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The role of mammals in Avian Influenza: a review

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Abstract

Avian influenza (AI) is an infectious viral disease of birds, including domestic poultry, which has been causing outbreaks worldwide, leading to several millions of dead wild birds and culled poultry. AI is mainly found in birds, but recently, there was an increase in reported infections in mammals, ranging from no symptoms to mass mortality events and some human cases. Epidemiologically of great concern, evidence of mammalian adaptations have been found, but the transmission routes and pathogenesis in mammals are still to be defined. Hence, it is paramount to address all facets of AI viruses epidemiology, including investigating taxa not customarily thought to be involved in the transmission and/or trafficking of AI, such as wild mammals. The scope of this report was to assess the role of mammals in AI epidemiology, virology and pathology, i.e. AI maintenance, reservoir role, immunity, role of mammals in a potential pandemic. To do so, we performed an all-encompassing review of the literature on the topic with a two-fold approach: a systematic review of the published AI cases in wild mammals and a narrative approach to provide an expert opinion on the role of mammals in AI spread. The final number of peer-reviewed papers included in the systematic literature review was 76, resulting in 120 unique infection records with AI in wild mammal species. The most represented taxa were included in the order Carnivora. The risk of infection was identified mainly as predation (or feeding) upon infected birds or contact with avian species. Evidence of mammal-to-mammal transmission in the wild is only circumstantial and yet to be confirmed. Cases of AI from the systematic review of experimental findings were discussed concerning epidemiology, pathology and virology. Knowledge gaps and potential pandemic drivers were identified. In summary, although a greater number of infections in wild mammals have been reported, there is no hard evidence for sustained mammal-to-mammal transmission in the wild. The factors contributing to the increased number of infections found in wild carnivores are not clear yet, but the unprecedented global spread of highly pathogenic avian influenza (HPAI) viruses creates ample opportunities for intense, mostly alimentary, contact between infected wild birds and carnivores.

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Close surveillance of circulating strains and continued assessment of new epidemiological situations are crucial to quickly identify strains with enhanced mammalian fitness.

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Key words: Avian Influenza, HPAI, wild mammals, spillover, wild birds, reservoir potential, bird flu

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Summary

Avian influenza (AI) is an infectious viral disease of birds, including domestic poultry, which has been causing outbreaks worldwide for decades, leading to several millions of dead wild birds and culled poultry. Avian influenza is mainly found in birds, but recently, there has been an increase in reported infections in mammals, ranging from no symptoms to mass mortality events, as well as some human cases. Epidemiologically of great concern are the cases of H5N1 infection reported in minks in Spain and dolphins in South America, as well as the H5N1 outbreak in harbour and grey seals in New England (USA), which coincided with a large number of avian infections in the region and the mass mortalities of sea lions and southern elephant seals in Argentina, Brazil and Uruguay. Evidence of mammalian adaptations has been found, but the transmission routes and pathogenesis in mammals are still to be defined. The extremely wide spread of the virus by wild birds and the increased risk of direct or indirect virus introduction into poultry or captive bird holdings have led to the largest HPAI epidemic in the EU so far. Hence, it is paramount to address all facets of AI viruses epidemiology, including investigating taxa not customarily thought to be involved in the transmission and/or trafficking of AI, such as mammals. Numerous genotypes of different subtypes have been detected in mammals so far, and a high percentage of European viruses collected from mammalian species contained molecular markers of mammalian adaptation, which might signal greater zoonotic potential than those without these molecular modifications.

In light of all this, a thorough epidemiological analysis of the role of wild mammals in the maintenance and spread of AI is needed by the Commission and Member States. The role of mammals, specifically wild mammals, in avian influenza maintenance, spread, pathology and virology is evaluated through an informative and all-encompassing review of the literature on the topic, in agreement with the Terms of Reference (ToR) specified in the SC1.

The literature review was performed with a two-fold approach. Firstly, a systematic review of the published AI cases in mammals to appraise the current epidemiological context and highlight transmission dynamics in the wild, AI subtypes and taxa involved and the relative interconnections. Secondly, a narrative approach to critically appraise the results from the systematic review and provide an updated expert opinion on the role of mammals in AI spread, as requested by the ToR.

The systematic search of literature took place in September 2023. The final number of peer-reviewed papers included in the systematic literature review was 76, resulting in 120 unique infection records with AI in wild mammal species. The most represented taxa were included in the order Carnivora, families Phocidae, Felidae, Mustelidae, and Canidae. Countries of detection with the highest records were the USA, China, Thailand, The Netherlands, and Germany. These papers mostly reported infections detected through passive surveillance. Very few studies described the impact of the infection on the population including quantitative epidemiological data. The risk of infection was identified as predation (or feeding) upon infected birds or contact with unspecified susceptible avian species. In a few studies, synanthropic bird species were found to be associated with mammal infection; these species were identified as *Corvus macrorhynchos*, *Cygnus olor*, *Gallus domesticus*, and *Phasianus colchicus*. The majority of the studies reported isolated cases of infection. Molecular markers of mammal mutations identified were predominantly E627K and D701N on the PB2 gene segment of the H5N1 subtype. Evidence of mammal-to-mammal transmission in the wild was hypothesised in *Otaria flavescens* in Chile and *Phoca vitulina* in the USA, but this has yet to be confirmed by experimental evidence. Less than 20% of the studies investigated the evidence of clustering of mammal cases.

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Given the broad scope and ongoing nature of the outbreak in birds and the increase of reported cases in mammals, it is likely that the reported cases are only a small percentage of the total number and species of mammals infected with circulating strains of HPAI viruses. In the wild, neurological signs, respiratory symptoms, weight loss, and other sporadic findings such as conjunctivitis were recorded, but probably neurological signs led to the oversight of mild respiratory symptoms, underestimating the number of infected individuals. Indeed, throat and anal swabs were less effective in detecting positivity to HPAI H5N1. Therefore, when possible, brain samples should be included in wildlife surveillance programs for reliable detection of the HPAI H5N1 virus in mammals, ideally coupled with serological investigations. This is to understand how species, age, and other intrinsic factors affect morbidity and mortality in wild mammal populations and develop preparedness and management measures, which may be host- and strain-specific. Since the pathology of the H5N1 virus in wild mammals differs between carnivore species (and potentially genotypes), it is impossible to generalise the pathogenicity of HPAI for wild mammals.

Infection risk factors identified for wild mammal species were exposure with potentially infected bird species (i.e. aquatic, migratory, peridomestic), scavenging habits (especially for foxes, although seals do also feed on dead birds), and the contact with guano, water, and other contaminated environmental matrices (i.e. no airborne transmission).

In wild settings, observations of potential mammal-to-mammal transmission were recorded for sea lions (*Otaria flavescens*) infected by the HPAI H5N1 in Chile and Peru; although epidemiological data suggested a potential transmission between sea lions, phylogenetic analyses pointed towards transmission from avian sources. Despite the current unprecedented spread of HPAI, there is comparatively little information about the clinical course and the pathology of avian influenza in wild mammals. Gradually increasing knowledge on the course of mammal infection comes from inoculation experiments with animal models, especially to evaluate the possibility of viral replication in human respiratory tract cells. The most valuable models for respiratory viruses are ferrets, given their similarities in lung physiology, distribution of sialylated glycan receptors throughout the respiratory tract, and the glycomic profiles of ferret respiratory tissue that are more similar with those of humans compared with other animal models. In ferrets, like in humans, viral RNA can be found in respiratory fluids and rectal swabs, suggesting potential routes of onward mammal-to-mammal transmission. Although molecular markers of mammal mutations were identified, such as the most abundant and relevant genetic change reported in our systematic literature review, the E627K mutation, to date, no mammal-adapted lineage of HPAIV H5Nx has emerged to circulate independently of the avian HPAIV reservoirs. Mammal-to-bird transmission was not reported in the wild, although a small-scale experiment highlighted a negligible but existing potential. We have not found any other evidence of this type of transmission.

There is a need to increase global surveillance for influenza infection in mammals (e.g. implementing a system for reporting of cases), especially in carnivores, to monitor virus adaptation to the mammalian host as an early proxy for an increase in pandemic potential. Surveillance of avian influenza viruses should include not only HPAI subtypes but also H9 and H3 subtypes circulating in wild bird reservoirs, which express frequent mutations that make them more easily adapted to mammals' respiratory tract cells. Future experimental studies are necessary to increase our understanding of avian influenza transmission beyond identifying single mutations but to characterise the underlying biochemical and biophysical properties responsible for aerosol transmission in mammalian hosts.

In addition, the exact geographic locations of infections in mammals are mostly unknown, so patterns of spatial distribution of mammal carcasses are seldom investigated. However,

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surveillance should be focused on the wildlife-livestock-domestic interface, which has long been recognised as a risk pathway.

With the current evidence, it is possible to state that zoonotic infections are certainly possible and reported. Yet, these infections do not represent an immediate threat to human health. Although there is a need for high vigilance, zoonotic pathogens must overcome a hierarchical series of barriers to cause human spillover infections. Potential intrinsic pandemic drivers might be host susceptibility and genetic adaptations within mammalian species, particularly domestic pigs and ferrets might play a crucial role in the transmission of AIVs. Host features that seemed to favour infection were certainly scavenging feeding habits (e.g. generalist mesopredators such as red foxes and mustelids), and, in general, carnivores were more exposed to infection and displayed more viral mammalian adaptations. Extrinsic risk factors include human-driven activities such as intensive farming practices that create environments conducive to cross-species transmission. Deforestation, urbanisation, and habitat changes contribute to increased interactions between domestic and wild species, influencing the transmission dynamics of avian influenza. Global trade and travel may facilitate the spread of AIVs, with infected avian and mammalian hosts potentially introducing novel strains to different regions.

In conclusion, although a greater number of infections in wild mammals have been reported, there is no hard evidence of mammal-to-mammal transmission in the wild. The factors contributing to the increased number of infections found in wild carnivores are not clear yet but the unprecedented global spread of HPAIV creates ample opportunities for intense, mostly alimentary, contact between infected wild birds and carnivores. Our data support the outcomes of other risk assessments on this topic, e.g. the CDC Influenza Risk Assessment Tool determination that states that HPAI H5N1 viruses do not pose a substantial risk to public health at this time. However, close surveillance of circulating strains, an efficient reporting system and continued assessment of new viruses are crucial to identify strains with enhanced mammalian fitness.

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1 Introduction

Avian influenza (AI) is an infectious viral disease of birds, including domestic poultry, which has been causing outbreaks worldwide for decades, leading to several millions of dead wild birds and culled poultry (Root and Shriner, 2020). Infections in poultry are distinguished by their virulence; the low pathogenic (LPAI) form generally only causes mild symptoms, while the highly pathogenic (HPAI) form results in very high mortality rates in most poultry species (Reperant et al., 2009). HPAI viruses have been causing more frequent outbreaks since the 90s, involving different avian species and spreading worldwide (CDC, 2022).

Avian influenza is mainly found in birds, but recently, there was an increase in reported infections in mammals, ranging from no symptoms to mass mortality events, as well as some human cases (Charostad et al., 2023). Epidemiologically of great concern are the cases of H5N1 infection reported in minks in Spain and dolphins in South America, as well as the H5N1 outbreak in harbour and grey seals in New England (USA), which coincided with a large number of avian infections in the region (Puryear et al., 2023) and the mass mortalities of sea lions and southern elephant seals in Argentina, Brazil and Uruguay. Evidence of mammalian adaptations has been found, but the transmission routes and pathogenesis in mammals are still to be defined (Charostad et al., 2023).

The capability of the virus to be carried by wild birds over long distances makes the epidemiological investigations and the containment of the spread more challenging (Venkatesan, 2023). Wild bird involvement in the spread of the various AI subtypes is remarkable; HPAI virus strains have been detected in wild migratory birds or captive birds worldwide, including the vast majority of European Member States, where poultry has been infected as well with significant economic loss (EFSA, ECDC and EURL, 2023). The number of infections recorded in migratory wild birds and the geographic extent of these findings pose a serious threat to virus introduction into poultry, captive birds, and other taxa such as mammals (Venkatesan, 2023). The spillover into mammals exacerbates the risk of infection of poultry farms and other bird species and the introduction of the virus into new regions, threatening wildlife, the poultry sector, and food safety and security (Root and Shriner, 2020). To give an idea of the extent of the HPAI threat, during 2015, only in USA, H5 AI viruses were responsible for the death or culling of tens of millions of birds and the initial costs associated with these outbreaks were estimated to be in the realm of billions of U.S. dollars (Root and Shriner, 2020). Further, in 2023, 250000 poultry were culled in 3 weeks between October and November 2023 (WOAH, 2023).

The extremely wide spread of the virus by wild birds and the increased risk of direct or indirect virus introduction into poultry or captive bird holdings has led to the largest HPAI epidemic in the EU so far (EFSA, ECDC and EURL, 2023). Hence, it is paramount to address all facets of AI viruses epidemiology, including investigating taxa not customarily thought to be involved in the transmission and/or trafficking of AI, such as mammals. For instance, the latest infections recorded in several fur farms in Finland (hosting multiple species), where animals exhibited very low mortality and clinical signs, are further evidence that mammals might act as reservoir system for these viruses, and infections could go undetected to passive surveillance (EFSA, ECDC and EURL, 2023). Additionally, the spread of AI in wild mammals is also of conservation concern; e.g. the virus has been detected in southern elephant seals in the Antarctic region, home to half of their global population (Charostad et al., 2023; EFSA, ECDC and EURL, 2023).

Numerous genotypes of different subtypes have been detected in mammals so far, and a high percentage of European viruses collected from mammalian species contained molecular markers of mammalian adaptation, which might signal greater zoonotic potential (Vreman et al., 2023). In light of all this, a reflection on and a re-evaluation of preparedness, risk assessment, and response is needed by the Commission and Member States. To do that, there is a need for a thorough epidemiological analysis based on available data, particularly

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regarding the role of wild mammals, whose role in the maintenance and spread of AI is yet to be clarified and assessed.

