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Susceptibility of Mammals to Highly Pathogenic Avian Influenza: A Qualitative Risk Assessment From the Belgian Perspective

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ABSTRACT

Aims: The world experienced a huge number of outbreaks of highly pathogenic avian influenza (HPAI) in birds, which could represent one of the largest registered epidemics of infectious disease in food-producing animals. Therefore, mammals, including humans, are continuously exposed to HPAI viruses leading to sporadic and sometimes unusual mammal infections. The aim of this paper is to assess the risk of crossing the avian/mammalian species barrier by the currently circulating HPAI viruses, focusing on the epidemiological situation of Belgium, a representative country for Western Europe.

Methods and Results: Information on transmission pathways and species susceptibility, based on the experimental and epidemiological data, was reviewed and weighted to assess the risk of mammal infection with HPAI A(H5N1) viruses of the circulating clade 2.3.4.4b. This risk is defined as the likelihood of mammal infection by birds crossed by the clinical consequences of this infection for this animal. From the Belgian perspective, it is concluded that this risk remains 'low' to 'moderate' for captive/domestic mammal species. However, this risk was categorised as 'high' for certain species, i.e. mammals that have the opportunity to have frequent direct or indirect close contacts with infected (dead) birds, such as wild felids, wild mustelids, foxes and wild marine carnivore mammals. For some mammal species, the uncertainty associated with the assessment remains high due to an ever-changing situation.

Conclusions: The longer the virus will continue to circulate in wildlife/the environment the stronger the probability of contact between infected birds and mammals will become. This will increase the related risk of viral adaptation for efficient transmission between mammal, posing concerns for public health. Regular reassessments based on the field and experimental data are therefore necessary to implement and adapt risk-based mitigation measures. This will require continuous monitoring of avian influenza viruses in both birds and mammals as well as sharing of sequence data.

Summary

- In parallel with the highly pathogenic avian influenza epidemic that has been affecting birds since 2021, the world is experiencing an unprecedented number of cases of infection in mammals, as well as a few cases in humans. Cross-species transmission to mammals can occur either through direct contact between mammals and birds reservoir of avian influenza virus or through indirect contact with bird faeces or a contaminated environment. In addition, there is increasing evidence of mammal-to-mammal transmission. This epidemiological situation is increasing the probability of interspecies transmission events, allowing the virus to adapt to mammalian hosts, including acquiring zoonotic potential.
- With the current outbreaks of H5N1, a significantly higher occurrence of spill-overs has occurred in an ever-increasing number of mammalian species. Moreover, a new susceptible species has been identified with infections of dairy cattle in the USA. In addition to the classical respiratory transmission pathway, a new transmission route has been described in these particular outbreaks, with viral replication in the udder and mechanical transmission from cattle-to-cattle via the milking machine.
- The risk of mammalian infection by the strains currently circulating in Belgium remains 'low' to 'moderate', except for animals that may have close contact with infected (dead) birds, such as wild felines, wild mustelids, foxes and wild marine carnivorous mammals. To reduce the likelihood of contact between infected birds and mammals, it is therefore necessary to reinforce biosecurity measures, including in the hobby breeding sector, to limit the spread of the virus from the wild animal compartment to captive animals and vice versa.

1 | Introduction

Influenza A viruses (IAV) are found in a wide range of hosts, including birds (avian IAV), humans (human IAV) and pigs (swine IAV) (Lee and Saif 2009; Abdelwhab and Mettenleiter 2023). Also known as 'bird flu', avian influenza is a contagious viral disease mostly affecting poultry and wild birds, often resulting in severe animal health, biodiversity and economic consequences (CMS FAO 2023; Imperia et al. 2023; Plaza et al. 2024; WOAH 2024). Avian IAV have been distinguished on their pathogenicity (i.e., clinical outcome) in poultry (Lee and Saif 2009; WOAH 2024). Influenza viruses are classified into subtypes based on the two surface proteins: the hemagglutinin (HA) and neuraminidase (NA). At least 16 hemagglutinin (H1–H16) and 9 neuraminidase (N1–N9) subtypes have been found in viruses from birds (Lee and Saif 2009; Mänz, Schwemmle, and Brunotte 2013; Suttie et al. 2019). These subtypes are divided in clades using H5 HA phylogenetic characterisation and nucleotide sequence divergence based on the nomenclature system designated by the WHO/OIE/FAO H5N1 Evolution Working Group to classify the A/goose/Guangdong/1/1996 lineage of Eurasian highly pathogenic H5N1 avian influenza viruses (WHO/OIE/FAO 2012). Highly pathogenic avian influenza virus (HPAIV) infection is a regulated disease in poultry and wild birds in Europe (Regulation EU 2016/429; Regulation EU 2018/1882). Although the World Organisation for Animal Health (WOAH) is requesting to communicate any outbreak of HPAI, regulation is often lacking in many countries where HPAIV infections are detected in wild birds, captive birds and, more importantly from a zoonotic perspective, in mammal species.

The world experienced a huge number of outbreaks of HPAI in birds which could represent, by any relevant measure such as the huge number of cases in birds or their geographical distribution in the whole world, one of the largest registered epidemics of infectious disease in food-producing animals in the history of animal domestication. Indeed, HPAIV became widely spread in wildlife with substantial losses in the wild bird population endangering some bird species, as well as frequent spill over to captive birds and poultry (CMS FAO 2023; PAHO 2023). The predominant strains involved in the current outbreaks belong to the clade 2.3.4.4b which shows a high variability and diversification with many reassorted subtypes (exchange of genetic segments between viral strains) and genetic lineages co-circulating globally as well as the sporadic occurrence of various mutations that could increase the risk of spill-over to and replication in mammals (Antigua et al. 2019).

Although HPAIV are considered species-specific, they have occasionally crossed the species barrier and have been isolated from mammalian species, including humans (Mänz, Schwemmle, and Brunotte 2013; WAHIS 2024; WHO 2024). The transmission of avian IAV to mammal species is of great concern because this may allow the virus to adapt to mammal hosts and acquire zoonotic potential. Moreover, some mammal species are expressing sialic acid (SA) receptors to both human and avian viruses. As such they could be susceptible to both and act as mixing vessels for them, leading to the emergence of new viruses. Pigs are the best known mixing vessel hosts for the generation of zoonotic influenza viruses but recent studies have shown that many other mammalian species possess the two types of SA receptors along their respiratory tract (Ma, Kahn, and Richt 2008; Abdelwhab and Mettenleiter 2023).

Although most of the reports on HPAIV A(H5N1) in wild and domesticated mammals consist of single or a few animals, there recently occurred some mass mortality events of mammals associated with HPAI A(H5N1), sometimes characterised by increased evidence of mammal-to-mammal transmission and/or highlighting some adaptative mammal mutations (Agüero et al. 2023; Domańska-Blicharz et al. 2023; Lindh et al. 2023; Puryear et al. 2023; Rabalski et al. 2023; Plaza et al. 2024; Rimondi et al. 2024; Tomás et al. 2024; Uhart et al. 2024). Moreover, many United States dairy herds were hit by unexpected HPAI outbreaks which seem to originate from a single wild bird-to-cattle spill over event and a subsequent cattle-to-cattle transmission chain (Nguyen et al. 2024).

