIV) and low pathogenic avian influenza virus (LPAIV) have been identified, with the recent H5N1 (2.3.4.4b clade) outbreak affecting poultry, wild birds and carnivores. Transmission likely occurs through contact with infected birds, their excretions or contaminated raw poultry, while cat-to-cat transmission remains unconfirmed. First reported in Thailand in 2003, H5N1 infections in cats have since occurred in multiple countries. In Poland, 25 confirmed cases were identified in June 2023, with genetic sequencing linking the virus to strains detected in local wild birds. The virus primarily replicates in the lower respiratory tract, spreading via viremia or nerve fibers, causing multi-organ failure. While avian influenza in cats is severe and often fatal, it should not yet be considered an epidemic. Further interdisciplinary research is essential to clarify transmission routes and assess the zoonotic risk. Additionally, differential diagnosis should include rabies, which presents similar neurological symptoms and remains a critical public health concern. This article presents the current knowledge of H5N1 virus infection in cats, especially the possible routes for its spreading, the current epizootic situation of the disease around the world, its pathogenesis, clinical course and methods of diagnosis.

Keywords: cats, clinical course, epizootic situation, H5N1, methods of diagnosis



Introduction

Avian influenza virus (AIV) infections in cats are a new and not fully understood problem in Poland. They have drawn the attention of both veterinarians and human medical practitioners, mainly because of their zoonotic potential, i.e. possible spreading to humans and other mammals (Vahlenkamp and Harder 2006, Yuen and Wong 2020). In wild felids as well as domestic cats, AIV can cause severe infections, often ending in death (Rimmelzwaan et al. 2006, Klopffleisch et al. 2007a).

The avian influenza virus belongs to the *Orthomyxoviridae* family. Its outer part is formed by two glycoproteins: haemagglutinin (HA) and neuraminidase (NA), and the differences in their structure lead to the division of avian influenza (AI) viruses into H and N subtypes. In turn, the clinical manifestations of the disease, as well as some genetic features, have led to distinguishing two pathotypes of AIV: low pathogenic avian influenza virus (LPAIV) and highly pathogenic avian influenza virus (HPAIV). Its primary reservoir is birds, in which 16 HA subtypes and 9 NA subtypes have been identified. They can occur in all possible combinations, while in poultry farming the biggest problem is the highly pathogenic AIV-H5 subtype AIV. In the last two decades, the largest epidemics have been caused by the HPAI virus, whose HA gene of subtype H5 is derived from the Gs/Gd/1/96 lineage. The H5N1 viruses containing HA of this lineage were first identified in Hong Kong in 1997 and described as the 2.2. clade (Xu et al. 1999). HPAIV H5 viruses have been continuously changing over the years due to antigenic drift (observed as H5 of individual clades) and reassortment (exchange of genome segments, particularly observed as different HA/NA combinations, e.g., H5N2, H5N5, H5N6 or H5N8) (WHO/OIE/FAO H5 Working Group 2015). The recent HPAI outbreak in poultry and wild birds in Europe and North and South America was caused by the 2.3.4.4b clade of the H5N1 subtype virus, which has also been identified in a wide range of wild carnivores (European Food Safety Authority et al. 2023). Reports have included foxes, lynxes, skunks, raccoons, bears, otters, polecats, badgers, ferrets, pumas, panthers, opossums, black bears, seals, porpoises, sea lions, bottlenoses, dolphins and, more recently, cats. It is likely that infection of cats, as well as the above-mentioned carnivorous mammals, occurs after contact with infected birds or their droppings. Raw meat from diseased birds can also be a source of infection in animals. Under laboratory conditions, cats have been infected by oral and endotracheal introduction of the virus into their bodies. Infection is also likely to spread between cats after contact between infected

and susceptible animals, although this route of transmission has not been definitively confirmed.

Cases of AI are most commonly found in cats that are free-roaming, in contact with birds and fed raw poultry meat, especially of unknown origin (Thiry et al. 2009).

The AI incubation period in cats is short and lasts for about 2 days. In contrast, viral shedding with nasal secretions and feces begins three days after infection and continues for at least 4 days (Thiry et al. 2009).

Affected cats first develop non-specific general symptoms, such as fever, apathy and loss of appetite. They are followed by respiratory distress, dyspnoea, serous and purulent discharge from the nose and conjunctival sac, prolapse of the third eyelid and, later, neurological abnormalities (Fig. 1). Over time, ataxia, convulsions and uneven pupil size appear. The disease generally ends with the collapse of infected individuals within a few hours to a few days after the first clinical signs. On post-mortem examination, petechiae can be observed in internal organs (tonsils, liver, lymph nodes). Histopathological examination may reveal inflammatory and necrotic lesions in the lungs, heart, brain and liver (Thiry et al. 2009).