1.1 Background and terms of reference

The contract entitled "Wildlife and One Health: wildlife ecology, health surveillance and interaction with livestock, human population, and environment (framework contract number: OC/EFSA/BIOHAW/2022/01) was awarded to the University of Turin by EFSA. From here, we refer to this framework contract as the ENETWILD project. The Specific Contract 1 (SC1) of the framework contract refers to "Wildlife ecology, health surveillance and interaction with livestock, human population and environment".

The role of mammals, specifically wild mammals, in avian influenza maintenance, spread, pathology and virology is evaluated through an informative and all-encompassing review of the literature on the topic, in agreement with the following terms of reference specified in the SC1:

"Literature review about the role of mammals in avian influenza (maintenance, spread, potential pandemic): species, epidemiology, pathology, virology."

1.2 Scope of the report

The scope of the literature review is to answer the ToR reported in the previous section that was translated into the following research questions:

1. Do wild mammals play a role in the maintenance of the Avian Influenza virus (HPAI and other subtypes different from H5 and H7) favouring current or future spill back to wild birds and spill over to humans or other taxa?

2. Can wild mammals acquire immunity and become a reservoir for the AI virus subtypes that are the object of this review?

3. According to epidemiological and experimental evidence, how likely is mammal-to-mammal transmission of AI virus?

4. Considering the answers to the above questions, what could be the role of mammals in a potential pandemic caused by AI viruses? Could this taxon represent a risk due to the recent increase in cases? And which could be the drivers for a potential pandemic?

2 Methodology

To answer the research questions in the previous section, we performed a literature review with a twofold approach. Firstly, a systematic review of the published AI cases in mammals to appraise the current epidemiological context and highlight transmission dynamics in the wild, AI subtypes and taxa involved and the relative interconnections. Secondly, a narrative approach to critically appraise the results from the systematic review (also in light of the updated list of cases in the periodic EFSA report "Avian Influenza overview") and provide an updated expert opinion on the role of mammals in AI spread, as requested by the ToR. Targeted taxa were wild mammalian species (captive e.g. in zoos, farms, exotic pets and free-living). The avian influenza viruses (AIVs) subtypes included in the analysis were, as of ToR, those mostly relevant to mammals. Specifically, we included HPAI virus (HPAIV) (i.e. H5Nx and H7Nx subtypes), H10Nx (mostly affecting marine mammals and causing mass mortalities), H3N8 and H9N2 because frequently found in wild mammals and recently identified as etiological agents in human cases (EFSA, ECDC and EURL, 2023) (see Table 1 for inclusion/exclusion criteria). This approach was justified by recent findings regarding the role of mammals in the spread of subtypes other than HPAI. For example, several mammalian

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adaptive mutations were found in H3N8 isolated in mammals (Li et al., 2021), potentially threatening animal and human health. In addition, the H9N2 subtype, which circulates endemically in poultry flocks in some regions of the world and has also been associated with cases of zoonotic infections, has been found to have molecular factors favouring the spillover and transmission through the respiratory route in mammalian species (Cáceres et al., 2021).

The systematic literature review, designed in agreement with Tsafnat et al. (2014), was performed in the following manner:

- Naïve search strings were created based on a priori knowledge about the topic and the review questions (see Table 2).
- Relevant databases were identified and selected: PubMed, Web of Science, Embase, Scopus, and Google Scholar.
- Naïve search was performed in September 2023 in a subset of databases using naïve strings, and results were exported into a unique dataset.
- This dataset was analysed using *litsearchr* package (Grames et al., 2019) in R (R Core Team, 2023) to extract keywords (after eliminating duplicates).
- Definitive string searches based on previous analyses were optimised (long composite strings elimination and manual analysis by terms group) (Jaspers et al., 2018).
- Final search was performed using definitive strings (Table 2) in all databases (searches performed in September 2023).
- Results were analysed in R (R Core Team, 2023) with the *revtools* package (Westgate, 2019): elimination of duplicates by title and DOI, elimination of citations, reviews, book chapters, conference papers, etc.
- Only papers with European languages were selected (for capability of translation reasons).
- The first screening was performed using a semi-automated methodology implemented in the revtools package (Westgate, 2019); i.e. articles were screened using an algorithm to distinguish the broad topic, and those not relevant were discarded (see Table 1 for inclusion/exclusion criteria and Figure 1).
- Screening by title and abstract was performed manually according to the inclusion and exclusion criteria (Table 1 and Figure 1).
- The remaining papers from the previous step were screened in full text allowing data collection from the relevant ones, as well as snowballing (follow citations from included papers to find additional ones) (Jaspers et al., 2018) (Figure 1).
- Data were extracted in a shared data extraction template (Table 3), which was filled with one row (i.e. record) per species (e.g. different rows in case one paper described infection in more than one species).
- Papers included at this stage were included in final synthesis, data synthesisation, and critical appraisal (Figure 1).

In the text, we adopt the internationally accepted naming convention for influenza viruses, when we mention subtype, clade, strain, etc. (WHO, 1980).

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Table 1: List of inclusion and exclusion criteria.

Inclusion criteria	*Exclusion criteria*
H5N1 or another subtype of HPAIV involved (report also H10 and others if reported in the same paper with HPAIV), as well as H9N2 and H3N8 (human cases)	*Non-HPAIV involved (e.g. LPAIV or swine), or any subtype not specified in the inclusion criteria*
Transmission bird-to mammal or mammal-to mammal, only cases in wild mammals (free-living or captive)	*Domestic mammals*
Natural infection in the wild or in captive/farming settings of wild mammal species	*Experimental infection or laboratory study on cultures or tissues*
Spillback mammal-to-bird, documented avian outbreak caused by mammalian host	*Outbreak in birds without documented mammalian origin*

Table 2: Summary table of the concepts to be addressed by the systematic literature review, the naïve string searches, and the definitive strings.

Review question	Target	Terms group	Naïve string	Definitive string
Subtypes of AI virus (HPAI or other relevant ones) found in wild mammals	topic (title, abstract, keywords)	pathogen	"avian flu" OR H5N1 OR HPAIV OR "avian influenza" OR "fowl plague" OR H7	"avian flu" OR "avian influenza" OR "fowl plague" OR H10N7 OR H5N1 OR "H7 subtype" OR "high pathogenicity" OR "highly pathogenic avian influenza" OR HPAIV OR "influenza a" OR "pandemic influenza" OR "reassortant influenza" OR "subtype avian" OR "subtype influenza"
Wild mammal species susceptible to AI virus	topic (title, abstract, keywords)	population	carnivor* OR seal* OR "marine mammal*" OR mammal* OR "farmed animal*"	carnivor* OR "mammalian host" OR "mammalian species" OR "marine mammal*" OR "other mammals" OR seal* OR wildlife
Mammals' potential of sustaining AI virus transmission (epidemiology, pathology and virology)	topic (title, abstract, keywords)	process	spillover OR transmission OR outbreak* OR infection OR high-mortality OR surveillance OR epizoo* OR spillback OR mammal-to-mammal OR bird-to-mammal	outbreak* OR "active surveillance" OR "adaptive mutations" OR "animal health" OR bird-to-mammal OR "cross-species transmission" OR "interspecies transmission" OR mammal-to-mammal OR "mammalian adaptation" OR spillback OR spillover OR conservation
Mammal related outbreaks of AI virus	topic (title, abstract, keywords)	process	spillover OR transmission OR outbreak* OR infection OR high-mortality OR surveillance OR epizoo* OR spillback OR mammal-to-mammal OR bird-to-mammal	outbreak* OR "active surveillance" OR "adaptive mutations" OR "animal health" OR bird-to-mammal OR "cross-species transmission" OR "interspecies transmission" OR mammal-to-mammal OR "mammalian adaptation" OR spillback OR spillover OR conservation

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Table 3: Example spreadsheet* to be provided to partners for data extraction (modified from Table 3 in EFSA, ECDC and EURL (2023)). Note: each row will be repeated for each strain and/or species in each reference.

User			Reference				Host							Sequence data				
search list no.	Initials/institute	Relevant	Reference APA style	DOI link	Link to database of paper dataset (if available) (E.g. GISAID/Zenodo/etc.)	Original report	Order	Family	Genus	Species	Age group (if specified)	sex	breed	Sequence available (Y/N)	Database in which the sequence is deposited	Sequence reference (e.g. GenBank accession number)	Genetic evidence for mammal adaptations; if so, which mutations	Genetic evidence for clustering of mammal case (Y/N)

Table 3: (continued).

Location						Timescale		Surveillance	Sampling design			Setting
ountry of detection	ountry of infection (if available)	egion/ ounty	ocality	abitat/major environmental features	oordinates (x;y)	ear(s)	omments on paper timeline (e.g. continuous outbreak, isolated cases in a specific time range)	Active/passive surveillance (or both)	Random sampling (1: present, 0: absent, empty: not reported)	Stratified sampling (1: present, 0: absent, empty: not reported)	Risk-based sampling (1: present, 0: absent, empty: not reported)	Wild/captive

Table 3: (continued).

Epidemiological data																	Snowballing	
# of sick individuals	# of dead individuals	# of susceptible individuals	# of tested	# positive	Proportion positive	Test performed (virus, serology, etc.)	Symptoms (if described)	Summary of post-mortem findings, if post-mortem available	Target organ(s) if available	Impact on host population (e.g. mass mortality event)	Outbreak origin (if known)	Spillover/ spillback details (if known)	First report in species? (Y/N)	First report in the country? (Y/N)	Implication for conservation? if so, summarise	Evidence of mammal-to-mammal transmission (Y/N)	Risk factors identified (if yes, please list)	References potentially useful for snowballing? If yes, list the relevant papers	Extra comments

* https://zenodo.org/records/10657384

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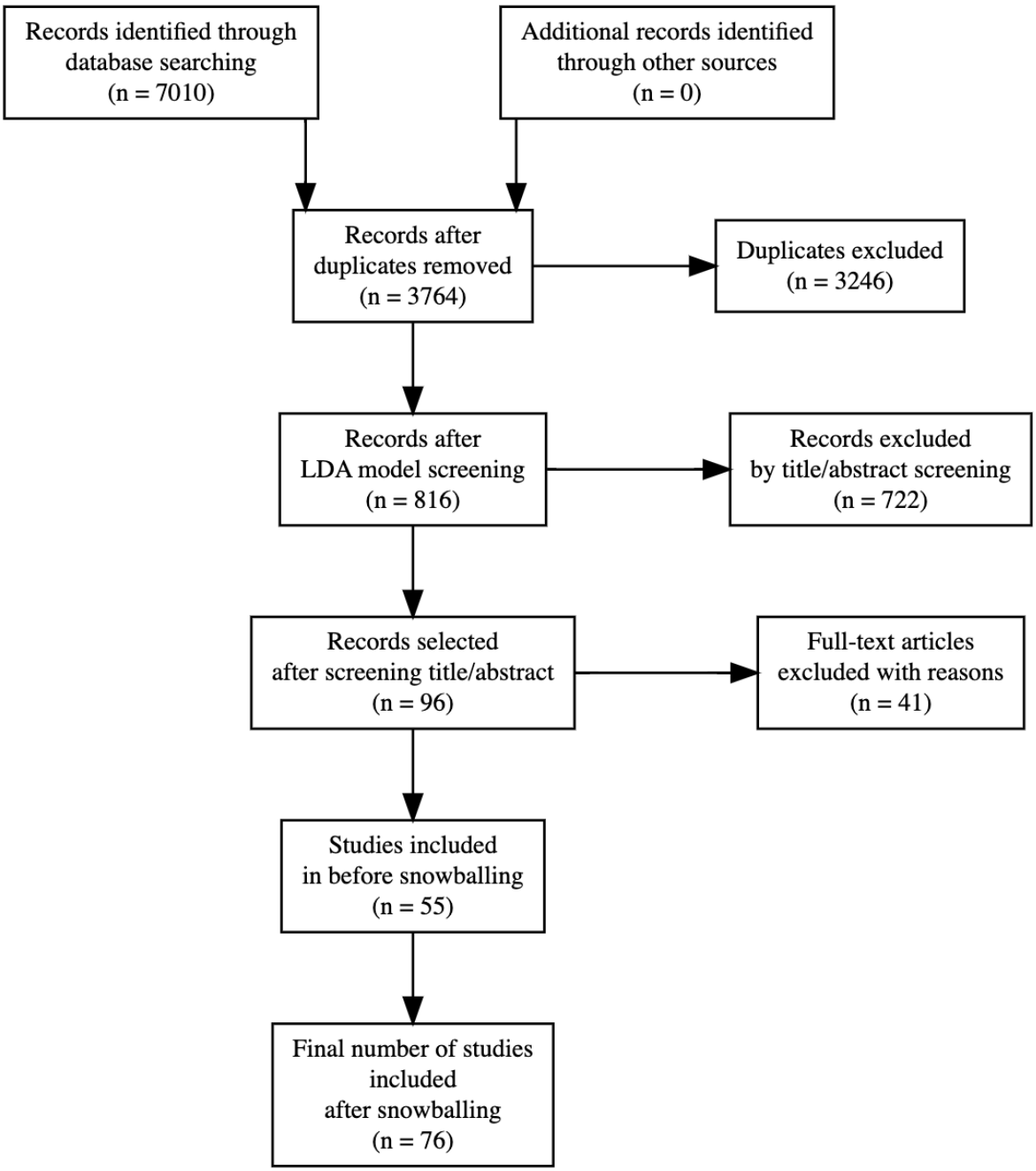

Figure 1: PRISMA figure. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

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3 Results

3.1 Descriptive statistics and qualitative results of data extracted

The final number of peer-reviewed papers included in the systematic literature review was 76 (Figure 1). The excluded papers from the systematic epidemiological analysis but relevant to gathering information about the broader topic were saved in a separate list to be accessed later. In total, from the 76 papers, we extracted 120 unique cases (records) of infection with AI of the selected subtypes, of which 106 were from the order Carnivora (other orders recorded were Artiodactyla, Didelphimorphia, Lagomorpha, Perissodactyla, Rodentia). The most represented families, in terms of records of infections (i.e. not the number of individuals infected), were Phocidae (23), Felidae (22), Mustelidae (22) and Canidae (20). The species of these families accounting for most records were harbour seal (*Phoca vitulina*) and *Halichoerus grypus* (grey seal) (11 and 8 records respectively), *Panthera tigris* (tiger) (10), *Neogale vison* (American mink) (6), and *Vulpes vulpes* (red fox) (11). Countries of detection with the highest records were the USA (25), China (15), Thailand (15), The Netherlands (12), and Germany (6), with other countries only being represented by sporadic records. These papers mostly reported infections detected through passive surveillance (55 records). Full data extracted from the papers included in this systematic review are shown in Annex A.