According to the available literature, human infections with HPAIV A(H5N1) have mainly been associated with close contact with sick birds and/or their environment. Recently, at least

two human cases were described in dairy farms workers after exposure to presumably infected dairy cattle (Garg et al. 2024) and a human case with no immediate known animal exposure was described in the USA (CDC 2024b). Up to now, the virus does not seem to be easily transmitted from person to person (Abdelwhab and Mettenleiter 2023; PAHO 2023). Considering the increased circulation of avian IAV in wild birds, HPAI outbreaks in poultry and backyard holdings, as well as virus spill over from birds to captive and wild mammal species, humans are currently more exposed to avian IAV than before.

The aim of this paper is to perform a qualitative assessment of the risk of transmission of HPAIV A(H5N1) clade 2.3.4.4b from avian to mammalian species. Starting from the epidemiological and molecular context in Europe, this assessment is focusing on the situation of Belgium, a representative Western European country where poultry are mainly raised indoor, with wild birds migration ways and the majority of poultry flocks sharing the same geographic area.

2 | Epidemiological Situation of HPAIV

2.1 | HPAIV in Birds in Europe

The HPAI epidemic observed during the 2021–2022 epidemiological year was the largest recorded in Europe so far with a total number of HPAI A(H5N1) outbreaks detections in birds (domestic and wild) of 5716. In comparison, the HPAI epidemic observed during the 2022–2023 epidemiological year was characterised by a higher total number of HPAI detections in wild birds (3873 compared with 3228) and a lower total number of HPAI detections in domestic birds (1173 compared with 2488) (Avian Flu Data Portal and EURL for Avian Influenza and Newcastle Disease 2024). The distribution of the HPAI A(H5N1) birds outbreaks in Europe for the epidemiological years 2021–2022 and 2022–2023 is represented in Figure 1.

In Belgium, there were 33 virus detections reported in the poultry and captive birds and 250 in wild birds between 1 October 2022 and 30 September 2023 (Sciensano Institute, NRL Avian Influenza and Coordination of Veterinary Activities/Veterinary Epidemiology Service 2024). The distribution of the HPAI A(H5N1/H5Nx) cases in Belgium for the epidemiological year 2022–2023 is represented in Figure 2.

The high infection pressure in wildlife increases the risk of infection for kept poultry and, following the first epidemic year, the awareness of the operators and the biosecurity measures were reinforced putatively, explaining the decrease of incidence of the disease in the poultry sector.

2.2 | HPAIV in Mammals in Europe

Since the beginning of the pandemic of HPAIV in wild birds, individuals from several mammal species have been found positive for HPAIV in Europe (EFSA 2023b; ESFA 2024;

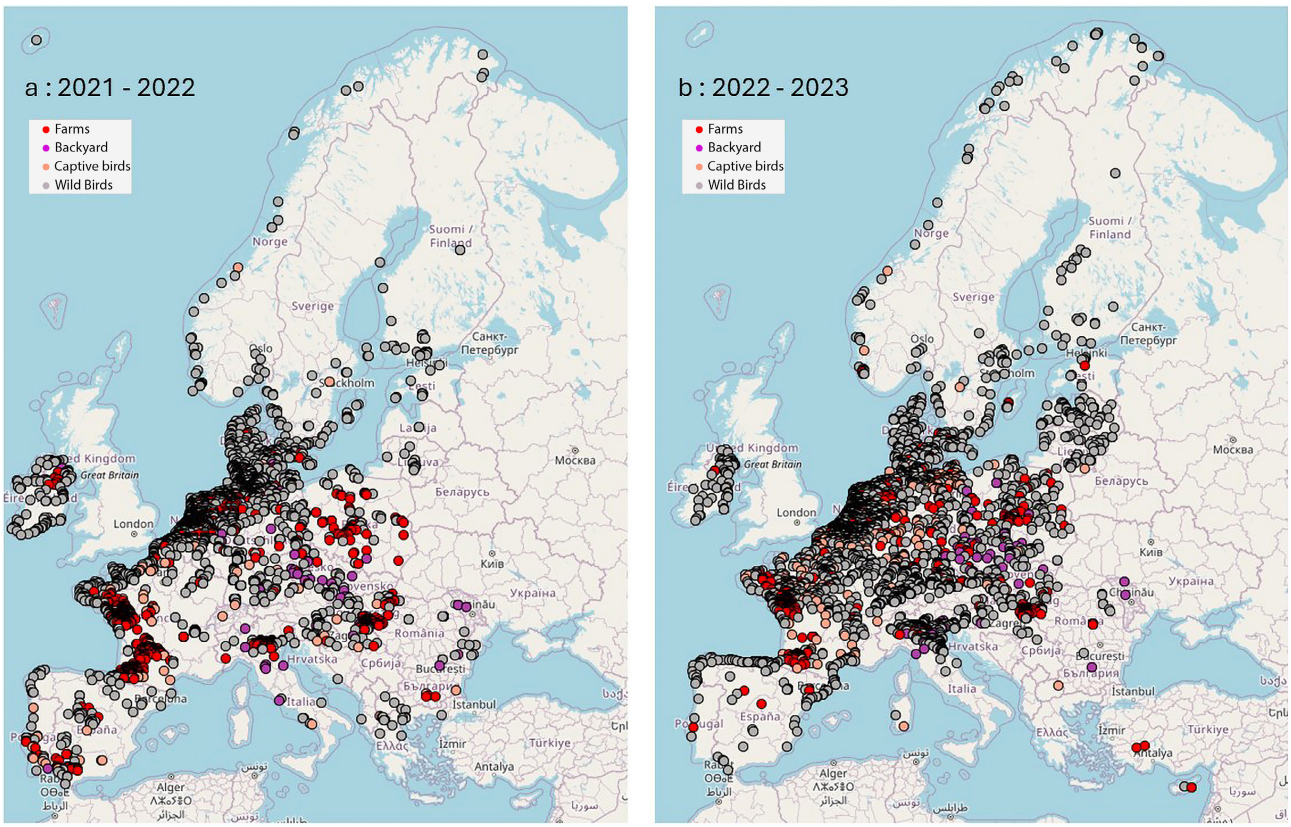

FIGURE 1 | Distribution of the HPAI A(H5N1) domestic and wild birds outbreaks notified by each member states/reporting countries in Europe during (a) the epidemiological year 2021–2022 and (b) the epidemiological year 2022–2023 (figure generated via Avian Flu Data Portal and EURL for Avian Influenza and Newcastle Disease 2024, available at https://eurlaidata.izsvenezie.it/epidemio.php#).