Epizootic situation of H5N1 influenza virus infection in cats worldwide

Until 2003, there were no reported cases of H5N1 influenza virus infections in cats. In the 1970s and 1980s, trials were conducted to experimentally infect cats with human subtypes of H3N2 and H7N3 viruses isolated from turkeys and H7N7 isolated from seals. The effect of these infections was a transient increase in internal temperature in the animals included in the study and periodic viral shedding with their secretions. However, none of the animals included in the study developed disease symptoms. Unfortunately, no data are available as to whether these viruses belonged to the low- or high-pathogenic AIVs (Paniker and Nair 1970, Paniker and Nair 1972, Hinshaw et al. 1981).

The first case of feline infection with the H5N1 strain of HPAI influenza virus was diagnosed in December 2003 in two tigers and two leopards at a Thai zoo (Keawcharoen et al. 2004). The animals were fed poultry meat from a local abattoir. The onset of disease in the felids coincided with cases of influenza in local birds. Both tigers and leopards were noted to have high fever and respiratory failure, which soon led to death. Post-mortem examination revealed inflammatory infiltrates and petechiae in the lungs, heart, thymus, stomach, intestines, liver and lymph nodes. In addition, encephalitis was noted in one tiger and one leopard.

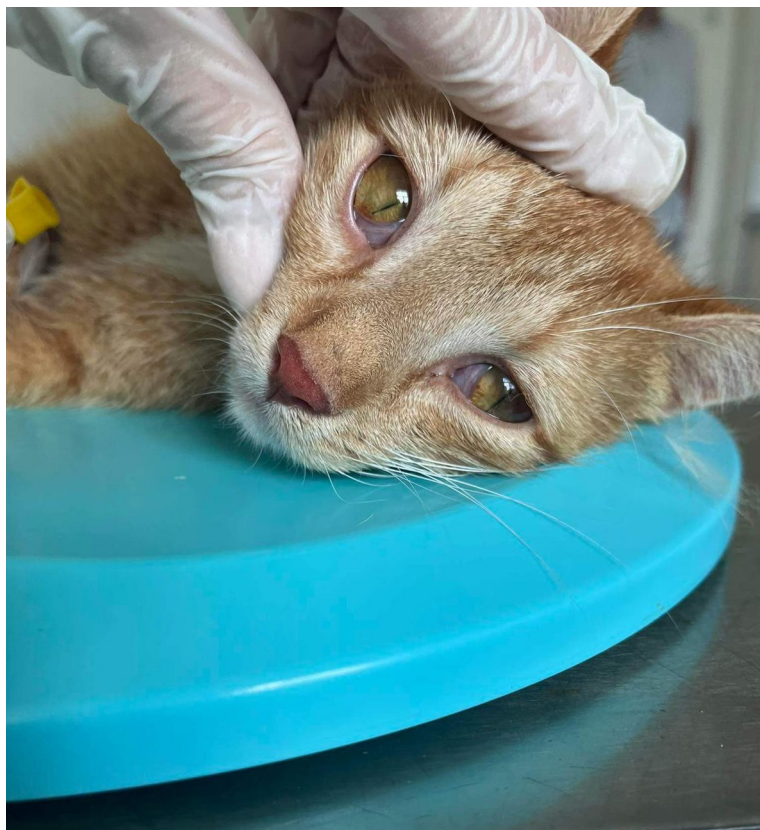


Fig. 1. Constricted pupils of a cat unresponsive to light.

The virus isolates obtained from these cases had a glutamine at position 222 (226 in H3) and a glycine at position 224 (228 in H3) in the HA protein, (also found in previously detected avian isolates of the H5N1 virus), determining the affinity of this pathogen for receptors located on the surface of avian cells (Li et al. 2004).

In the same year, Kuiken et al. (2004) published in *Science* the results of their observations on cats experimentally infected with the HPAIV strain. The animals were infected intratracheally or by feeding them contaminated poultry meat. This resulted in the development of the severe disease with respiratory symptoms and viral shedding with secretions and excretions.

Further cases of H5N1 influenza virus infection in felines were also reported a year later in Thailand in 14 domestic cats and 147 tigers kept in a zoo. Of the infected animals, only one tiger survived the disease. The rest died, having developed such symptoms as fever, shortness of breath, unsteadiness and convulsions (Thanawongnuwech et al. 2005, Songserm et al. 2006, Marschall and Hartmann 2008).