Data revealed that taxa with the most records also showed a higher diversity of subtypes recorded (Tables 4 and 5). Figure 2 shows that the HPAI subtype H5N1 was, by far, the most represented in the data, followed by H9N2 and H10N7. Figure 3 shows the number of records of each subtype by host Family, confirming the higher diversity of subtypes in families with a higher number of records of infection. Similarly, this can be appreciated when data is analysed by Genus (Figure 4), with *Vulpes* and *Phoca* displaying infection with several AI subtypes (Table B1). Nevertheless, the Genus *Panthera* is an exception, showing infections only with the H5N1 subtype. Sequences were made available in databases such as GenBank and GISAID in 38 infection records (and in some cases, there was more than one sequence per case of infection).

Studies included very little quantitative epidemiological data, and although for some taxa many records of infection were available, no precise information could be inferred either on the outbreak features or prevalence. There was a high variability of the testing regime, as well as most records were accidental findings (Figure 5 and Figure A3). The most represented families, in terms of number of records, did not present high numbers of individuals tested, skewing their positivity proportion (Figure 5 and Figure A3). Very few studies described the impact of the infection on the population, namely the number of sick individuals; while, when the number of tested individuals was available, it was rarely possible to infer how this number related to the population under study (i.e. the proportion of susceptible individuals tested, or sick, or positive). Data regarding age of infection were collected in a few studies, resulting in 39 records, with no clear indication of higher risk in adult versus young individuals (probably also an effect of the difficulty of creating homogenous age classes and the vagueness of the reported age data in most cases). In total, 49 records (41%) reported the presence of symptoms, of which the majority were respiratory (27 records) and neurological (19 records), alone or in conjunction, with some sporadic reports of fever, lethargy, emaciation, dehydration and conjunctivitis.

Most reports were infections observed in the wild (~71%), while the remaining were detected in captive settings. Out of the 120 records, around 30% reported more detailed information regarding the habitat where the wild population was infected (Figure 6). The most represented habitats were coastal areas (due to the many cases of marine mammals infected), savannah-steppe and finally, captive settings (e.g. zoos, wildlife sanctuaries, etc.) (Figure 6). These infections were characterised by different subtypes, likely due to the species infected and the

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transmission route. In coastal areas, the predominant subtypes were H10 and H7, while in savannah-steppe environments, H5N1 dominated. In captive settings, H5 was most abundant (Figure 6). When the risk of infection was investigated or clearly mentioned/hypothesised, this was linked to predation (or feeding in captive settings) upon (25 records), cohabitation (shared habitat/contact in captive settings/high density of birds) with infected birds (15 records), or contact with unspecified avian species in general (5 records). In a few studies, synanthropic bird species were found to be associated with mammal infection; these species were identified as *Corvus macrorhynchos* (large-billed crow), *Cygnus olor* (mute swan), *Gallus domesticus* (chicken), and *Phasianus colchicus* (common pheasant). The majority of the studies reported isolated cases of infection. It was possible to retrieve information regarding outbreak duration from around 60% of the records, of which more than half (53%, corresponding ~33% of the total reports) were considered isolated cases, and the majority of the rest were estimated during from 1 to 6 months (rarely reporting a one year or multi-year outbreak).

Studies involving investigations of molecular markers of mammal mutation (~20 records) revealed that the most frequent mammal mutations were E627K and D701N on the PB2 gene segment. Species hosting these mutations were predominantly infected by the subtype H5N1 and included those with the highest number of cases (*Vulpes vulpes*, and various mustelids) (Figure 7). Species suggested to have displayed mammal-to-mammal infection were *Vulpes vulpes*, *Nyctereutes procyonoides* (common raccoon dog) and *Neogale vison* in captive settings in China, although evidence was only circumstantial and the risk of infection in these cases was identified as feeding on infected bird meat. Similarly, it was supposed that captive tigers in Thailand could transmit the infection, but it was also likely that they were fed on infected poultry meat. In wild settings, circumstantial evidence was observed for *Otaria flavescens* in Chile and *Phoca vitulina* in the USA, but this has yet to be confirmed by experimental evidence. Less than 20% of the studies investigated the evidence of clustering of mammal cases (~25 records), as shown in Figure 8, where it can be appreciated the species and the subtype involved.

Table 4: List of orders recorded with the associated number of unique subtypes recorded.

Order	***Number of subtypes recorded***
Artiodactyla	3
Carnivora	12
Didelphimorphia	1
Lagomorpha	3
Perissodactyla	1
Rodentia	2

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Table 5: List of all species reported with associated number of records for each subtype recorded (H5, H7, and H10 are aggregated by hemagglutinin type). N/A: unspecified HPAI.

Order	Family	*Species*	H5	H7	H10	H3N8	H9N2	other	N/A
Artiodactyla	*Bovidae*	*Connochaetes taurinus*	0	0	0	1	0	0	0
Artiodactyla	*Cervidae*	*Hydropotes inermis*	1	0	0	0	0	0	0
Artiodactyla	*Delphinidae*	*Delphinus delphis*	1	0	0	0	0	0	0
Artiodactyla	*Delphinidae*	*Globicephala melaena*	0	0	0	0	0	1	0
Artiodactyla	*Delphinidae*	*Tursiops truncatus*	1	0	0	0	0	0	0
Carnivora	*Ailuridae*	*Ailurus fulgens*	0	0	0	0	0	0	1
Carnivora	*Canidae*	*Canis latrans*	1	0	0	0	0	0	0
Carnivora	*Canidae*	*Canis mesomelas*	1	0	0	0	0	0	0
Carnivora	*Canidae*	*Nyctereutes procyonoides*	2	0	0	0	3	0	0
Carnivora	*Canidae*	*Urocyon cinereoargenteus*	1	0	0	0	0	0	0
Carnivora	*Canidae*	*Vulpes lagopus*	0	0	0	0	1	0	0
Carnivora	*Canidae*	*Vulpes vulpes*	9	0	0	0	2	0	0
Carnivora	*Felidae*	*Caracal caracal*	1	0	0	0	0	0	0
Carnivora	*Felidae*	*Catopuma temminckii*	1	0	0	0	0	0	0
Carnivora	*Felidae*	*Lynx lynx*	1	0	0	0	0	0	0
Carnivora	*Felidae*	*Lynx rufus*	1	0	0	0	0	0	0
Carnivora	*Felidae*	*Neofelis nebulosa*	1	0	0	0	0	0	0
Carnivora	*Felidae*	*Panthera pardus*	4	0	0	0	0	0	0
Carnivora	*Felidae*	*Panthera tigris*	10	0	0	0	0	0	0
Carnivora	*Felidae*	*Prionailurus bengalensis*	1	0	0	0	0	0	0
Carnivora	*Hyaenidae*	*Crocuta crocuta*	1	0	0	0	0	0	0
Carnivora	*Mephitidae*	*Mephitis mephitis*	1	0	0	0	0	0	0
Carnivora	*Mustelidae*	*Enhydra lutris kenyoni*	0	0	0	0	0	0	1
Carnivora	*Mustelidae*	*Lutra lutra*	2	0	0	0	0	0	0
Carnivora	*Mustelidae*	*Martes foina*	4	0	0	0	0	0	0
Carnivora	*Mustelidae*	*Meles meles*	2	0	0	0	0	0	0
Carnivora	*Mustelidae*	*Mellivora capensis*	0	1	0	0	0	0	0
Carnivora	*Mustelidae*	*Mustela lutreola*	0	0	2	0	0	0	0
Carnivora	*Mustelidae*	*Mustela putorius*	2	0	0	0	0	0	0
Carnivora	*Mustelidae*	*Neogale vison*	3	0	1	0	2	0	0
Carnivora	*Mustelidae*	*Pekania pennanti*	1	0	0	0	0	0	0
Carnivora	*Odobenidae*	*Odobenus rosmarus divergens*	0	0	1	0	0	0	0
Carnivora	*Otariidae*	*Arctocephalus australis*	1	0	0	0	0	0	0
Carnivora	*Otariidae*	*Otaria flavescens*	4	0	0	0	0	0	0
Carnivora	*Phocidae*	*Halichoerus grypus*	3	0	1	4	0	0	0
Carnivora	*Phocidae*	*Phoca groenlandica*	0	0	0	1	0	0	0
Carnivora	*Phocidae*	*Phoca hispida*	0	1	0	0	0	0	0
Carnivora	*Phocidae*	*Phoca vitulina*	2	2	6	1	0	0	0
Carnivora	*Phocidae*	*Pusa caspica*	0	0	1	0	0	0	0
Carnivora	*Phocidae*	*Pusa hispida*	0	1	0	0	0	0	0
Carnivora	*Procyonidae*	*Procyon lotor*	4	0	1	0	0	0	0
Carnivora	*Ursidae*	*Ursus arctos*	0	0	0	0	0	0	1
Carnivora	*Ursidae*	*Ursus thibetanus*	1	0	0	0	0	0	0
Carnivora	*Viverridae*	*Chrotogale owstoni*	2	0	0	0	0	0	0
Didelphimorphia	*Didelphidae*	*Didelphis virginiana*	1	0	0	0	0	0	0
Lagomorpha	*Ochotonidae*	*Ochotona curzoniae*	2	1	0	0	2	0	0
Perissodactyla	*Rhinocerotidae*	*Diceros bicornis*	1	0	0	0	0	0	0
Rodentia	*Muridae*	*Rattus norvegicus*	0	0	0	0	0	0	1
Rodentia	*Muridae*	*Mus musculus*	1	0	0	0	0	0	0

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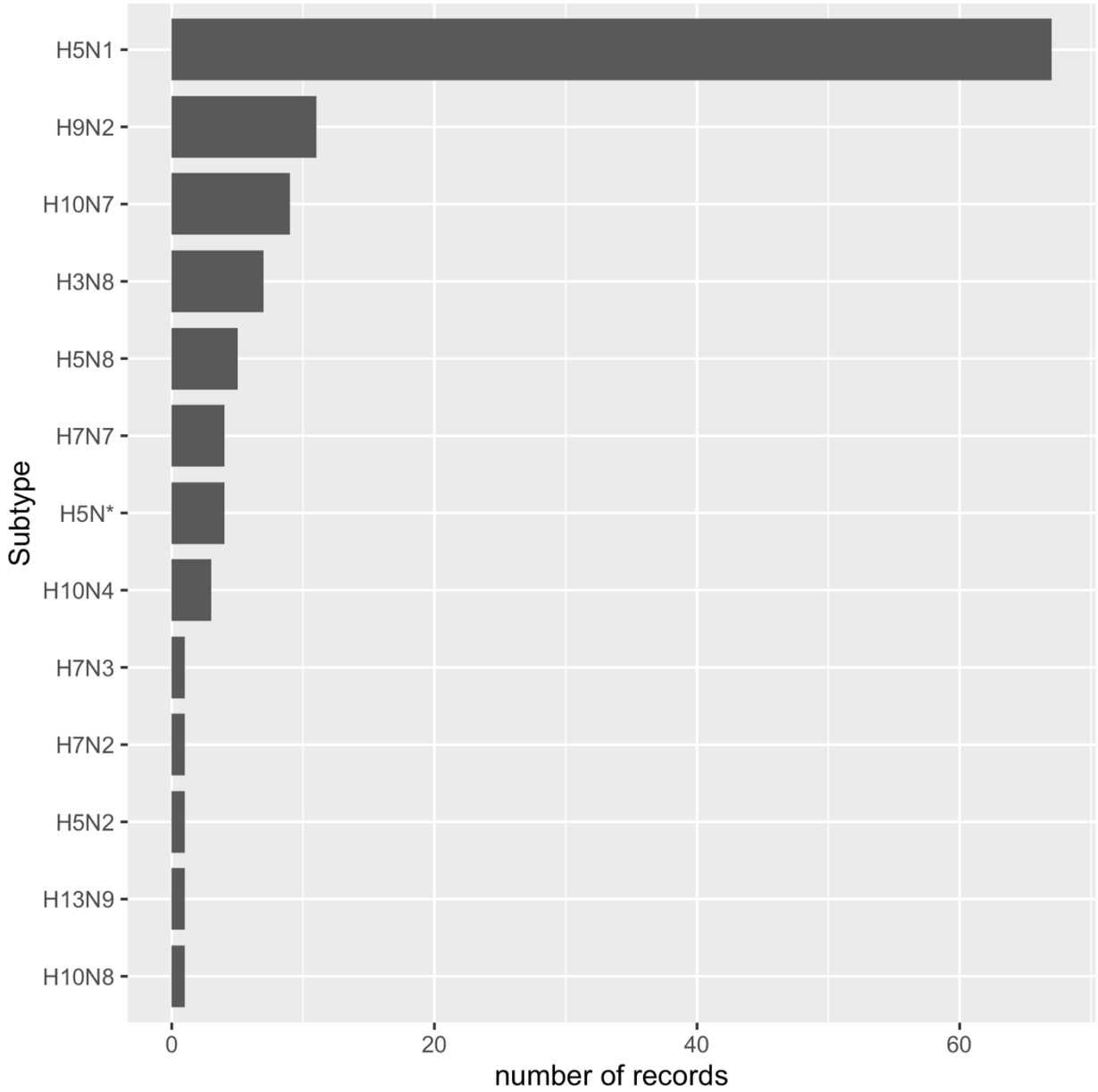

Figure 2: Number of records for each subtype reported.