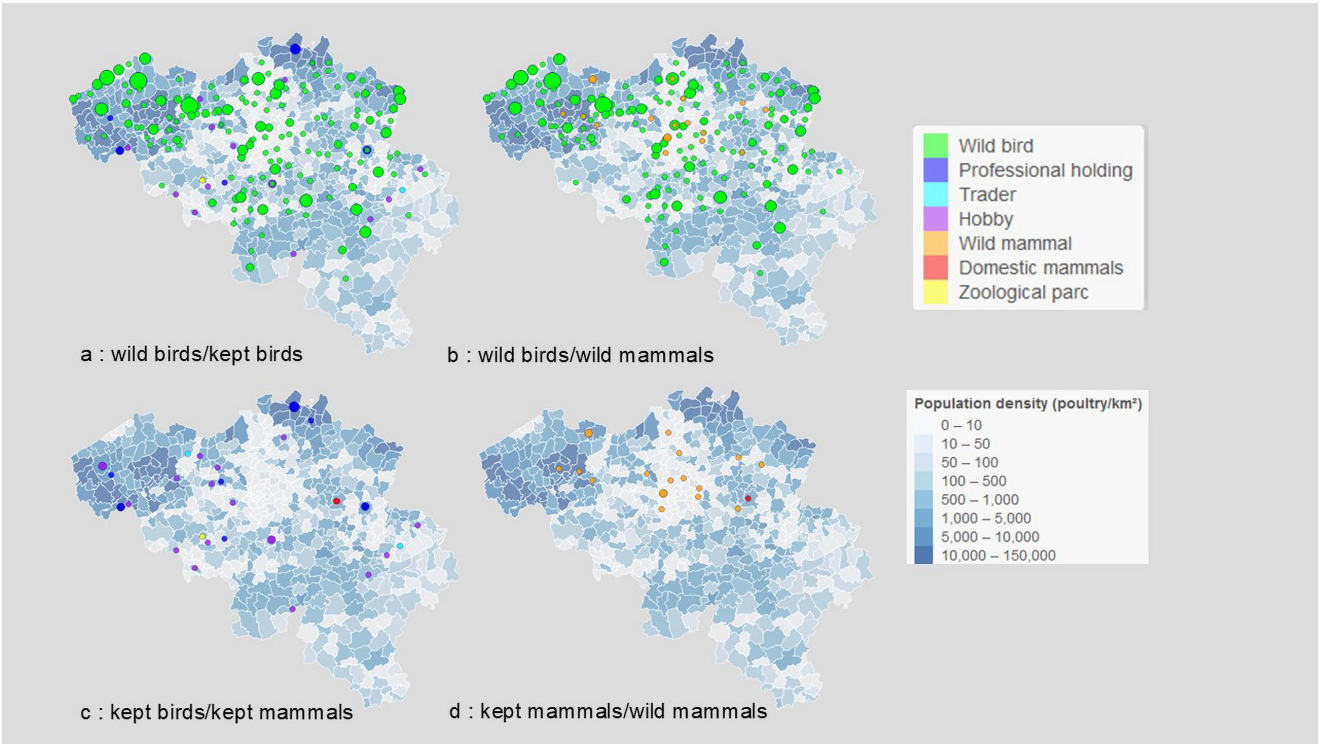

FIGURE 2 | Distribution of HPAI H5N1/H5Nx cases reported in birds and mammals in Belgium during the epidemiological year 2022–2023 (1 October 2022–30 September 2023). The number of cases notified during this period is proportional to the size of the circles (from 1 to 9). Kept birds include the following subcategories: 'Professional holding' which refers to people who keep poultry professionally, 'Trader' which refers to people who specifically trade poultry, 'Hobby' which refers to people who keep poultry as a leisure activity and 'Zoological park' which refers to birds held in zoos with the courtesy of Dr. M. Steensels and M. Driesen (Sciensano Institute, NRL Avian Influenza and Coordination of Veterinary Activities/Veterinary Epidemiology Service 2024, available at https://moriskin.sciensano.be/shiny/avianinfluenza/).

WAHIS 2024; FAO 2024). The vast majority of the HPAI A(H5Nx) cases reported in mammals involved wild carnivores (Order *Carnivora*) that predate wild birds and/or feed on wild birds carcasses, whereas fewer events were reported in captive/domestic species such as ferret (*Mustela furo*), cat (*Felis catus*), American mink (*Neogale vison*) and dogs (*Canis lupus familiaris*). However, it is important to note that the monitoring and the sampling could be different between domestic and wild populations of animals and that we do not know the true prevalence in both of populations. Between 1 October 2022 and 28 March 2024, virus detections were reported in 15 wild red foxes, 3 wild European polecats and in one outbreak in domestic ferrets in Belgium ($n = 6$) (Sciensano Institute, NRL Avian Influenza and Coordination of Veterinary Activities/Veterinary Epidemiology Service 2024). Birds were the common suspected source of contamination for mammals.

Between October 2020 and December 2023, the complete genome sequences ($n \sim 250$) of HPAIV A(H5) clade 2.3.4.4b collected in 16 European countries from 18 distinct mammalian species (i.e., American mink (*Neogale vison*), Arctic fox (*Vulpes lagopus*), Asian black bear (*Ursus thibetanus*), beech marten (*Martes foina*), bush dog (*Speothos venaticus*), caracal (*Caracal caracal*), cat (*Felis catus*), common raccoon dog (*Nyctereutes procyonoides*), European badger (*Meles meles*), Eurasian lynx (*Lynx lynx*), Eurasian otter (*Luttra luttra*), European polecat (*Mustela putorius*), ferret (*Mustela furo*), grey seal (*Halichoerus grypus*), harbour porpoise (*Phocoena phocoena*), harbour seal (*Phoca vitulina*), red fox (*Vulpes vulpes*) and South American coati (*Nasua nasua*)) have been generated (EFSA 2023b).

2.3 | Molecular Epidemiology of HPAIV in Europe

Several mutations have been described in the literature as being associated with (i) enhanced polymerase activity and replication in mammals or mammalian cells, (ii) increased virulence in Galliformes or mammals, (iii) increased/conferred resistance towards antiviral drugs, (iv) increased in vitro binding to human-type receptors alpha2,6-SA, (v) decreased antiviral response in ferrets, (vi) evasion of the human defence mechanism mediated by butyrophilin subfamily 3 member A3 (BTN3A3), (vii) disruption of the second SA binding site on the neuraminidase protein and (viii) overcoming of MxA restriction factors via mutations in the nucleoprotein (Mänz et al. 2013; Du et al. 2018; Suttie et al. 2019; Pinto et al. 2023; EFSA 2024). Some of these mutations have been detected in viruses from birds and mammals collected in different European countries since October 2022 (summarised in Table 1). Their net effect on the biological characteristics of the viruses is still unknown (EFSA 2023b), as other less monitored mutations could also affect it.

Among the identified mutations, molecular markers associated with an increased replication and/or virulence in mammals (PB2-E627K, PB2-D701N, PB2-T271A, PB2-526R) have

TABLE 1 | summary of mutations identified in HPAIV A(H5N1) circulating in birds in Europe between October 2022 and December 2023 and in mammals between 2020 and 2023 (adapted from Suttie et al. 2019; EFSA 2023b; Plaza et al. 2024).