In Europe, cases of H5N1 infection in cats were reported in Germany, Austria and, in December 2022, in France. While in the first of the above-mentioned countries, reports described the death of 3 H5N1-infected cats from the island of Rügen, in the case of Austrian

cats, the genetic material of the virus was detected in tracheal swabs from three of forty asymptomatic randomly tested cats living in a shelter that had close contact with a swan that had died due to H5N1 infection (Klopfleisch et al. 2007a). These individuals were isolated and monitored for a period of 50 days. None of the cats developed signs of AI, indicating that they had developed a subclinical infection (Leschnik et al. 2007). In France, on the other hand, one cat was initially diagnosed with a general disorder, including apathy and mild hyperthermia, but, after a few days, it developed clear neurological and respiratory symptoms (dyspnoea) and was consequently euthanised. The cat belonged to a family running a commercial duck farm next to the house, which had seen a 20% drop in egg production due to HPAIV H5N1 infection two weeks earlier (Briand et al. 2022). Indonesian reports that H5N1 antibodies were found in 20% of 500 cats tested confirmed that cats may be subclinically infected with H5N1 (Mackenzie 2007).

Overall, AI virus infections have so far been reported in cats and felines in China, Thailand, Vietnam, Indonesia, Iraq, Cambodia, France, Austria and Germany (Harder and Vahlenkamp 2010). According to the US Department of Health, H5N1 infections have been confirmed in 6 cats in the United States since October 2022 (Fig. 2).



Fig. 2. International distribution of avian influenza virus infections in cats and felines.

The cases of the disease in cats appear to overlap with its areas of occurrence in birds. Phylogenetic analysis of strains of the pathogen in question isolated from cats and tigers revealed its high genetic similarity with viruses circulating in poultry and wild birds. Point mutations have been detected in the genome of viruses isolated from cats, which may have influenced the greater H5N1 virulence to mammals, although none of them appear to be crucial for increasing the affinity of the virus exclusively to cats (Amonsin et al. 2006, Weber et al. 2007).

In Poland, the first cases of the 2.3.4.4b clade of the H5N1 virus infection in 25 cats in the Lublin, Masovian, Kuyavian-Pomeranian, Lower Silesian, and Pomeranian voivodeships were confirmed at the National Veterinary Research Institute in Puławy in June this year (Domańska-Blicharz et al. 2023, Rabalski et al. 2023). Complete genome sequences of 19 HPAI H5N1 virus-positive cats indicate that the viruses showed close affinity to one another and, according to the nomenclature used by the European Union Reference Laboratory (EURL) in Padua, belonged to clade 2.3.4.4b, genotype CH (H5N1-A/Eurasian_Wigeon/Netherlands/3/2022-like). The cat viruses were clustered with a virus of the same genotype detected at the beginning of June in a white stork in Poland. When infections appeared in cats, it was the BB genotype that was initially suspected as the cause of infection, considering that the EURL had issued a warning stating that the infection in poultry with this genotype could go undetected. In April 2023, it was

reported that infection with the BB genotype could give anomalous disease signs in some poultry species such as turkeys and commercial layers, characterised by low mortality, very low prevalence of infection and almost the total absence of the typical HPAI signs, i.e. egg drop or reduced feed consumption. When there was a definite dominance of this genotype over others detected in wild birds, the veterinary inspectorate and poultry veterinarians in Poland received this information requesting very close monitoring of the health status of poultry flocks.

Pathogenesis and clinical symptoms

Initially in cats, the virus multiplies locally in the lower respiratory tract and can lead to the development of severe pneumonia (Yingst et al. 2006, Klopffleisch et al. 2007a). Since the virus is not able to attach to the receptors on cells in the upper respiratory tract, H5N1 shedding with respiratory aerosol is relatively low (Kuiken et al. 2004, Klopffleisch et al. 2007b). Over time, the pathogen spreads to other tissues, inducing the development of inflammatory and necrotic lesions in many organs. H5N1 can spread in the body of the infected animal with the blood (viremia), or via nerve fibres (Rimmelzwaan et al. 2006).

Infected cats shed the virus with respiratory aerosol (small amounts), feces and urine (Rimmelzwaan et al. 2006, Songserm et al. 2006, Yingst et al. 2006, Klopffleisch et al. 2007a) even before the onset of clinical signs (Paniker and Nair 1972).