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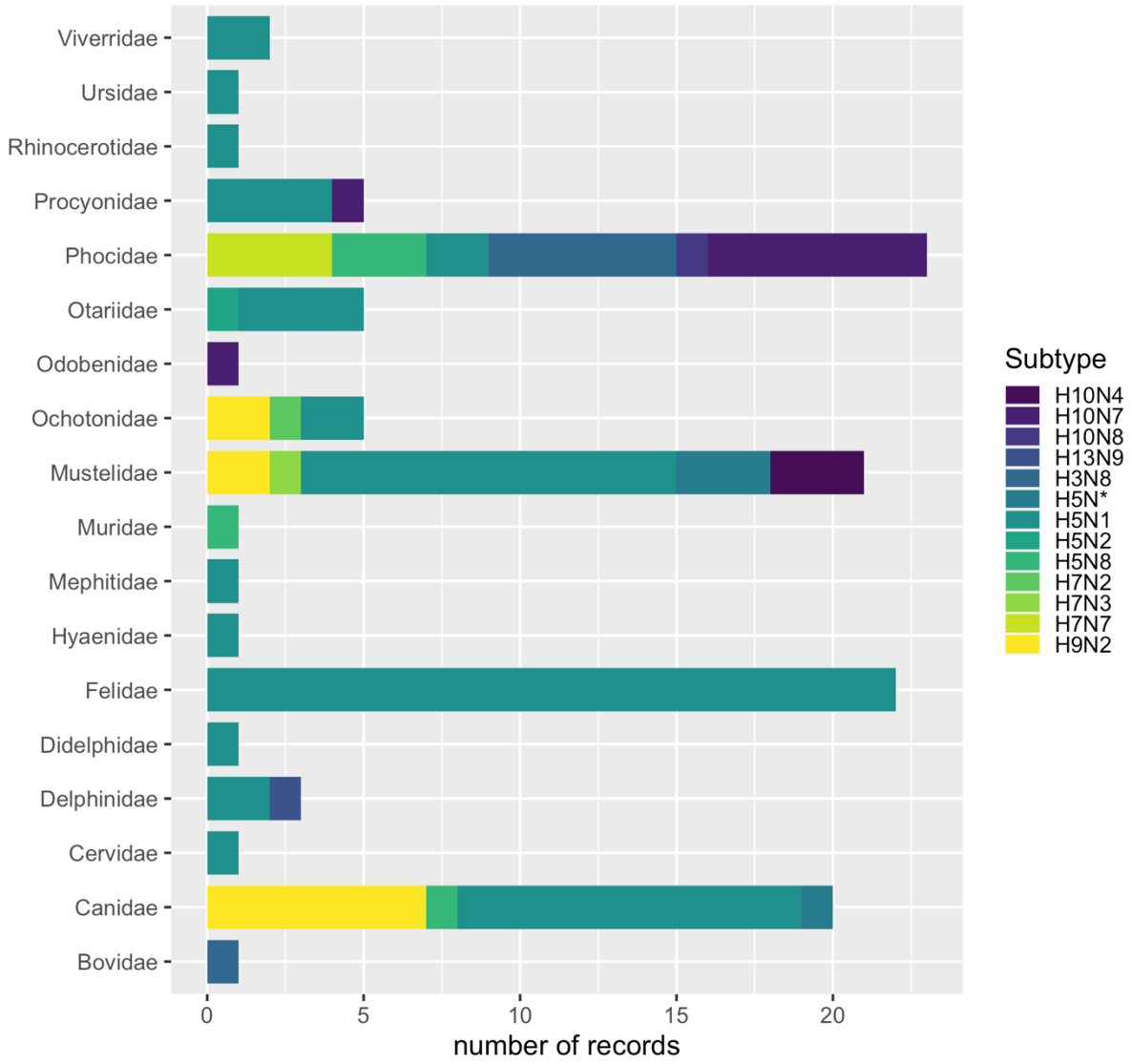

Figure 3: Number of records of unique subtypes recorded by host Family. H5N*: neuraminidase not specified in the study.

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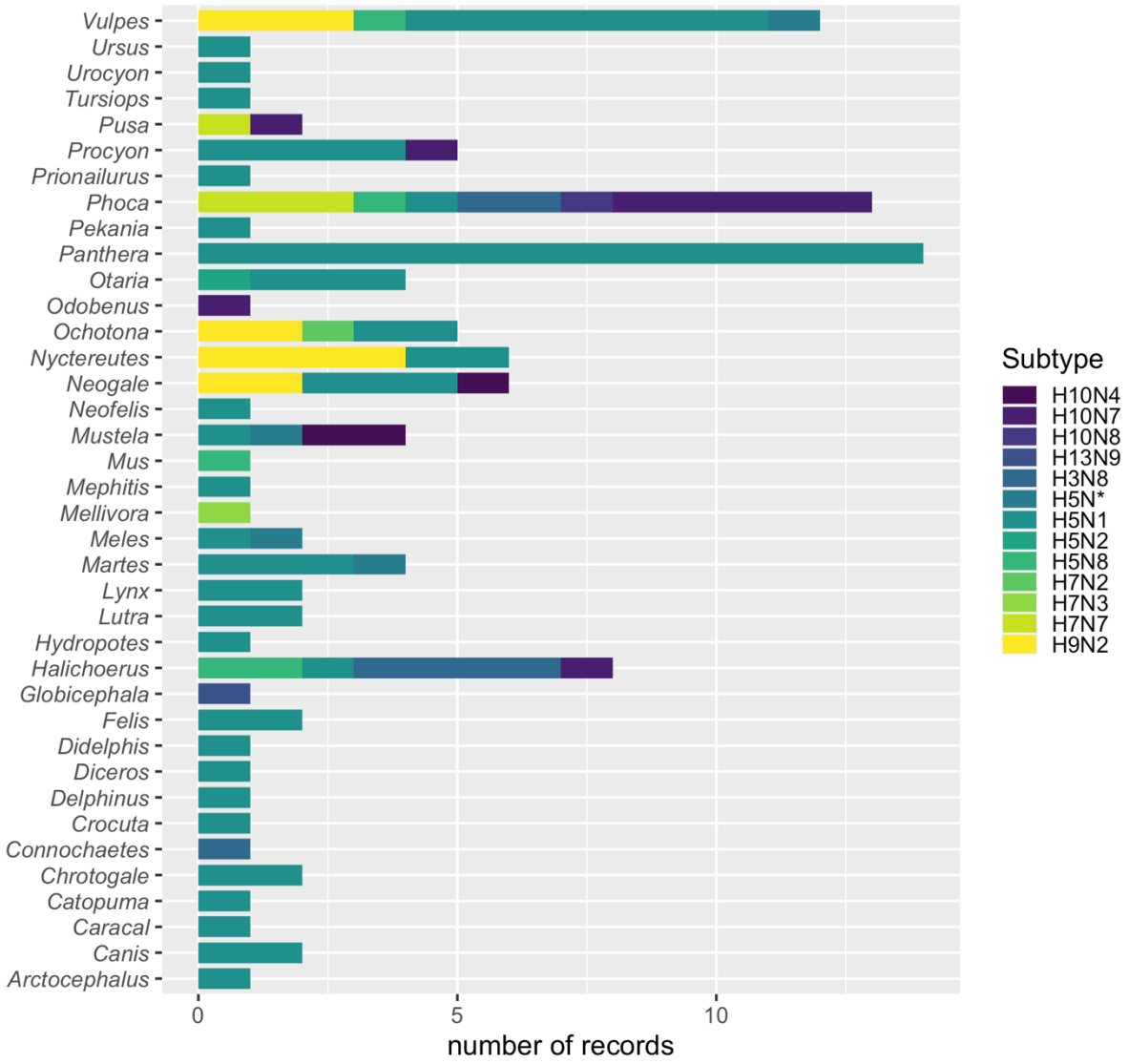

Figure 4: Number of records of unique subtypes recorded by host Genus. H5N*: neuraminidase not specified in the study.

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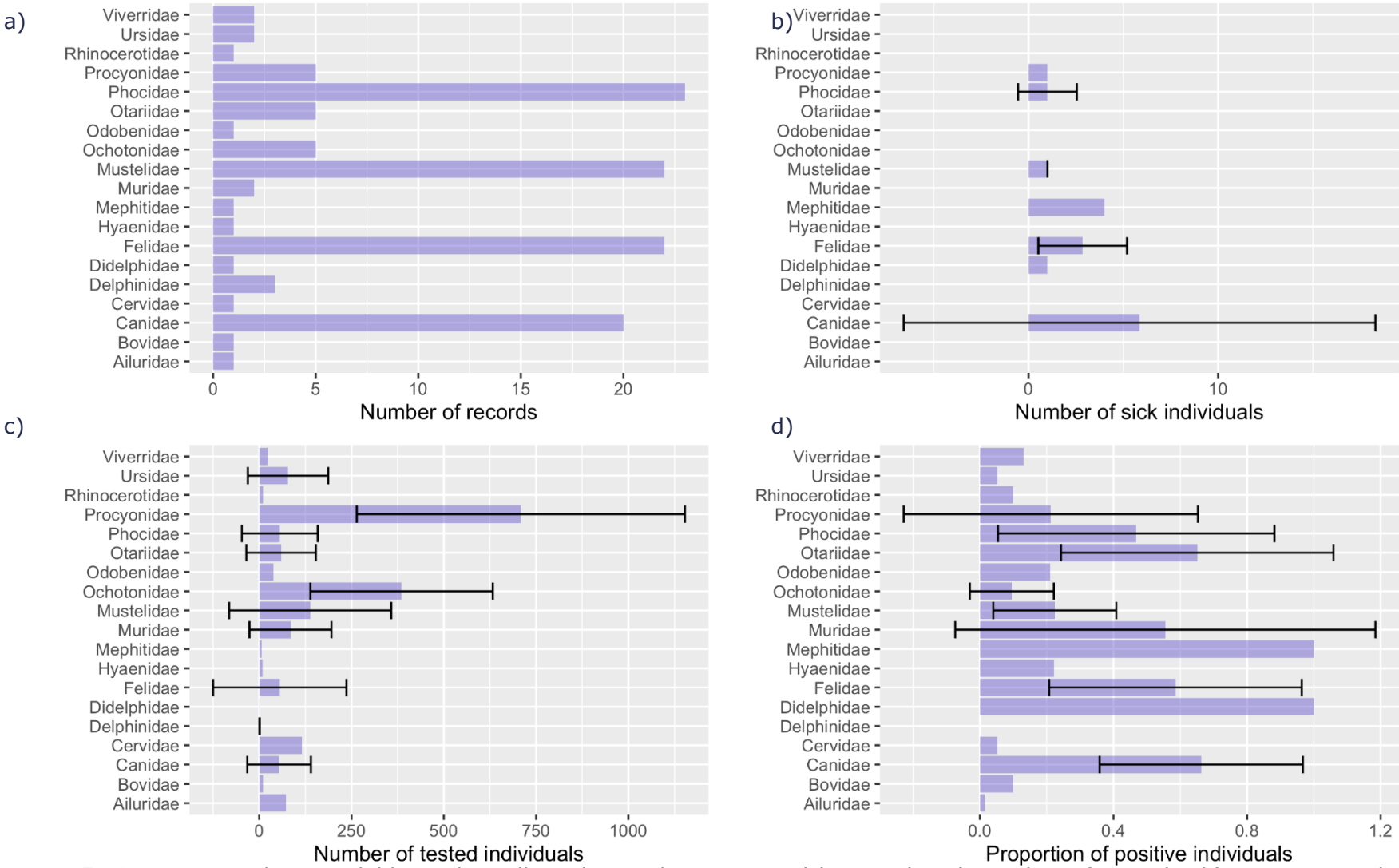

Figure 5: Quantitative data available in the collected records aggregated by Family: a) number of records; b) average number of sick individuals reported; c) average number of individuals tested; d) proportion positive. Values are average across records; error bars represent standard deviation.