Mutation/motif	Phenotype (subtype H5N1)	Frequency of detection in birds in UE	Mammal species in which mutations were detected (2020–2023)
Polymerase basic protein PB2—Polymerase activity, virulence and transmissibility			
E627K	Enhanced polymerase activity, increased virulence in mice, contributes to air- borne pathogenicity of IAVs in ferrets and contact transmission in guinea pigs. Decreases polymerase activity and replication in avian cell lines. Decreases virulence in chickens	Sporadically observed	Canidae (*Vulpes vulpes*), Felidae (*Felis catus*, *Lynx lynx*), Mustelidae (*Lutra lutra*, *Mustela putorius*), Phocidae (*Halichoerus grypus*, *Phoca vitulina*[a]), Procyonidae (*Procyon lotor*[a])
D701N	Increased polymerase activity, enhanced replication efficiency, increased virulence and contact transmission in guinea pigs, increased virulence in mice.	Sporadically observed	Canidae (*Vulpes vulpes*), Felidae (*Lynx lynx*), Otariidae (*Otaria flavescens*[a]), Phocidae (*Phoca vitulina*[a]), Ursidae (*Ursus americanus*[a])
T271A	Increase polymerase activity in avian and mammalian cell line	Sporadically observed	Mustelidae (*Mustela putorius*, *Neovison neovison*)
K526R	Increased polymerase activity in mammalian cell line.	Sporadically observed	Felidae (*Felis catus*)
K526R, E627K	Increased polymerase activity and viral replication in mammalian cell lines, increased virulence in mice	Sporadically observed	Felidae (*Felis catus*)—Outbreak in Poland
E627K, D701N	Increased polymerase activity, viral replication in cell lines, increased virulence in mice		Felidae (*Lynx lynx*), Phocidae (*Phoca vitulina*[a])
Hemagglutinin (H5 numbering)—Receptor binding specificity			
S133A, S154N, TI56A	Increased virus binding to α2-6[b]	Majority of A(H5N1) viruses circulating in birds in EU since October 2022	
D94N, S155N, V210I	Increased virus binding to α2-6	Sporadically observed	

[a]Outside Europe.
[b]α2-6-linked SA (galactose C6), commonly referred to as 'human receptor'.

been rarely detected in viruses collected from wild and domesticated birds. In contrast, about half of the characterised viruses isolated in mammals contain at least one of the adaptive markers associated with an increased virulence and replication in mammals in the PB2 protein (E627K, D701N, T271A or K526R) (Suttie et al. 2019; EFSA 2023b, 2024). Moreover, the mammalian adaptative amino acid substitution Q591K/D701N in PB2, which is associated with increased virulence, transmission and adaptation to mammalian hosts, was detected in strains isolated in South America in 2023. Phylogenetic analysis suggested that these strains have a mammalian origin and there is growing evidence that they could be able to transmit efficiently among pinnipeds while retaining the ability to infect wild birds and poultry (Tomás et al. 2024; Rimondi et al. 2024; Rivetti et al. 2024; Uhart et al. 2024). These observations suggest that mutations with potential public health implications are preferentially selected after transmission to mammals and the first replication within the new host species, i.e. during virus adaptation (EFSA 2023b, 2024). The analysis of a strain isolated from a human case in Chile showed that this strain possessed also the two amino acid substitutions Q591K/D701N in PB2 and was able to replicate very efficiently in mammalian cells, to cause fatal diseases in the ferret model and to transmit between co-housed ferrets (Pulit-Penaloza et al. 2024). Therefore, mass outbreaks with the HPAI A(H5N1) virus in mammals where mammal-to-mammal transmission is suspected may facilitate virus adaptation and represent a potential zoonotic risk.

On the other hand, mutations in the protein hemagglutinin (HA), which initiates influenza virus infections by recognising and binding to sialylated glycan's on the surface of host cells,

have been identified either frequently or sporadically in birds (see Table 1) (EFSA 2023b). The impact of these HA mutations on the biological characteristics of the circulating viruses is still unknown. Host adaptation of influenza virus is partly dependent on the SA isoform bound by the viral hemagglutinin (with avian influenza viruses preferentially bind the α-2,3 SA isoform and human influenza viruses the α-2,6 SA isoform) (Shelton et al. 2011; Abdelwhab and Mettenleiter 2023) but none of these mutations has been demonstrated to cause a shift from avian-like to human-like receptor binding preference. Moreover, mutations NP-Y52N and NA-S3691 are detected in almost all the HPAIV A(H5N1) belonging to the BB genotype (H5N1 A/gull/France/22P015977/2022-like). These mutations may increase the zoonotic potential of these viruses (EFSA 2023b). Mutations associated with antiviral resistance were occasionally identified in the circulating strains (Suttie et al. 2019; EFSA 2023b).

Even if HPAIV A(H5Nx) continue to spread and diversify worldwide, molecular analyses of circulating strains in birds in Europe during the 2022–2023 epidemiological year indicate that they retain a preferential binding for avian-like receptors, making that they continue to be well-adapted to avian species and transmit and replicate best in birds (ECDC 2024a; EFSA 2023b).

Considering the many different IAV circulating in the wild and that reassortment events was demonstrated to be constitutive in IAV (Lowen 2017), events will likely continue to occur. This will probably lead to an increasingly complex and unpredictable situation regarding the predominant strains and their virulence in the future.

3 | Transmission Pathways to Be Considered for Mammal Infection With HPAI A(H5N1) Clade 2.3.4.4b

Contacts between infected birds and susceptible mammals are a precondition for avian influenza spill over into mammals. Cross-species transmission of avian IAV to mammals may occur through either direct contacts between mammals and bird reservoirs of avian IAV (including predation of infected bird carcasses, mucosal contact and inhalation of aerosolised viral particles) or indirect contact with bird faeces or a contaminated environment (including mucosal contact or ingestion of contaminated food/feed or water) (Reperant et al. 2008). Figure 3 summarises the avian-to-mammals infection pathways and the subsequent inter-mammal transmission pathways currently identified or suspected for HPAIV A(H5N1) clade 2.3.4.4b.