Experimental results have shown that shedding begins three days after infection and continues until day seven (Kuiken et al. 2004). Subclinically infected cats may shed the virus with secretions and excretions up to 14 days after infection (Leschnik et al. 2007).

Respiratory symptoms observed in infected cats are associated with severe lung damage (hemorrhages, edema) (van Riel et al. 2006). Histopathological examination in the lungs of dead individuals shows inflammatory and necrotic lesions as well as alveolar damage. Neurological abnormalities result from damage to the brain and cerebellum and the development of non-tropical meningitis (Fig. 1) (Thanawongnuwech et al. 2005, Marschall and Hartmann 2008).

Hematological examinations of the infected cats reveal severe leukopenia and thrombocytopenia, while serum biochemical tests show an increase in AST and ALT activity, which may be a consequence of hepatic necrosis. On the other hand, the presence of serous-bloody nasal discharge in affected cats may be the result of the development of thrombocytopenia (Thanawongnuwech et al. 2005, Marschall and Hartmann 2008).

Diagnosis and treatment

The most sensitive method for detecting the virus in individuals is molecular methods, i.e. reverse transcription (RT) combined with polymerase chain reaction (PCR), both one step RT-PCR and real time RT-PCR. This enables detection of the pathogen in nasal, pharyngeal, and rectal swabs and in organ sections (Songserm et al. 2006, Yingst et al. 2006). It is interesting that in cats subclinically infected with H5N1 virus, viral RNA was detected only in throat swabs (Leschnik et al. 2007). Other techniques that can be used to diagnose the disease are: infection of cell cultures, chicken embryos, hemagglutination test, hemagglutination inhibition and post-mortem immunohistochemistry (Marschall and Hartmann 2008).

Definitive virus identification is only possible based on sequencing of its genome and the results of a comparative phylogenetic analysis of the sequence obtained with those available in the gene database. However, these are labour- and time-consuming, and the pathogenicity of the detected virus requires, according to classical virology, *in vivo* testing (experiments on 6-week-old chickens infected intravenously with the isolated pathogen, i.e. determination of the intravenous virulence index). Currently, pathogenicity is determined by sequencing the cutting site of the HA gene. However, definitive virus identification and accurate epidemiological investigation is only possible based on whole-genome

sequencing and the results of phylogenetic analysis of the obtained sequence with sequences available in the public gene database (determination of the clade or genotype of the virus, as well as the zoonotic potential – point mutations that increase the potential of the virus to infect mammals).

A causal treatment for H5N1 infections in cats has not yet been developed and described. The neuraminidase inhibitor, oseltamivir, which has been used to treat influenza in humans, mice and ferrets (Leneva et al. 2000, Govorkova et al. 2007, Schunemann et al. 2007), has not been shown to be effective in the treatment of tigers infected with avian influenza virus. Thus there is only symptomatic treatment with the hope that the sick cat will itself be able to overcome the infection.

Nor is there a vaccine against the disease, so preventive methods include only non-specific prophylaxis by keeping cats indoors to minimise their contact with infected birds and other infected cats in the area, and avoiding feeding raw meat to cats. Infected cats should be kept in isolation rooms, contact with them should be kept to a minimum, and veterinary surgeons and support staff in veterinary clinics caring for such patients should carefully adhere to the principles of asepsis and hygiene.

Conclusion

This article presents the current knowledge of H5N1 virus infection in cats. In this review article, the current state of knowledge on avian influenza in cats is presented based on available literature sources. It is a secondary clinical and contextual review based on previously published data and does not include any new experimental findings. There are still many uncertainties, especially regarding the routes of infection spread in the cat population, and therefore further interdisciplinary research is needed, also taking into account the potential zoonotic aspect of the disease. Within its preventive measures, the State Sanitary Inspectorate of Poland has taken up epidemiological surveillance of the owners/carers of cats with confirmed avian influenza virus infection (Notice of 04.07.2023). The disease cannot be underestimated, especially as its course is violent and fatal in most cases; nevertheless it should not be considered as an epidemic at this point. By 04/07/2023, several dozen suspected clinical cases of AI in cats or felines (one caracal) had been diagnosed in Poland. Certainly this number is not small, but every month in our surgeries and clinics, we far more frequently diagnose other fatal infectious diseases in cats, such as, for example, leukaemia, FIV and FIP. A separate issue in terms of AI in cats and the accompanying nervous

symptoms is rabies. We need to ask whether, while “chasing” cases of AI in cats and trying at all costs to diagnose the disease in our patients, we are equally careful in the differential diagnosis of rabies, which produces similar symptoms, being a fatal and notifiable zoonosis.

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