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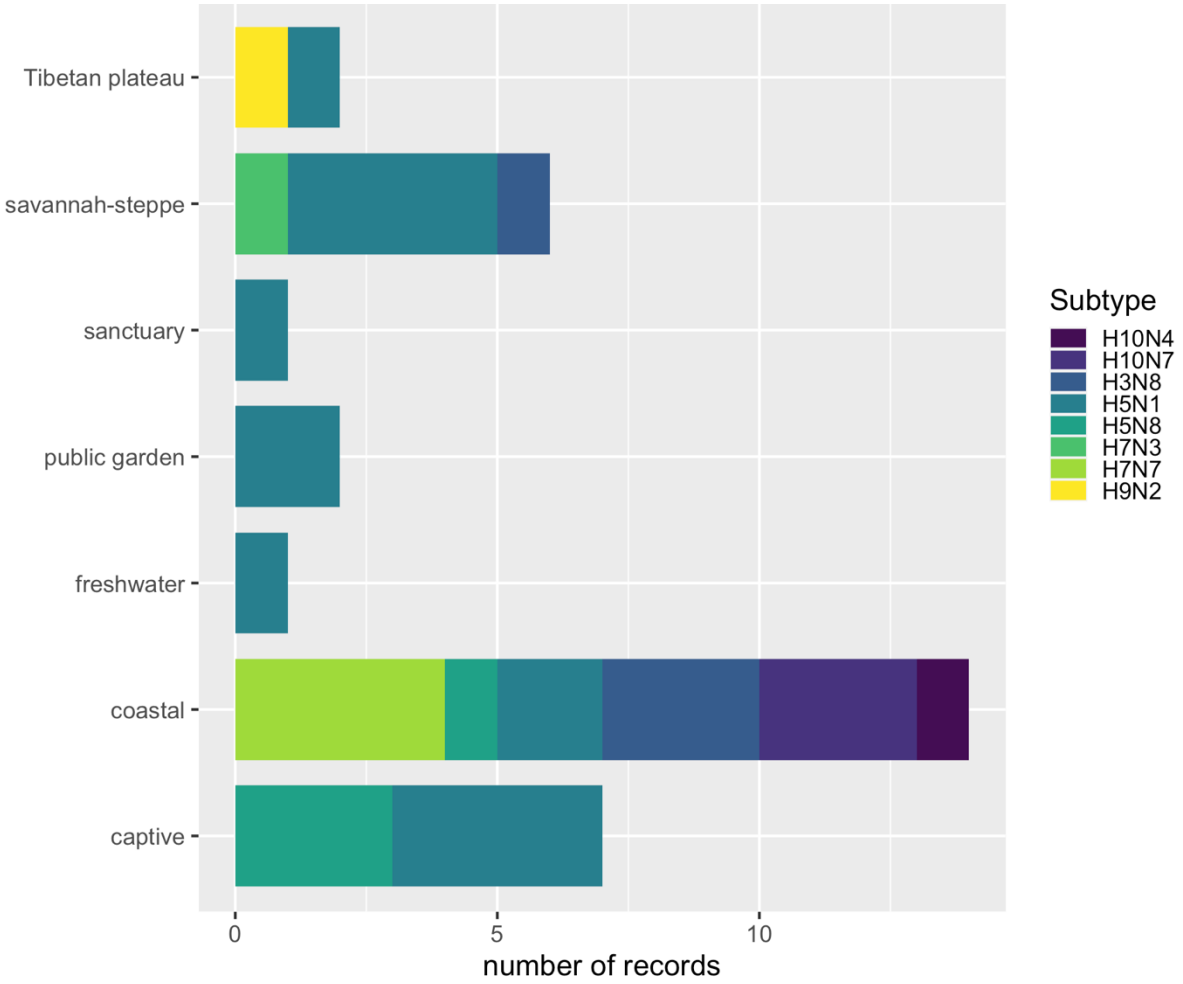

Figure 6: Habitat information reported with associated subtype. X-axis represents the number of records (records without explicit information regarding habitat are not shown); Y-axis represents the type of habitat reported aggregated in homogenous categories.

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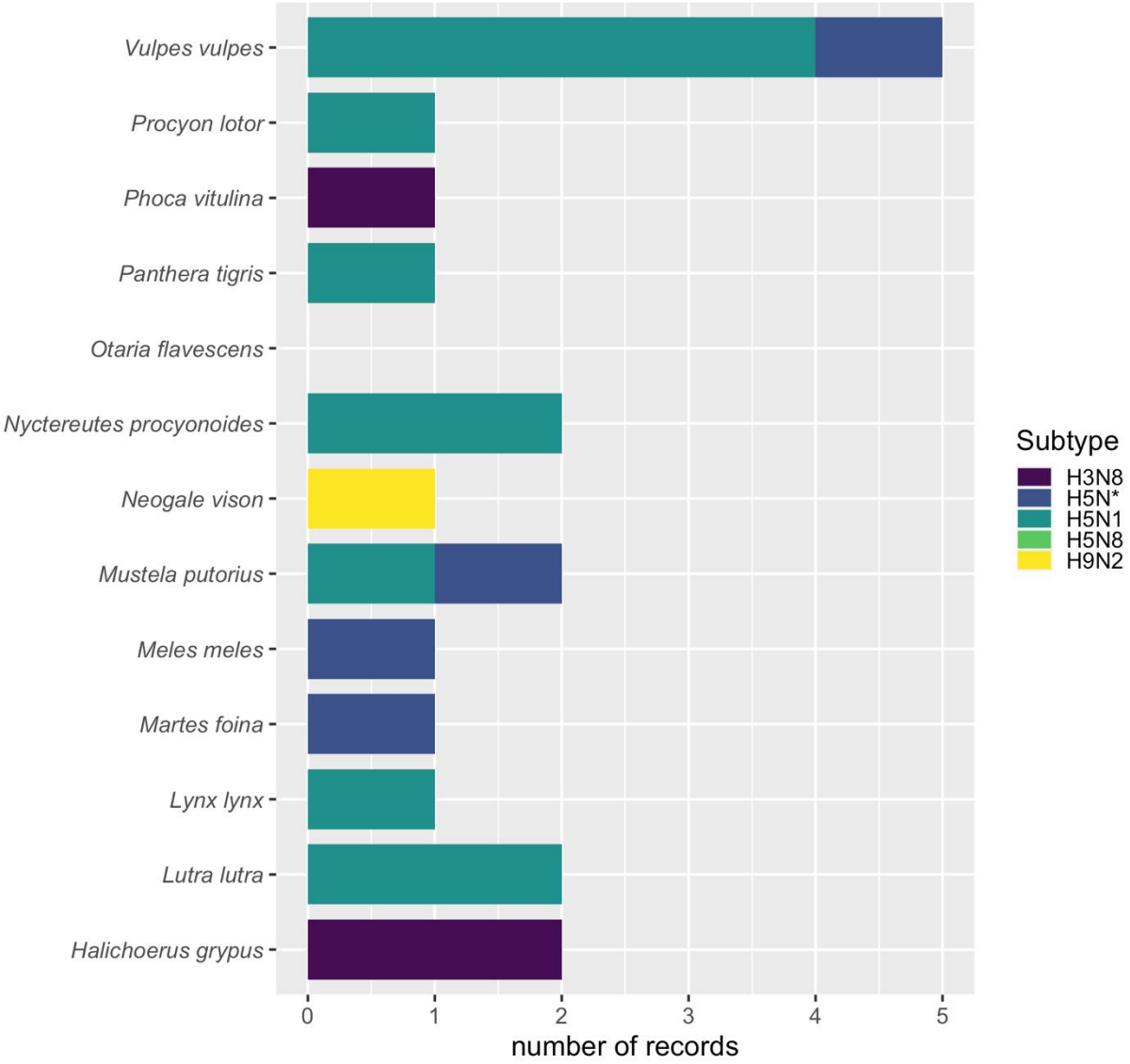

Figure 7: The number of records where molecular markers of mammal mutation were investigated in the study by host species (if no evidence of mammal mutation was found, species are shown without bar). X-axis represents the number of records; Y-axis represents the species investigated.

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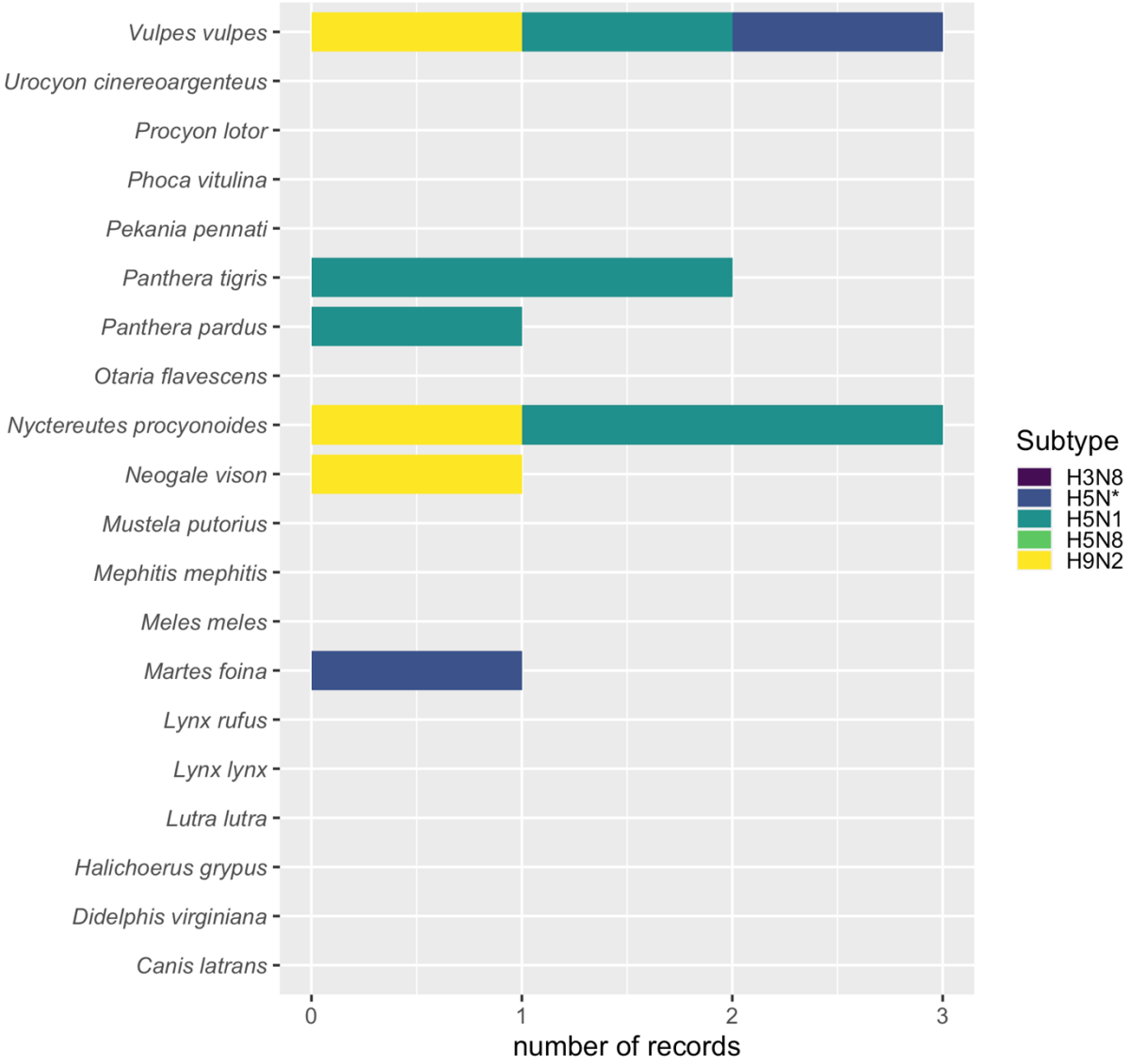

Figure 8: The number of records where mammal clustering was investigated, showing the species and the subtype involved (if no evidence of clustering was found species are shown without bar). X-axis represents the number of records; Y-axis represents the species investigated.

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4 Discussion

4.1 Epidemiology of avian influenza in wild mammals

The assessment of epidemiological features of outbreaks, or cases, of AI in wild mammals was challenging due to the inhomogeneity of data reported in the literature, the underestimation of cases compared to grey literature (e.g. reports from WHO, and other official organisations), and the current lag between publications and the newly discovered cases. Indeed, many data categories included in the data extraction spreadsheet (Table 3) were rarely recorded and could not be analysed. Nevertheless, data from the systematic review gathered information on all available viral sequences associated with records of infections in wild mammals (up to September 2023), which represent an essential and readily compiled dataset for wider phylogenetic analyses (beyond the scope of this review) to further assess virological features of AIV infecting mammals. In particular, quantitative epidemiological data were scarce, making it difficult to formulate hypotheses on the impact of infection on the populations involved (e.g. degree of conservation threat). This might be connected to identifying cases through passive surveillance and the lack of assessment of the "susceptible population". Therefore, active surveillance efforts in wild mammals are recommended to improve the characterisation of AIVs circulating in wild populations, both for public health and conservation purposes (Elsmo et al., 2023; Leguia et al., 2023; Rosone et al., 2023). Recent cases in the United States marked the first detections of the highly pathogenic avian influenza virus Eurasian lineage H5 clade 2.3.4.4b in wild terrestrial mammals, involving numerous species (Elsmo et al., 2023). Given the broad scope and ongoing nature of the outbreak in birds and the increase of reported cases in mammals, it is likely that those are only a small percentage of the total number and species of mammals infected with circulating strains of HPAI viruses in the United States (Elsmo et al., 2023), and worldwide.

Our evidence suggested that HPAI subtype H5N1 was most frequently found in wild mammals, compared to other subtypes of interest: H7Nx, H10Nx, H3N8, and H9N2. It is still unclear whether this is a bias towards testing individuals/populations displaying symptoms, as the vast majority of the infection identification was through passive surveillance or due to the greater circulation of this subtype among wild mammals. This subtype is known to be responsible not only for respiratory but also for neurological symptoms in the wild (Elsmo et al., 2023; Tammiranta et al., 2023; Roberton et al., 2006; Thanawongnuwech et al., 2005; Keawcharoen et al., 2004) and in experimental settings in ferrets and mice (Siegers et al., 2023; Plourde et al., 2012; Bodewes et al., 2011; Jang et al., 2009; Hoffmann et al., 2008); although this seems to be dependent on the specific strains (Pulit-Penaloza et al., 2022; Plourde et al. 2012). Neurological symptoms might be more severe, leading to the death of infected individuals or leading to a clearer identification of affected individuals, which makes these individuals (dead or visibly sick) targeted by passive surveillance (Vreman et al., 2023). Nevertheless, our review and evidence in experimental settings revealed that other subtypes were also responsible for neurological signs in wild mammals. Respiratory symptoms, weight loss, and other sporadic findings such as conjunctivitis were recorded, but probably neurological signs led to the oversight of mild respiratory symptoms. Nevertheless, it has been reported that throat and anal swabs were less effective in detecting positivity to HPAI H5N1; therefore, when possible, brain samples should be included in wildlife surveillance programs for reliable detection of the HPAI H5N1 virus in mammals (Vreman et al., 2023).