Given the high circulation of avian influenza viruses during HPAI outbreaks in wild birds, in poultry farms and backyard holdings, as well as virus spill over from birds to domesticated and wild mammal species, people working in some specific sectors (e.g., slaughterhouses, farms or wildlife rescue centres) can be highly exposed to IAV. Therefore, sporadic zoonotic events cannot be excluded especially in case of direct

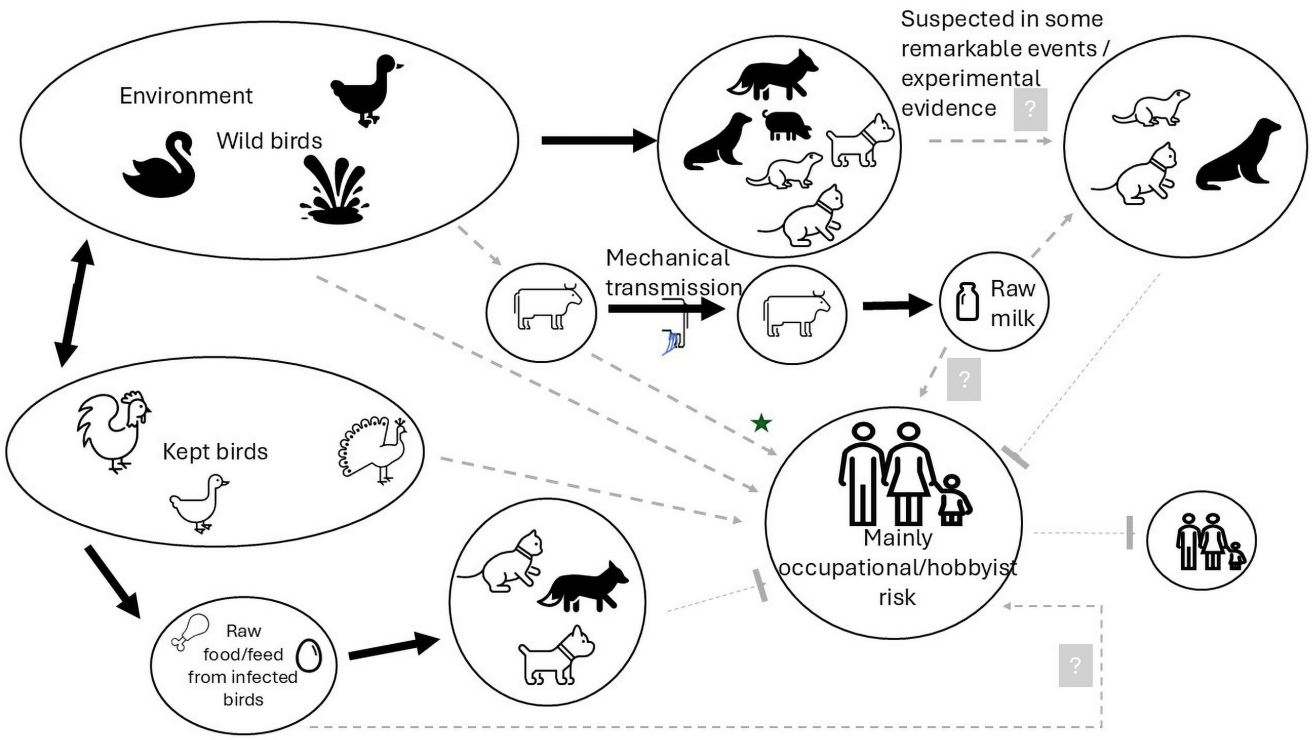

FIGURE 3 | Graphical model of avian-to-mammals infection pathways as currently identified or suspected for HPAIV A(H5N1) clade 2.3.4.4b. These infection pathways could evolve along time and should be reassessed depending on emergence of viral strains better adapted to mammals. Wild animals are represented in black and captive animals are represented in white. Main infection pathways are represented by solid lines while uncommon infection pathways are represented in broken lines with an arrow. Undescribed infection pathways are represented in broken lines with a trait. Question mark represents suspected pathway based either on remarkable field events, experimental evidence or in evidence in other species. A circumstantially pathway for bovine in the outbreak of April 2024 in the USA is represented by the star.

and unprotected contact between humans and infected animals. This aligns with the literature in which it is reported that human infections with HPAIV A(H5N1) are typically linked to close contact with infected birds and/or their environment. Moreover, H5N1 infection in farmers working in close contact with infected cattle has been detected (Garg et al. 2024). The infection pathway (direct vs. indirect vs. alimentary) is up to now unclear.

Although the most plausible source of infection for mammals in Europe is currently direct or indirect close contact with infected birds, including predation or carcass scavenging (Plaza et al. 2024), some experimental studies have shown that the HPAIV A(H5N1) virus could spread from mammal-to-mammal (Kuiken et al. 2004; Thiry et al. 2007; Pulit-Penaloza et al. 2024). Moreover, increased field evidence for mammal-to-mammal transmission has also been described in some massive HPAI outbreaks such as in farmed minks and in fur farms in Europe or in marine mammals in South America (Agüero et al. 2023; Kareinen et al. 2024; Uhart et al. 2024). More recently, cattle-to-cattle transmission was described in dairy cattle HPAI outbreaks in the USA as well as suspicion for interspecies transmission from cattle-to-cats and raccoons (Caserta et al. 2024).

Finally, the digestive route should also be considered since it has been shown that ferrets can be experimentally infected through intragastric inoculation with a A(H5N1) strain that differ from those actually circulating in Europe (Lipatov et al. 2009; Shinya et al. 2011; Edenborough et al. 2016). This transmission pathway should therefore be considered in case of ingestion of raw poultry products (e.g., meat or eggs). Moreover, it was recently described that milk from infected cows in the USA contained large number of viruses underlined that raw milk (and maybe raw dairy products) should also be considered as a source of infection (Burrough et al. 2024). Of note, even if intragastric inoculation in ferrets can experimentally produce systemic infection with virus dissemination to organs including pancreas, liver and lungs, viral titres in the nasal wash were lower than after an infection via the respiratory route (Lipatov et al. 2009; Shinya et al. 2011; Edenborough et al. 2016). As no further viral excretion through the respiratory route does occur following the digestive route, it should be considered as a dead-end infection.

4 | Susceptibility of Mammalian Species and Humans to Infection With HPAIV A(H5N1) Clade 2.3.4.4b and Clinical Consequences

4.1 | Canids

The susceptibility of canids to HPAI viruses has been demonstrated following experimental infections in both dogs and foxes (Maas et al. 2007; Giese et al. 2008; Reperant et al. 2008; Chen et al. 2010). There are also reported cases of natural infection in dogs (Songserm et al. 2006; Moreno et al. 2023) and foxes and some cases are described in foxes in Belgium (Chestakova et al. 2023; Cronk et al. 2023; Vreman et al. 2023). This is not surprising as foxes are omnipresent in Belgium and as they predate wild birds and/or scavenge on them. Their infection may result in a variation of outcomes from asymptomatic (Chestakova et al. 2023) to subclinical infection or serious illness with respiratory and neurological signs (Songserm et al. 2006; Cronk et al. 2023; Moreno et al. 2023; Vreman et al. 2023). Infections may remain undetected.

4.2 | Cattle, Sheep and Goats

Experimental infection with IAV has been successful in cattle and demonstrated this species as permissive (Kalthoff et al. 2008). However, despite the growing mammal host range of IAV over the last century, the incidence of IAV in bovine species remained low until recently and one hypothesis to explain why this species has been spared until this period was the presence of some host-dependent restriction factors in the bovine respiratory tract, which possibly hinder/interfere with IAV replication and further adaptation (Sreenivasan et al. 2019).

In 2024, HPAIV A(H5N1) was reported in 10 neonatal goat kids displaying neurological signs and mortality and then in alpacas from premises with a history of HPAI affected poultry, marking the first natural infection of A(H5N1) virus in any ruminant species (suborder Ruminantia) worldwide (FAO 2024; EFSA 2024; Animal and Plant Health Inspection Service—U.S. Department of Agriculture (USDA) 2024; Sreenivasan, Li, and Wang 2024).

At the same time, but unrelated to the cases in goats and alpacas and involving another genotype, cases have also been reported in dairy cattle in the USA (Nguyen et al. 2024). In these particular outbreaks, clinical signs included a drop in milk production, loss of appetite, changes in manure consistency, thickened or colostrum-like milk, low-grade fever, lethargy and dehydration (APHIS 2024; Caserta et al. 2024). A preferential tropism of the virus to the mammary gland tissue as well as high levels of H5N1 viruses in raw milk were also described, underlying a new cattle-to-cattle transmission pathway implicating virus-contaminated milking machine (Le Sage et al. 2024; Sreenivasan, Li, and Wang 2024). Moreover, evidence for interspecies transmission from cattle to cats and raccoons were also described. One hypothesised pathway of transmission is raw milk ingestion leading to a neurotropism in cats (Caserta et al. 2024).

These cattle outbreaks seem to be due to a single wild bird-to-cattle spill over event of a USA strain and a subsequent cattle-to-cattle transmission mainly through milking machine (Nguyen et al. 2024). Moreover, a recent study showed that other HPAIV H5N1 strains have also the potential to replicate in the udder of cows underlying that cows infections with subsequent transmission through milk could also occurred with European strains (Halwe et al. 2024). If cases were to be described in cattle in Belgium, it is conceivable that intra-herd transmission via the milking machine would be similar to that in the USA, while the inter-herd transmission that enabled the virus to spread in many American states should be limited in view of the organisation of the dairy sector in Belgium, in which trade of lactating animals remains limited.

4.3 | Felids

The susceptibility of felids to HPAIV has been well demonstrated during experimental infections showing that these animals could excrete large amounts of virus and that horizontal transmission between cats (with strain A/Vietnam/1194/04) occurs (Kuiken et al. 2004; Govorkova et al. 2005; Rimmelzwaan et al. 2006; Thiry et al. 2007). Virus isolation from pharyngeal, nasal and rectal swabs of intratracheally inoculated cats, from cats fed on virus-infected chicks and from sentinel cats has been demonstrated (strain A/Vietnam/1194/2004). Some studies suggested that infection could be transmitted from infected cats to contact cats (or tigers) through contaminated saliva, urine and faeces. Cat-to-cat transmission probably depends on the level of virus excretion, assumed to be higher in experimentally infected cats exposed to high infectious doses (Kuiken et al. 2004; Thanawongnuwech et al. 2005; Rimmelzwaan et al. 2006; Thiry et al. 2007).

There are many reported cases of natural infection in cats and large felids with HPAI A(H5N1) viruses which have occurred with clinical signs ranging from sub-clinical infection to severe deadly illness including neurologic and respiratory symptoms (Thanawongnuwech et al. 2005; Hu et al. 2016; Briand et al. 2023; Domańska-Blicharz et al. 2023; Moreno et al. 2023; Rabalski et al. 2023). An important outbreak with several cases of HPAIV infection in domesticated cats occurred in Poland in June 2023 (WHO 2023). Cat viral sequences ($n = 21$) were highly similar, suggesting a potential common infection source. HPAIV was detected in one sample from poultry raw meat used as cat feed. There was no indication of further transmission from cat to cat or to their owner (Domańska-Blicharz et al. 2023; Rabalski et al. 2023). In that time frame, no case was described in cats in Belgium. However, based on the high susceptibility of felids to HPAIV and the fact that a significant proportion of the cat population in Belgium has access to the outdoors and may therefore come into contact with birds, future outbreaks in Belgium cannot be ruled out. Moreover, as animals can present a wide range of clinical signs including being asymptomatic, a number of cases can go unnoticed.

4.4 | Marine Mammals

Concomitant with waves of HPAI A(H5N1), many outbreaks in marine mammals such as harbour and grey seals or sea lions have been described around the world, resulting sometimes in massive mortality events. Transmission from wild birds to marine mammals probably occurred through environmental transmission of shed viruses (e.g., accidental ingestion of faeces or feathers from infected birds, drinking of faecal-contaminated water or inhalation of aerosols or respiratory fomites from infected avian carcasses). Another possible route of transmission is through predation or scavenging infected birds even if birds are not a typical food source for harbour and grey seals (Scharff-Olsen et al. 2019; Puryear et al. 2023). There is growing evidence of sustained-transmission between marine mammals in the United States and in South America (Gamarra-Toledo et al. 2023; Leguia et al. 2023; Puryear et al. 2023; Tomás et al. 2024; Rivetti et al. 2024; Uhart et al. 2024). If this direct transmission pathway was confirmed in Europe, it could be of concern due to the colonial breeding behaviour of these animals that are sometimes congregated by hundreds in the same area (Gamarra-Toledo et al. 2023). Furthermore, seals may act as a reservoir for the virus and a driver for mammalian adaptation (Puryear et al. 2016).

4.5 | Mustelids

The susceptibility of mustelids to HPAIV is well documented as ferrets are susceptible to infection with human and avian IAV and constitute one of the most used experimental model for studying HPAIV infection. The pathogenesis of H5N1-induced disease in ferrets resembles, in many characteristics, that described for H5N1 infection in humans (Lipatov et al. 2009). A recent study showed that the virus A/Chile/25945/2023 HPAI A(H5N1) isolated from a severe human case in Chile was capable of transmission between co-housed ferrets (Pulit-Penaloza et al. 2024).

Field data highlighted the susceptibility of mustelids with two recent reported outbreaks: one in intensively farmed minks in 2022 in Spain and another involving several herds of farmed fur animals in Norway in 2023. As animal density and the mode and frequency of interactions with congeners are probably influencing the risk of further propagation within the same species (Frymus et al. 2021; Chestakova et al. 2023), intensively farmed fur animals are at higher risk for mammal adaptation and interspecies transmission.

In Belgium, some cases in wild European polecats and by a hobby holder who kept birds and a few ferrets captive in different outdoor enclosures were reported (EFSA 2023a). The ferrets, in contact with infected captive birds and fed with eggs from them, showed respiratory signs and mortality.