From the systematic literature review, there was a balance between infections in young and adult individuals, although the first might be more detectable as, in the wild, they might display harsher symptoms or higher prevalence (e.g. in North-Atlantic seals: Puryear et al.

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2016), and more often reported to rehabilitation centres (Tammiranta et al., 2023; Lukesova et al., 2022; Venkatesh et al., 2020). However, observational and experimental studies in mammals do not clarify whether clinical infections might be higher in younger individuals. Juveniles/young individuals might be more susceptible due to their naive immune system, but adults displayed increased inflammatory responses, leading to higher disease severity in older individuals (e.g. Rioux et al., 2021; Gholipour et al., 2017; Maines et al., 2008). Different exposure risks and the likelihood of co-infections might exist across age groups, and behavioural differences may make infected or clinically ill adult mammals less likely to be encountered (Elsmo et al., 2023). There is a scarcity of data from serological investigations of apparently healthy wild carnivores against LPAI or HPAI subtypes, which might shed light on morbidity and mortality in wild mammals and the role of factors such as age. Understanding how age affects respiratory virus disease is essential for developing preparedness and management measures, which may be host- and strain-specific.

In the wild, we found that the greater the number of infection records in a particular species, the higher the diversity of subtypes recorded. This finding might suggest a higher probability of exposure to a wider range of subtypes from multiple sources due to species ecology and distribution (e.g. scavenging habits), or a higher susceptibility of those species to be infected with different AIVs (e.g. immunity features). This is yet to be demonstrated, as experiments are restricted to a few combinations of subtypes/strains and mammal species, mostly ferrets (*Mustela furo*) (but see Xiang et al., 2020; Lina et al., 2019; Root et al., 2016; Romero Tejada et al., 2015; Root et al., 2014). Ferrets are recognised as an excellent model of influenza as they are susceptible to human and avian influenza viruses, exhibit disease signs and severity resembling those in humans, and these are dependent on host factors such as obesity, pregnancy, immune status, age, and viral strain (Cáceres et al., 2021; Rioux et al., 2021; Maines et al., 2008; Maher and DeStefano, 2004). Yet, they cannot represent the diversity of species infected in the wild. Although mustelids were represented by ten species in our records, harbour seals, grey seals, and red foxes were reported to be infected with more subtypes (Table B1). Infection risk factors identified for these species were a high probability of coming into contact with potentially infected bird species (i.e. aquatic, migratory, peridomestic), scavenging habits (especially for the fox, although seals do feed on dead birds), and the contact with guano, water, and other contaminated environmental resources (i.e. no airborne transmission) (Leguia et al., 2023; Root et al., 2014; Reperant et al., 2008). The confirmed case of H5N1 in a harbour porpoise, a species that does not feed on birds, strongly suggests that there can be a high enough infection burden of H5N1 in marine environments for spillover to mammals (Thorsson et al., 2023). In captive settings, even in taxa with high reported infections, such as tigers and mustelids, the most frequent subtypes were H5N1 and H9N2. In these cases, the source of infection was generally identified as feeding on raw chicken meat from infected poultry (Agüero et al., 2023; Yong-Feng et al., 2017; Reperant et al., 2009; Amosin et al., 2006; Keawcharoen et al., 2004). Avian influenza infections have been found several times in domestic cats and large felids in zoos (Thiry et al., 2007). Indeed, most studies reported isolated cases of infection in Carnivora, suggesting a connection to feeding behaviours.

Mammal-to-mammal transmission has been hypothesised in red foxes, raccoon dogs, and American minks in captive settings in China, involving H5N1 and H9N2 subtypes, yet only circumstantial evidence was available (Qian et al., 2021; Yong-Feng et al., 2017; Qi et al., 2009). In wild settings, observations of potential mammal-to-mammal transmission were recorded for *Otaria flavescens* in Chile and Peru infected by the HPAI H5N1 (Ulloa et al., 2023; Gamarra-Toledo et al., 2023); although epidemiological data suggested a potential transmission between sea lions, phylogenetic analyses pointed towards transmission from avian sources (Pardo-Roa et al., 2023). During an outbreak of H7N7 involving *Phoca vitulina* in the US (Webster et al., 1981a), humans handling the dead seals developed purulent

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conjunctivitis, raising concerns about the zoonotic potential of the virus (Webster et al. 1981b); yet, this could not be considered effective mammal-to-mammal transmission, but rather through a fomite (the dead animals). Experimentally, a wild bird-origin H5N6 avian influenza virus was found to be transmissible in guinea pigs, with horizontal transmissibility observed only with a specific genotype (SW8) (Xiang et al., 2020). Similarly, experimental infection studies showed that raccoons (*Procyon lotor*) seroconvert and could become infected with avian and human influenza A viruses, potentially shedding and transmitting the virus to virus-free animals (Hall et al., 2008). However, evidence of airborne transmission in animal models such as ferrets is still under investigation with variable efficacy from low to moderate in respiratory droplet and/or direct contact, especially for HPAI subtypes, and seemed to be strictly related to the specific strain (see review in Belser et al., 2020). Recently, Vreman et al. (2023) found none to very little shedding at the time of death of infected carnivores in the Netherlands, and although it cannot be excluded that infected mammals might have shed the virus at an earlier time point, no evidence of transmission between animals was found based on phylogenetic analysis.

In terms of transmissibility among mammals, the H9N2 subtype has significantly contributed to the internal gene segments of more virulent zoonotic strains, such as H5N1/N6, H7N9, and H10N8/N3, which were involved in human infections and displayed some ability to transmit via aerosol (Cáceres et al., 2021). Figure 9 shows a summary of H9 subtype influenza A viruses (IAV) reassortant viruses and their transmission ability in ferrets, which shows that minimal amino acid changes in the HA and/or the combination of H9N2 surface genes with internal genes of human influenza viruses are enough for the generation of H9N2 viruses with the ability to transmit via aerosol (Cáceres et al., 2021). Nonetheless, human-to-human transmission has not yet been reported (Cáceres et al., 2021). In addition, Li et al. (2022) demonstrated that a novel reassortant avian influenza A(H3N8) virus XJ47 isolated from wild birds exhibited enhanced α2,6 receptor binding and adaptation in mammals, posing a potential threat to animal and human health. Hence, surveillance of avian influenza viruses should include HPAI subtypes and H9 and H3 subtypes circulating in wild bird reservoirs.

Molecular markers of mammal mutations were found mostly on the H5N1 subtype and included E627K and D701N on the PB2 gene segment (Hatta et al., 2007), which were identified in red foxes and various species of mustelids (in natural infection in the wild and in farming contexts) (Elsmo et al., 2023; Tammiranta et al., 2023; Vreman et al., 2023; Yong-Feng et al., 2017), and a lynx (Tammiranta et al., 2023), raising particular concerns for spread in high-density settings (Root et al., 2015). These mutations were observed in conjunction, which is considered an extremely rare finding in nature, in an outbreak involving mammalian species and connected to mass mortality of pheasants in Finland (Tammiranta et al., 2023). The cases in mammals were spatially and temporally connected with avian mass mortalities, suggesting increased infection pressure from birds to mammals. Both mutations in the same strain have been demonstrated to be associated with increased polymerase activity, increased replication in human cells and enhanced virulence in mice (Zhu et al., 2015). Nevertheless, this finding might be biased, as these are the most commonly investigated mammal mutations, concealing other important molecular features determining adaptation to human respiratory tract cells and effective airborne transmission. Several markers were identified when more mutations were investigated (e.g. 22 substitutions in H3N8 isolated in a grey seal: Venkatesh et al., 2020; see Table B2). In the viruses isolated from a mass mortality event of sea birds and sea lions in Peru, PB2 E627K or D701N mutations were not detected, but at least eight novel polymorphic sites warrant further examination, as well as 40 variable sites previously linked to altered polymerase activity and replication efficiency were reported (Leguia et al., 2023).

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Despite the debate regarding this practice, gain-of-function studies should be carefully considered in identifying the contributions of molecular mutations to the avian influenza host range and relative adaptations due to the significant diversity of H5N1 and other influenza subtypes/strains in nature and the unpredictable combinations with hosts, leading to different transmission outcomes (Imperiale et al., 2018). Future experimental studies are necessary to increase our understanding of avian influenza transmission beyond identifying single mutations and to characterise the underlying biochemical and biophysical properties responsible for aerosol transmission in mammalian hosts (Imperiale et al., 2018; Kaplan and Webby, 2013).

In experimental settings, Root et al. (2015) investigated transmission from mammals to birds, finding that striped skunks (*Mephitis mephitis*) and cottontail rabbits (*Sylvilagus* sp.) were able to transmit avian IAV to mallards (*Anas platyrhynchos*) through indirect contact with shared resources (no via aerosol). The experiment was at a small scale, but it was possible to observe that individuals able to transmit to birds were not the ones shedding the most but the most active, suggesting that in nature, a risk factor might be the ecological/behavioural features of mammalian hosts. To date, we have not found any other evidence of this type of transmission; this might be due to the fact that this route of transmission is largely overlooked. Hence, mammals associated with and around poultry-rearing facilities should be considered in biosecurity plans, and further investigations are needed to investigate potential transmission pathways.

Finally, another knowledge gap identified by our review was related to the spatial distribution patterns of mammal outbreaks. We identified that countries with higher records might be on major migratory flyways, but we did not investigate whether outbreak locations shared similarities in terms of farming systems, density of poultry, biosecurity measures, abundance/density of scavenging species, abundance of wetlands, habitat connectivity (for the species of interest), and other environmental factors. All these elements might be important in detecting areas at risk of infection for the mammal population to prioritise for surveillance. Currently, the exact locations of infections are mostly unknown, and therefore, this analysis would have been unreliable. Thus, it is encouraged to have a more precise recording of spatial information on infections to develop such an analysis. Nonetheless, surveillance should be focused on the wildlife-livestock-domestic interface, which has long been recognised as a risk pathway (Moreno et al., 2023; Shriner et al. 2016; Caron et al., 2014; Thiry et al., 2007).

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VIRUS	DESCRIPTION	TRANSMISSION - DIRECT CONTACT OR AIRBORNE			REFERENCE
Avian-isolate 226L H9N2	H9N2 viruses isolated from avian species carrying 226L in the HA	Direct contact (66%) No Aerosol transmission	N/D	Direct contact depending on strain	71
Avian-isolate 226Q H9N2	H9N2 viruses isolated from avian species carrying 226Q in the HA	No transmission	N/D	Direct contact depending on strain	71
2WF10:6M98 H9N2	Reassortant H9N2 virus with internal genes from a seasonal H3N2 IAV	Direct contact No Aerosol transmission	N/D	N/D	71, 98
2WF10:6pdm H9N2	Reassortant H9N2 virus with internal genes from a pandemic H1N1 IAV	Direct contact Aerosol transmission	N/D	N/D	88
1WF10:7pdm H9N1	Reassortant H9N1 virus with internal genes and NA from a pandemic H1N1 IAV	Direct contact No Aerosol transmission	N/D	N/D	88
P10 H9N2	Reassortant H9N2 virus with internal genes from a seasonal H3N2 IAV adapted by 10 serial passages in ferrets	Direct contact Aerosol transmission	N/D	N/D	98
1P10:7pdm H9N1	Reassortant H9N1 virus (HA from P10 virus) with internal genes and NA from a pandemic H1N1 IAV	Direct contact Aerosol transmission	Direct contact	No Direct contact	88, 99
2P10:6pdm H9N2	Reassortant H9N2 virus (HA and NA from P10 virus) with internal genes from a pandemic H1N1 IAV	Direct contact Aerosol transmission	Direct contact	Direct contact	88, 99
2P10:6M98 H9N2	Reassortant H9N2 virus (HA and NA from P10 virus) with internal genes from a seasonal H3N2 IAV	Direct contact Aerosol transmission	N/D	N/D	98

Figure 9: Summary of H9 subtype IAV reassortant viruses and their transmission ability in ferrets. Schematic representation of the different H9 subtype IAVs tested for transmission in ferrets by our group and described in this review, description of each virus, and type of transmission observed. Results compiled from [71: Wan et al., 2008; 88: Kimble et al., 2011; 98: Sorrel et al., 2009; 99: Obadan et al., 2015]. From Cáceres et al. (2021).

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4.2 Avian influenza virus pathology and virology in mammals

Wild birds of the order Anseriformes (ducks, swans, geese) have long been known to be the main reservoir of most of the yet-identified Influenza A virus subtypes of low pathogenicity (Xie et al., 2023). While those infections are usually not associated with disease, they can be transmitted to domestic birds, where a highly pathogenic form can evolve from the low pathogenic progenitor virus of subtypes H5 and H7. This has been demonstrated to be associated with a specific mutation (multiple basic amino acids) at the endoproteolytic cleavage site of the haemagglutinin gene, which gives rise to high mortality due to their ability to cause systemic infection in domestic birds (Escalera-Zamudio et al., 2020). In contrast, H9 influenza A viruses do not evolve a high pathogenicity phenotype but are associated with airborne transmission in mammals (Cáceres et al., 2021) and have recently sparked concerns due to their signature of mammalian adaptation (reviewed in Halwe et al., 2023; Carnaccini and Perez, 2020).

Until 2002, highly pathogenic avian influenza (HPAI) was a rare but notifiable animal disease causing an acute, usually fatal illness in poultry. In 1996, an HPAI virus (H5N1) emerged in China, which has spread regionally since 2002 and worldwide since 2005; today, the descendants of this virus are spreading at an unprecedented scale. The early strains of this virus had an increased zoonotic potential and proved to be highly prone to reassortment, producing a plethora of genotypes. In addition, genetic drift also caused substantial antigenic drift, leading to the emergence of at least ten phylogenetically defined clades of the haemagglutinin protein with subclades down to the fifth order (Xie et al., 2023; Xu et al., 1999). Today, the descendants of this virus are spreading at an unprecedented scale: not only has the virus managed to spread to almost all continents and cause devastating losses in the poultry industry, but it has also established itself in various regions of the world in the long term and repeatedly leads to mass deaths in wild bird populations including endangered species (Chen et al., 2006). In the first years of its spread, waterfowl were particularly affected. Still, since 2020, the virus has also been introduced into breeding colonies of coastal birds (e.g. gulls and terns), which in some cases has led to the complete collapse of their breeding populations.