4.6 | Suids

Since decades, pigs have been presented as the mixing vessel for interspecies transmission among birds, mammals and humans because these animals were shown to harbour both a-2,6- and a-2,3-SA in their upper respiratory tissues. This dogma is now recognised as a simplistic model of host species determinism (for a review on SA/HA interactions, see Byrd-Leotis, Cummings, and Steinhauer 2017) and of the peculiar pig susceptibility to both avian and mammalian IAV since both a-2,6- and a-2,3-SA moieties have been detected in the respiratory tract of several other species including human (Shinya et al. 2006; Wan and Perez 2006; Nicholls et al. 2008; Kimble, Nieto, and Perez 2010). Essentially, pigs are combining several characteristics of receptor binding specificity (beyond the terminal SA structures), glycosylation profile and distribution, as well as other host factors facilitating avian IAV adaptation to mammals and cell co-infection with both avian and mammalian IAV. As reassortment is a constitutive output of influenza virus replication during cell coinfection, pigs are constituting an adaptative link between avian and mammalian IAV and are contributing to the risk of reassortment. In such, pigs have been blamed for the emergence of at least one pandemic (A(H1N1)pdm2009 strain).

Experimental infection of pigs with HPAI (A)H5Nx clade 2.3.4.4b strains demonstrated low susceptibility of pigs to these specific strains (Kaplan et al. 2017; Arruda et al. 2024). However, A(H5N1) viruses clade 2.3.4.4b containing the PB2-E627K mutation were able to replicate in the nose of inoculated pigs and to be transmitted to contact pigs ($n > 1$) (Arruda et al. 2024; EFSA 2024). Moreover, serologically positive asymptomatic free-ranging pigs were reported in Italy after direct contact with infected poultry (Rosone et al. 2023) and wild boars exposure to IAV of diverse origin as well as the increasing variability of swine-IAV present in the field was also confirmed (Schülein et al. 2021). Due to the increasing presence of wild boar in Belgium in an environment that can be shared with wild birds, the presence of asymptomatic serologically positive animals in Belgium cannot be ruled out and particular attention should be paid in the event of an epidemic in birds to detect a possible appearance of mutations enabling viral strains to be better adapted to swine.

4.7 | Humans

Globally, from 1 January 2003 to 27 September 2024, 904 cases of human infection with avian influenza A(H5N1) virus were reported from 24 countries with a case fatality rate of 51% (WHO Western Pacific Region 2024).

Despite occupational hazards with a higher risk of exposition for some categories of the population as for example dairy or poultry workers, human infection remains a rare event and sustained human-to-human transmission have not yet been reported (ECDC 2024b; Pulit-Penaloza et al. 2024). However, the number of human cases is probably under-estimated as only the severe clinical cases are mostly detected as such, also probably explaining the high percentage of mortality.

To date, there is little epidemiological evidence to indicate that people have been infected with the HPAIV A(H5N1) following consumption of not properly cooked poultry or eggs. A recent study from the CDC suggested that not properly cooked poultry and poultry products (like blood) could have been the source of a small number of IAV infections in people in Southeast Asia (CDC 2024a). A UK qualitative assessment on the risk of acquiring IAV from poultry products concluded that the likelihood of human infection from handling and consuming commercial poultry products (like chicken or turkey) in the UK was 'negligible' (i.e., 'so rare that it does not merit to be considered' in their qualitative scale) with low uncertainty, while the likelihood of human HPAIV infection via the consumption of game birds was very low (i.e., 'very rare but cannot be excluded' in their qualitative scale) with medium uncertainty. The likelihood of human infection in the UK from handling and consuming hen table eggs was evaluated as 'very low' (i.e., 'very rare but cannot excluded' in their qualitative scale) with low uncertainty (Kintz et al. 2023).

Concerning raw milk, it has been shown that it could contain large number of viruses but that pasteurisation is efficient to avoid transmission through milk (Burrough et al. 2024; Guan et al. 2024; Spackman et al. 2024). However, limited data is available on the persistence of infectious viral particles in products prepared from raw milk, such as certain cheeses. Given the consumption of these products in Belgium, studies are needed on this risk.

5 | Assessment of Risk of HPAI A(H5N1) Clade 2.3.4.4b Spill-Over From Infected Birds to Mammals

This qualitative risk assessment evaluates the risk of spill-over from infectious birds to wild and captive mammals focusing on the Belgian situation as a case study.

Risk assessments for animal health usually involve two main steps, namely the assessment of the likelihood of occurrence (which results from the combination of the release of the hazard from its source and the exposure to the hazard) and the consequences of the occurrence of the hazard (Dufour et al. 2011). This was carried out in consensus between the authors of this study. All the assessments are related to the risk of infection which is defined here as 'the first event of HPAIV A(H5N1) clade 2.3.4.4b infection transmitted from an infected bird to a mammal'. They are based on the currently available epidemiological or experimental data. The risk of further virus spread, i.e. mammal-to-mammal infection and the environmental and/or economic consequences thereof have not been assessed. Moreover, this risk assessment was based on the classical organisation of the Belgian farming system and mixed species farms where poultry and livestock are raised outdoors and rotated on the same land are extremely rare and were not envisaged.

The likelihood of occurrence of mammal infection was assessed using a two-step approach. First, the susceptibility of the species of interest (i.e., the probability of a mammal being infected when exposed to an infectious bird) was assessed based on the field data and experimental data when available. Second, the likelihood of infection was assessed by combining the susceptibility of the species of interest with the likelihood of exposure to the virus through infectious (wild and domesticated) birds. The parameters taken into account to assess the likelihood of exposure were (1) the possibility of having close contact with wild birds, (2) the type of diet (whether or not including raw poultry products) and (3) the presence of reported cases in Belgium. The likelihood of exposure was determined as the highest value set for these parameters.

Table 2 summaries the susceptibility of the different species of interest, the likelihood of exposure (as well as the parameters taken into account to assess it) and the likelihood of infection in Belgium. Tables S1–S6 in detail the levels of the scales used to assess the species susceptibility, the likelihood of contact with infectious wild birds, the likelihood of ingestion of raw infected product, the clinical consequences and the uncertainty, as well as the matrix used to determine the likelihood of infection by combining the likelihood of exposure and the species susceptibility.

The risk of infection was estimated by combining the likelihood of infection of each mammal species and the evaluation of the clinical consequences of this infection in a risk-ranking matrix adapted from the Guidelines for the opinions of the Scientific Committee established at the FASFC, as already described in similar assessments for SARS-CoV-2 infections in animals (Table S7; Logeot et al. 2022). Four risk levels were set: 'very low', 'low', 'moderate' and 'high'. Table 3 summarises the results of the risk assessment.

TABLE 2 | Overview on the species susceptibility, the likelihood of exposure (as well as the parameters taken into account to assess it) and the likelihood of infection of mammals species of interest in Belgium.

Category of animals	Animal species	Species susceptibility	Likelihood of exposure		Reported cases in Belgium by April 5 2024	Likelihood of infection
			Likelihood of contact with infectious wild birds (direct or indirect)	Likelihood of ingestion of raw infected product		
Captive mammals						
Canids	Dogs	High	Low	Very low/low[a]	No	Low
			Low			
Felids	Cats	Very high	Very low[b]/moderate[c]	Very low[a,b]/moderate[c]	No	Very low[b]/High[c]
			Very low[b]/moderate[c]			
	Large felids	Very high	Low	Low	No	Low
			Low			
Mustelids	Ferrets	Very high	Very low	Very low	Yes[d]	Very low
			Very low to low[d]			
	Minks	Very high	Very low	Very low	No	Very low
			Very low[e]			
Phocids	Captive seals	Very high	Low	Very low	No	Low
			Low			
Ruminants	Cattle	Very high[f]	Moderate	Very low	No	High
			Moderate			
	Goats/Alpacas	Low	Moderate	Very low	No	Low
			Moderate			
	Sheep	Very low	Moderate	Very low	No	Very low
			Moderate			
Suids	Swine	Low	Low[g]/moderate[h]	Very low	No	Very low[g]/low[h]
			Low[g]/moderate[h]			

(Continues)

TABLE 2 | (Continued)

Category of animals	Animal species	Species susceptibility	Likelihood of exposure		Reported cases in Belgium by April 5 2024	Likelihood of infection
			Likelihood of contact with infectious wild birds (direct or indirect)	Likelihood of ingestion of raw infected product		
Wild mammals						
Canids	Foxes	High	Moderate/high[i]	Moderate/high	Yes	High
			Moderate/high[i]			
Felids	Wild cats	Very high	Moderate/high[i]	Moderate/high	No	High/Very high[i]
			Moderate/high[i]			
Mustelids	Wild mustelids	Very high[j]	Moderate/high[i]	Moderate/high	Yes	High/Very high[i]
			Moderate/high[i]			
Phocids	Seals	Very high	Moderate/high[i]	Moderate/high	No	High/Very high[i]
			Moderate/high[i]			
Suids	Wild boar	Low	Moderate/high[i]	Low	No	Low
			Moderate/high[i]			

Note: These parameters could evolve and should be reassessed with the potential emergence of viral strains better adapted to mammals.
[a]European regulation is strictly banning infected animals from the food chain.
[b]Indoor cats.
[c]Cats with free outdoor access.
[d]The likelihood of exposure could be moderate in some particular conditions (co-housing with birds) in correlation with the outbreak in domestic ferrets in Belgium.
[e]If good biosecurity practices are respected.
[f]Based on the current outbreaks in the USA.
[g]Pig farms without outdoor access.
[h]Open-air pig farming.
[i]For area with a high prevalence of HPAI A(H5N1) clade 2.3.4.4b.
[j]Assumed to be very high in correlation with data in ferrets and minks.

TABLE 3 | Summary of the risk assessment elements associated with infection of mammals by infected birds with HPAIv A(H5N1) clade 2.3.4.4b.

Category of animals	Animal species	Likelihood of infection	Clinical consequences	Risk of infection	Uncertainty
Canids	Dogs	Low	Minor to major	Low to high[a]	Low
Felids	Cats	Very low[b]/High[c]	Medium to major	Low[b] to high[c]	Low
	Large felids	Low	Medium to major	Moderate to high	Moderate
Mustelids	Ferrets	Very low	Medium to major	Low to moderate	Very low
	Minks[d]	Very low	Medium to major	Low to moderate	Moderate
Phocids	Captive seals	Low	Medium to major	Moderate to high	Moderate
Ruminants	Cattle	High	Minor to medium	Low to moderate	High
	Goats/Alpacas	Low	Marginal to minor	Very low to low	High
	Sheep	Very low	No data	Very low	High
Suids	Swine	Very low[e]/low[f]	Marginal	Very low	Very low
Canids	Foxes	High	Minor to major	Low to high[a]	Low
Felids	Wild cats	High/very high[g]	Medium to major	Moderate to high	Low
Mustelids	Wild mustelids	High/very high[g]	Medium to major	Moderate to high	High
Phocids	Seals	High/very high[g]	Medium to major	Moderate to high	Moderate
Suids	Wild boar	Low	Marginal	Very low	Moderate

[a]Large window of risk of infection due to the range of clinical consequences (from asymptomatic to serious illness).
[b]Indoor cats.
[c]Cats with free outdoor access.
[d]Belgium has banned the breeding of fur animals (minks) following the Covid-19 crisis and there are no longer active fur farms in Belgium in 2023.
[e]Pig farms without outdoor access.
[f]Open-air pig farming.
[g]For area with a high prevalence of HPAI A(H5N1) clade 2.3.4.4b.

The assessed risk for the different categories of animals ranges from 'very low' for species as pigs or sheep to 'high' for wild cats, wild mustelids, foxes or seals. There are several explanations for this risk variation. The first one is due to the housing conditions of the animals. Indeed, the risk of infection was considered higher for animals which have the opportunity to have close direct or indirect contact with potentially infected birds. This explains why the risk assessed for wild mammals is higher than for the corresponding captive species in Belgium. The second source of risk variation between individuals from the same species is the variability of clinical signs described in the literature, from which the level of 'consequences' of the infection were derived. Hence, for cats and dogs, both asymptomatic and lethal infection have been reported in experimental or epidemiological studies. The pre-infection conditions of the animal, concomitant infections and the viral strain constitute all explanatory factors for this variability.

6 | Conclusions

Overall, the risk of infection of mammals with HPAIV of the currently circulating HPAIV A(H5N1) clade 2.3.4.4b in Belgium remains 'low' to 'moderate'. However, this risk can be assessed as 'high' for certain animal profiles which have the opportunity to have closed contacts with infected (dead) birds such as wild felids, wild mustelids, foxes and wild marine carnivore mammals. Despite the outbreaks in dairy cattle in the USA, the risk of infection for cattle is still assessed as 'low' to 'moderate' in Belgium at this time but there is a high level of uncertainty in this rapidly evolving situation and a continuous monitoring of the situation including cattle testing to allow rapid detection of virus circulation is necessary.

As avian influenza A(H5N1) viruses infect multiple species, their geographical range increases and more viral variants emerged

from genetic evolution (mutation, reassortment). These variants could show new biological properties including mammal adaptation and zoonotic concerns. To date, there have only been a limited number of human cases. In contrast, mammal infections are increasingly detected, inducing a higher probability for the virus to acquire mutations that could enhance efficient infection, replication and spread in mammals.

This situation results in a high uncertainty for any risk assessment for animals and highlights the need for continuous genetic monitoring of circulating strains in the most exposed animal species (poultry, captive birds, wild birds) and in mammals that are particularly at risk. In Belgium, these animals still remain wild carnivores that can predate infected birds. However, events in the world are highlighting that this virus is a very unusual one. Episodes such as suspected spread of infection through raw meat fed to cats in Poland, the cases in cattle and goats in the USA, the massive mortalities of farmed fur animals are clearly indicating the need for a continuous vigilance in all exposed and susceptible mammals, particularly in the captive ones where animal densities could drive viral adaptation. Moreover, captive animals are also in closer contact to humans enhancing the risk of zoonotic spread.

Strong compliance with relevant biosecurity measures is therefore necessary, including in the hobbyist sector, to avoid spillover of HPAIV from the wild animal compartment to captive animals and vice versa.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.