According to our systematic review, around 100 wild mammal infections with HPAI virus clade 2.3.4.4b have been reported in more than 40 species. Despite the current unprecedented spread of HPAI, there is comparatively little information about the clinical course and the pathology of avian influenza in wild mammals. Gradually increasing knowledge on the course of mammal infection comes from inoculation experiments with animal models, especially to evaluate the possibility of viral replication in human respiratory tract cells, e.g. ferrets (Belser et al., 2020).

Being a standard model for studying zoonotic IAV in vivo, the pathogenesis and pathology of different reassortants of LPAI and HPAI viruses in ferrets (*Mustela putorius*) are well-defined in experimental settings after infection by direct contact (Driskell et al., 2012) or, more rarely, aerosols (Guan et al., 2019). AIVs originating from wild birds efficiently replicated in the upper and lower respiratory tract, leading to pulmonary lesions and nasal shedding with minimal clinical signs (but see Englund et al., 1986: mink affected by H10 AIV developed interstitial pneumonia and showed high morbidity). Reassortment after co-infection with different

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influenza A viruses (IAV) was successfully shown in ferrets (Ganti et al., 2022; Richard et al., 2018). American mink (*Neogale vison*), another species of the family Mustelidae, are farmed intensively, often under crowded and poor conditions (Fenollar et al., 2021). They are often fed raw poultry by-products, potentially exposing them directly to the main source of circulating HPAIV and LPAIV reassortants, of farmed poultry (Agüero et al., 2023). Several reports of infection with AIVs with clinical signs ranging from respiratory distress, depression and loss of appetite over bloody snouts and hypersalivation to neurological manifestation are known from farmed (Agüero et al., 2023) as well as wild minks (Reperant et al., 2009), suggesting high susceptibility of these mammals (Sun et al., 2021). Like in ferrets, viral RNA can be found in respiratory fluids and rectal swabs, suggesting potential routes of onward mink-to-mink transmission (Agüero et al., 2023).

An analysis of two IAV sequence databases, Influenza Virus Resource of NCBI and the Global Initiative on Sharing Avian Influenza Data (GISAID) (Zhao et al., 2019), discussed semi-aquatic mammals as a potential mixing vessel for the reassortment of LPAI and HPAI. Although most reassortments are likely happening in the avian hosts, the study highlights minks (*Neogale vison*) as being infected with influenza viruses with the highest species/subtype diversities. Our systematic literature review on wild mammal infection with HPAI and other subtypes of interest found similar results for seals and red foxes (as well as for mustelids if considered together), indicating that semiaquatic mammals and mesopredators[2] are frequently exposed to multiple subtypes that circulate in wild birds (Figure 4). Importantly, high numbers of highly susceptible species kept on farms function as applicators and incubators for the influenza virus, which significantly increases the health risk for humans in close contact. The introduction of the virus can come from feeding with raw poultry and pork by-products. Poor housing conditions and stress likely contribute to the virus spread within affected farms, and cautious monitoring and surveillance, combined with increased biosecurity measures, should be applied (Abdelwhab and Mettenleiter, 2023). Apart from farmed wildlife, a fatal, nationwide disease outbreak in domestic cats occurred in Poland from June to July 2023. The source of infection was suggested, but not confirmed, to come from fed raw poultry meat (Rabalski et al., 2023). Unfortunately, further virological or phylogenetic analyses were not conducted to investigate the origin, spread, and clustering of these cases in depth.

The first emergence of HPAI H5N1 in 1996 in Hong Kong in poultry coincided with human infection (Kaplan and Webby, 2013). Since then, natural infections of domestic and wild carnivores with the HPAI virus have occurred, usually through close contact with infected birds or contaminated carcasses, as shown by our systematic literature review (Figure B1). Sustained intra-species transmission has not yet been convincingly demonstrated, although it was assumed on rare occasions. Mass mortality events in wild mammals have been recently reported in the harbour (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) (Puryear et al., 2023). Obviously, the spatial relationship of diseased and dead seals with wild bird AI infection is suggestive of virus introduction via avian sources, which is also supported by the phylogeny of the virus strains (Puryear et al., 2023; Pardo-Roa et al., 2023). One seal was infected with

[2] A mesopredator is a predator that occupies a mid-ranking trophic level in a food web. Mesopredators are usually medium-sized carnivorous or omnivorous animals, such as raccoons, foxes, or coyotes, usually preying prey on smaller animals. They are often defined by contrast from apex predators or prey in a particular food web.

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an HPAI strain with the E627K substitution in the PB2 segment, raising concerns about the potential for (future) mammal-to-mammal infection. The E627K mutation is reported to be important for mammalian adaptation (Suttie et al., 2019; Hatta et al., 2007) and represents the most abundant and relevant genetic change in our systematic literature review on wild mammal infection with HPAI (in section 4.1 we further discuss mutations and related subtypes in which these were detected).

Further mass mortality events associated with HPAI virus H5N1 circulation in wild birds are reported in South American sea lions (*Otaria flavescens*) and Southern elephant seals (*Mirounga leonina*) (e.g., The Americas; EFSA, ECDC and EURL, 2023). However, investigations are ongoing and further evidence is needed based on a greater sample size. To date, no mammal-adapted lineage of HPAIV H5Nx has emerged to circulate independently of the avian HPAIV reservoirs.

Red fox (*Vulpes vulpes*) cubs naturally infected with avian influenza virus A (H5N1) clade 2.3.4.4b presented with a predominant neurological component paired with signs of respiratory distress (Rijks et al., 2021). Brain samples from both cubs tested positive for the virus. Infection was likely from contact with infected wild birds since phylogenetic analysis indicated a high genetic similarity with the viruses isolated from barnacle geese (*Branta leucopsis*) found dead in the same area. Yet, the HPAI strains were unrelated to zoonotic strains infecting humans in Asia and had no mutations known to promote adaptation to the mammalian host. Additional laboratory-confirmed cases are reported in stone marten (*Martes foina*) and Owston's civets (*Chrotogale owstani*) (reviewed in Kaplan and Webby, 2013).

In a recent study dealing with pathological findings in wild terrestrial mammals, 67 wild mammals from ten states in the USA infected with avian influenza virus Eurasian lineage goose/Guangdong H5 clade 2.3.4.4b were investigated (Elsmo et al., 2023). Species included red foxes (*Vulpes vulpes*), striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), bobcats (*Lynx rufus*), Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), fisher (*Pekania pannanti*) and a grey fox (*Urocyon cinereoargenteus*). In most cases, the 57 live-found animals had moderate to severe neurological abnormalities ranging from seizure, ataxia, and tremor. Loss of fear against humans was also reported. Interestingly, respiratory signs (dyspnoea) were exclusively recorded in skunks, bobcats, and red foxes, although most animals (n=49/58) revealed gross lung lesions, including congestion, oedema, and failure to collapse, suggesting a differential ability of the virus to replicate in upper and lower respiratory tract of different species. Lesions in the brain (e.g. haemorrhage and congestion), liver (e.g. haemorrhage and congestion), kidney (e.g. cortical haemorrhage), and heart (e.g. pericardial effusion) were frequently reported. Occasional mild ocular discharge was found in red foxes. In the same study, almost all examined animals had inflammatory (necrotizing mostly non-suppurative meningoencephalitis) brain lesions in histopathology. Only brains from skunks were notably less affected, suggesting potentially lower pathogenicity of the particular strain involved in this species. The second most common finding in histopathology was multifocal necrotizing interstitial pneumonia, followed by myocardial necrosis, acute hepatic necrosis, and depletion of the lymphoid organs. Avian influenza antigen associated with lesions could be demonstrated by immunohistochemistry (IHC).

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Notably, the study reported nine different H5N1 genotypes with notable combinations of Eurasian and North American gene segments caused by reassortment of the Newfoundland-like H5N1 2.3.4.4b virus, which arrived in the Americas via the Atlantic flyway. Contrary to this, one unreassorted virus isolated from a red fox in Alaska presented a separate introduction to the Americas, likely from Asia via the Bering Strait (Elsmo et al., 2023). The E627K substitution was present in three examined animals (raccoon and red fox). Although – as previously outlined – horizontal transmission has been experimentally shown for domestic cats (Kuiken et al., 2004) and ferrets (Sun et al., 2023; Pulit-Penaloza et al., 2022; Belser et al., 2020), the authors did not report outbreaks that suggest mammal-to-mammal transmission. Instead, the sequence data suggested local spillovers from infected wild birds. The latter could also explain the temporal and spatial association of the reported mammal infections (but this might only be confirmed by a thorough analysis of the spatial distribution patterns of mammal outbreaks, which has been identified as a knowledge gap in the previous section).

Since the pathology of the H5N1 virus in wild mammals differs between carnivore species (and potentially genotypes), it is impossible to generalise the pathogenicity of HPAI for wild mammals. Moreover, the study conducted by Elsmo et al. (2023) did not find HPAI virus infection in adult red foxes, striped skunks, coyotes, and Virginia opossums in the USA, which raises the question of whether young animals are more susceptible due to their reduced immunocompetence. As discussed in section 4.1, our data analysis from the systematic literature review could not answer this question either, which warrants more studies incorporating age as a variable for HPAI susceptibility. Also, as mentioned before, there is a considerable risk of overlooking minor to moderate symptoms caused by AI infections in wild mammals. Epidemiological investigations based on serological testing might shed light on the actual circulation of AI subtypes in the wild, overcoming some confounding factors. Detection of H5-specific antibodies has recently been demonstrated in certain carnivore species in the Netherlands, where a high prevalence indicates that a substantial proportion of animals had been in contact with HPAIV H5 (Chestakova et al., 2023). Since HPAI-infected birds and carcasses are easily preyed upon, it is unsurprising that carnivores are the primary exposed group of wild mammals that are reported host species, and show high positivity to serological analyses. The susceptibility and exposure to HPAI in carnivores is equally reflected in the outcome of our meta-analysis of current literature on HPAI infection in wild mammals (Figure 3, Figure 4 and Table 5).

Thus, there is a need to increase global surveillance for influenza infection in mammals, especially in carnivores, to monitor virus adaptation to the mammalian host as an early proxy for an increase in pandemic potential. Additionally, multiple cases of zoo animals: tigers (*Panthera tigris*), leopards (*Pathera pardus*), lions (*Panthera leo*), Asiatic golden cats (*Catopuma temminckii*) and clouded leopard (*Neofelis nebulosa*) (reviewed in Kaplan and Webby, 2013), as well as the recent case of a fatal H5N1 infection in a polar bear in Alaska (https://dec.alaska.gov/eh/vet/announcements/avian-influenza-outbreaks/; last accessed 23.01.2024), have outlined concerns for the conservation of endangered wild carnivores. Post-mortem findings in infected captive tigers were equal to what has been reported in wild carnivores, associated with a multiorgan spread of the pathogen and respiratory lesions (Keawcharoen et al., 2004). Even in zoo animals, infection correlated with the epidemiological

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spread of avian influenza in wild birds, making intramammalian transmission less likely as the major infection route in captivity.

4.3 Ability of mammals to be reservoir for AI: potential pandemic role and drivers

The persistent threat of avian influenza viruses (AIVs) extends beyond their primary avian hosts, raising concerns about the potential reservoir role of mammalian species and the risk of a pandemic. Only in China, as of December 2021, 65 human cases of clade 2.3.4.4 H5N6 viruses have been reported, with a 55% fatality rate, indicating the severity of the zoonotic potential (Zhu et al., 2022). While sustained human-to-human transmission has not occurred, the evolving nature of these viruses raises the question of whether these might represent a pandemic risk (EFSA, ECDC and EURL, 2023). The main natural reservoirs for avian influenza A viruses are wild waterfowl (Venkatesh et al., 2018). However, influenza viruses circulating in birds can also spill over to mammals, as described in the multiple cases of avian influenza virus transmission, especially H5N1 in this review. As mentioned above, some studies have shown that some clade 2.3.4.4 A(H5) viruses have dual-receptor specificity and can transmit between ferrets in direct contact. Furthermore, some A(H5N6) viruses isolated from humans have molecular signatures related to mammalian adaptation. Yet, it is uncertain what other changes are necessary for these viruses to become transmissible via aerosol human-to-human , i.e. being the transmission way to possibly generate a large-scale epidemic or a pandemic.

To briefly summarise the picture arising from the systematic review and previous sections, mammalian cases found in the wild were in areas where a large number of HPAI infections in wild birds have been noticed. Thus, it might be difficult to distinguish whether the transmission occurred through mammal-to-mammal infection, or via preying on infected avian carcasses and/or environmental transmission. Countries of detection of infection in wild mammals? with the highest records were the USA (n=25), China (n=15), The Netherlands (n=12), and Germany (n=6), with other countries only represented by sporadic records. Evidence for mammal-to-mammal transmission was missing or scarce and mostly hypothetical. Since September 2023, when the search was performed, new findings have been published, especially on the occurrence of H5N1 in foxes (Elsmo et al., 2023). Yet, the proportion of these carnivores being infected with influenza viruses is low (e.g. less than five percent in a study of foxes in Germany; Baechlein et al., 2023). Additionally, in experimental settings, ferrets could mostly transmit influenza viruses to their conspecifics, depending on the subtype, genotype, and strain.

Amongst mammals, pigs are important from the virological point of view, as they can be infected by both avian and mammal influenza viruses (Zhang et al., 2020). If the species is simultaneously infected with both types of influenza virus, gene reassortment may take place, potentially leading to increased transmission efficiency of AIVs amongst mammals (Harrington et al., 2021). However, no records were included in the systematic review reporting H5 or H7 infections in wild boar. Infection seemed to be a rare event. In a study in Bavaria (Germany), only 4 out of 375 examined wild boar samples showed seropositivity against H5N8 (Schülein et al., 2021). For a mammalian species to be a maintenance host, it

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has to be naturally infected with an avian influenza-type virus and be able to directly transmit the virus to its conspecific. Through this, the avian influenza virus can undergo genetic selection that allows a better compatibility with mammalian species (Lipsitch et al., 2016). For example, such transmission has been described for H3N2 and H3N8 in dogs (Tangwangvivat et al., 2022). In experimental settings, infections with HPAIV have been described in pigs and ferrets, as a model for human transmission, but also in domestic cats and dogs. As previously described, these experiments did not lead to solid evidence of direct transmission among these animal models. Although rarely, HPAIV H5 antibodies have been detected in pigs (Schülein et al., 2021); yet, in experimental settings, pigs seem to be only marginally susceptible (Graaf-Rau et al., 2023).

Hence, with the current evidence it is possible to state that zoonotic infections are certainly possible, and reported (EFSA, ECDC and EURL, 2023), although these infections are not representing an immediate threat of pandemic. Although there is the need for high vigilance, zoonotic pathogens must overcome a hierarchical series of barriers to cause spillover infections in humans (Plowright et al., 2017). Zoonotic infections do not immediately mean pandemic. The ability to infect humans does not mean ability to sustain transmission among humans, e.g. airborne transmission. During the last HPAI H5N1 panzootic, no adaptations specific to humans were found in the human cases, but only mammalian mutations that might have a moderate activity in humans (e.g.E627K) (EFSA, ECDC and EURL, 2023). Current H5N1 strains may not replicate efficiently in human cells due to sensitivity to human reception factors, although the mammalian adaptations found might suggest potential mammal-to-mammal transmission risks. The factors to be evaluated to assess pandemic potential involve replication in mammalian hosts (adaptation of the polymerase e.g. PB2 E627K mutation), overcoming human restriction factors (Cimiski et al., 2021), hemagglutinin receptor binding adaptations, and pH stability of hemagglutinin (Long et al., 2019). Indeed, in the transmission from bird-to-bird or bird-to-mammal through environmental contamination (i.e. faecal-oral cycle) emerges as a crucial factor, surpassing primary contact importance (Breban et al., 2009; Rohani et al., 2009).

In examining intrinsic drivers, host susceptibility and genetic adaptations within mammalian species, particularly domestic pigs and ferrets, might play a crucial role in the transmission of AIVs. Some clade 2.3.4.4 A(H5) viruses exhibit dual-receptor specificity and limited transmission among ferrets; reassortant viruses may arise from HPAI and human seasonal A (e.g. H3N2), raising concerns about their potential to adapt to mammalian hosts (Chen et al., 2012). Although the current potential pandemic role of HPAI H5 might seem low due to extremely low airborne transmission in ferret models, limited pathogenicity and transmissibility (Herfst et al., 2018; Noh et al., 2018; Richard et al., 2015), other avian influenza subtypes circulate and may play a role (Yamai et al., 2020). Intrinsic drivers regarding the virus-host interaction are related to the wide variety of host species and viral genotypes found in combination in many regions of the world, as supported by the results of the systematic literature review. Hence, additional studies are instrumental to better elucidate the potential mechanism that allows some viruses to cross the interspecies barriers (Gortazar et al., 2014). Host features that seemed to favour infection were certainly scavenging feeding habits (e.g. generalist mesopredators such as red foxes and mustelids), and in general carnivores were more exposed to infection and displayed more viral mammalian adaptations

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(Chestakova et al., 2023; Rijks et al., 2021). Surveillance of wild carnivore populations and notification to the Veterinary Authority are important from a one-health perspective, and might be part of a pandemic preparedness strategy (Vreman et al., 2023). Carnivores were more exposed if they had access to shared resources with migratory or synanthropic birds, such as aquatic mammals, companion mammals, fur farmed carnivores, or found at wildlife-livestock interface (Shriner et al., 2016). Additional potential intrinsic factors reported by Elsmo et al. (2023) in red foxes were abundance of immunologically naive animals present during the onset of the avian outbreak, and potentially increased susceptibility to infection in this species. Wildlife-livestock interfaces and surveillance efforts have increased over time, driven by the rise of H5N1 HPAI in Asia. The identification of potential synanthropic wildlife species and bridge host species is crucial for understanding transmission pathways (Shriner et al., 2016). At this stage, it might be possible that wild mammals might serve as bridge hosts, defined as non-maintenance host species capable of transmitting a pathogen from a reservoir population to a target population (e.g. from wild birds to poultry) (Caron et al., 2015). By this definition, bridge hosts for AI come into contact with both maintenance hosts (aquatic birds) and/or their habitat and poultry and/or their environment. These contacts might occur via shared resources, such as water in streams, drainages, or ponds or via shared use of fields surrounding poultry barns which are used by many waterfowl hosts feeding on corn and other crops (Shriner et al., 2016; Caron et al., 2014).

In general, extrinsic risk factors include human-driven influences such as intensive farming practices that create environments conducive to cross-species transmission. Deforestation, urbanisation, and changes in habitats contribute to increased interactions between domestic and wild species, influencing the transmission dynamics of avian influenza. Global trade and travel facilitate the spread of AIVs, with infected avian and mammalian hosts potentially introducing novel strains to different regions. Nonetheless, in areas with high contacts between wild birds, poultry, and wild mammals, including farms previously involved in HPAIV outbreaks, synanthropic wildlife was not found to be highly prevalent, and no infected wild synanthropic mammals were detected (Shriner et al., 2016). It is likely that other environmental drivers are involved in AI transmission. Si et al. (2013) found that poultry outbreaks increased with an increasing human population density combined with close proximity to lakes or wetlands, increased temperatures and reduced precipitation during the cold season, suggesting spread to poultry via both poultry and wild birds. In the same study, the environmental factors most significant in wild bird species outbreaks differed, being an increased Normalised Difference Vegetation Index (NDVI), lower elevation, and same climatic variables as poultry, suggesting that wild birds alone drive outbreaks in wild birds. The proximity of farms or trade areas with wild birds habitat has been widely recognised as a risk factor for outbreaks in poultry, whereas outbreaks in wild birds were mainly found in areas where food and shelters are available (Si et al., 2013). Similarly, in wild and farmed mammals the proximity with wild birds habitat and/or the contact with infected poultry together with the appropriate climatic conditions might be the most important extrinsic drivers of infection, especially considering that to date all the infections reported in mammals had avian origin. The periurban preferences of some species, especially scavengers such as red foxes, might lead to a higher reporting in those species, but also to a higher exploitation of highly anthropogenically modified habitats associated with abundant food resources, leading to higher infection potential (Root et al., 2014).

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In recent HPAI outbreaks in marine mammals, another potential risk of infection has been identified in bird guano concentrations (Leguia et al., 2023). Considering that the main route of transmission seems to be mainly environmental, guano represents not only a risk for wild mammals through proximity and scavenging behaviour, but also a potential zoonotic route of infection (Leguia et al., 2023). This is used for fertilising crops, it is abundant on fishing docks, and it is used to attract large concentrations of animals as touristic attractions (Leguia et al., 2023).

In farmed and domestic mammals, trading between farms and human movements might also represent a risk pathway for transmission, as well as the habit of feeding these species with raw poultry meat, which has been confirmed to be a route of infection in mammal hosts. In particular, domestic and wild pigs should be targeted for surveillance as they are a "mixing vessel" (being able to be infected with avian, human, and swine influenza viruses) (Harrington et al., 2021; Zhang et al., 2020). Although they seem little susceptible to HPAIV (Graaf-Rau et al., 2023; Schülein et al., 2021; Luo et al., 2013), the reassortment opportunities in this species seem higher.

In conclusion, although a greater number of infections in wild mammals have been reported, there is no hard evidence of mammal-to-mammal transmission in the wild. The factors contributing to the increased number of infections found in wild carnivores are not clear yet but the unprecedented global spread of gs/GD HPAIV creates ample opportunities for intense, mostly alimentary, contact between infected wild birds and carnivores. Considering that HPAI H5N1 clade 2.3.4.4b led to encephalitis (Cronk et al., 2023; Elsmo et al., 2023), this could determine easier detection of such affected animals in the wild with subsequent investigations; this coupled with active surveillance at relevant wildlife-livestock-interface might represent a good starting point for pandemic preparedness, which should also include H3 and H9 subtypes.

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5 Conclusion and recommendations for surveillance

- Mammal-to-mammal transmission was not detected in wild mammals, although some experimental evidence suggested that this might be possible, although not very effective, for some combinations of viral genotypes-hosts.
- In wild mammals, sustained mortality events due to H5N1 have thus far only been reported in seals (Puryear et al., 2023). No seal-to-seal transmission was identified as a primary route of infection. Yet, the high likelihood of continuous contact of this species with potentially infected birds requires ongoing vigilance, as this increases potential opportunities for further reassortment or adaptation of these viruses to mammalian hosts.
- In experimental settings, the transmission route was mostly similar to that occurring in birds, i.e. sharing environmental resources, not airborne. Nonetheless, mammal species may play a role in the circulation of the Avian Influenza virus (HPAI and other subtypes).
- It might be possible that wild mammals, especially synanthropic and periurban species, might serve as bridge hosts, favouring the potential reassortment of various AIVs.
- Beyond HPAIV, subtypes H3N8, and H9N2 should be monitored as more easily replicating in mammalian (including humans) respiratory tract cells, favouring potential spillover to humans.
- Information on species-specific susceptibility, and morbidity and mortality of viral genotype-host combinations are scarce; further studies on the susceptibility of mammalian species to infection with the currently circulating strains of the HPAI H5N1 may be warranted, especially in light of the unprecedented reassortment of the Newfoundland-like virus with North American wild bird origin influenza viruses.
- Subclinical infections are of great importance, while current investigations mostly focus on mass mortality events of marine mammals and other species of conservation interest. Yet, these have been documented experimentally and in the wild, making it very challenging to implement an active surveillance strategy in wild animals.
- From this review it can be observed that viral mutations are happening, especially with regards to the polymerase activity and the ability to escape mammalian restriction factors, which might favour infections in humans, but no reassortant with human viruses were isolated.
- Evaluations of North American HPAI H5N2 and H5N8 isolates in human airway cells demonstrated that these were capable of replication, but at reduced titers compared with H5N1 and H1N1 viruses.
- Currently, there is not enough evidence to determine whether wild mammal species might represent a reservoir for AIVs.
- Understanding the complex interplay of intrinsic and extrinsic drivers is imperative for pandemic preparedness. Intrinsic drivers might be summarised in host susceptibility and genetic adaptations within mammalian species. Host features that seemed to favour infection were certainly scavenging feeding habits (e.g. generalist mesopredators such as red foxes and mustelids), and, in general, carnivores were more exposed to infection and displayed more viral mammalian adaptations. Broadly, extrinsic risk factors include human-driven influences such as intensive farming practices that create environments conducive to cross-species transmission. Deforestation, urbanisation, and changes in habitats contribute to increased interactions between domestic and wild species, influencing the transmission dynamics of avian influenza. Global trade and travel facilitate the spread of AIVs, with infected avian and mammalian hosts potentially introducing novel strains to different regions.

Considering the above conclusive points, active monitoring is recommended for early detection of AIVs mutations and/or adaptations favouring the spread in mammals, including humans. Vigilant surveillance, research on transmission mechanisms, and proactive measures to mitigate human-wildlife interactions are critical components in averting potential

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pandemics. Surveillance actions might include testing synanthropic and peridomestic birds as well as syndromic surveillance of "at risk" individuals/workers, e.g. people at poultry farms or dealing with infected deceased animals (Leguia et al., 2023; Burns et al., 2012). Coupling PCR-based diagnostics and/or serology of suspected cases is also highly recommended for early detection of clinical infections and transmission (Leguia et al. 2023). Active surveillance should focus on the wildlife-livestock-domestic animal interface, particularly investigating periurban/peridomestic mammals (multiple samples from different apparatus are recommended if possible). Nonetheless, these species are usually difficult to monitor, e.g. synanthropic wildlife such as rodents are very abundant and it is challenging to test an adequate proportion of the population. Additionally, diagnostic tests with high sensitivity and specificity are not necessarily available for every single species (e.g. serologic tests might have different sensitivity/specificity in different species). Mice have been commonly observed in rearing poultry facilities and at farms in general and might represent bridge hosts, so rodent control programs might be an addition to current biosecurity programs in poultry farms, specifically for AIVs control (Shriner et al., 2016). In general, rodents are considered reservoirs for zoonotic diseases and thrive in anthropogenically modified habitats (Mendoza et al., 2020; Plourde et al., 2017). Other management actions to reduce the risk of transmission between synanthropic wildlife and poultry, at a farm level might be: removing and/or reducing wildlife attractants such as ponds, standing water, food sources, and waste/carcasses; preventing wildlife access to poultry facilities; increasing wildlife deterrents (Shriner et al., 2016).

Finally, capacity building in disease prevention, outbreak investigations, and controlling the spread of disease in wildlife (e.g., through carcass removal) should be priorities. Our data support the outcomes of other risk assessments on this topic, e.g. the CDC Influenza Risk Assessment Tool determination that states that HPAI H5N1 viruses do not pose a substantial (although highly potential) risk to public health at this time (CDC, 2022). However, close surveillance of circulating strains and continued assessment of new viruses are crucial to ensure strains with enhanced mammalian fitness are quickly identified.

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Annex A – Data from systematic literature review

Annex A1.xlsx (raw data from data extraction) and Annex A2.csv (cleaned data used for performing analyses and creating figures) are available at https://zenodo.org/records/10733872.

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Annex B – Extra figures and tables from systematic literature review results

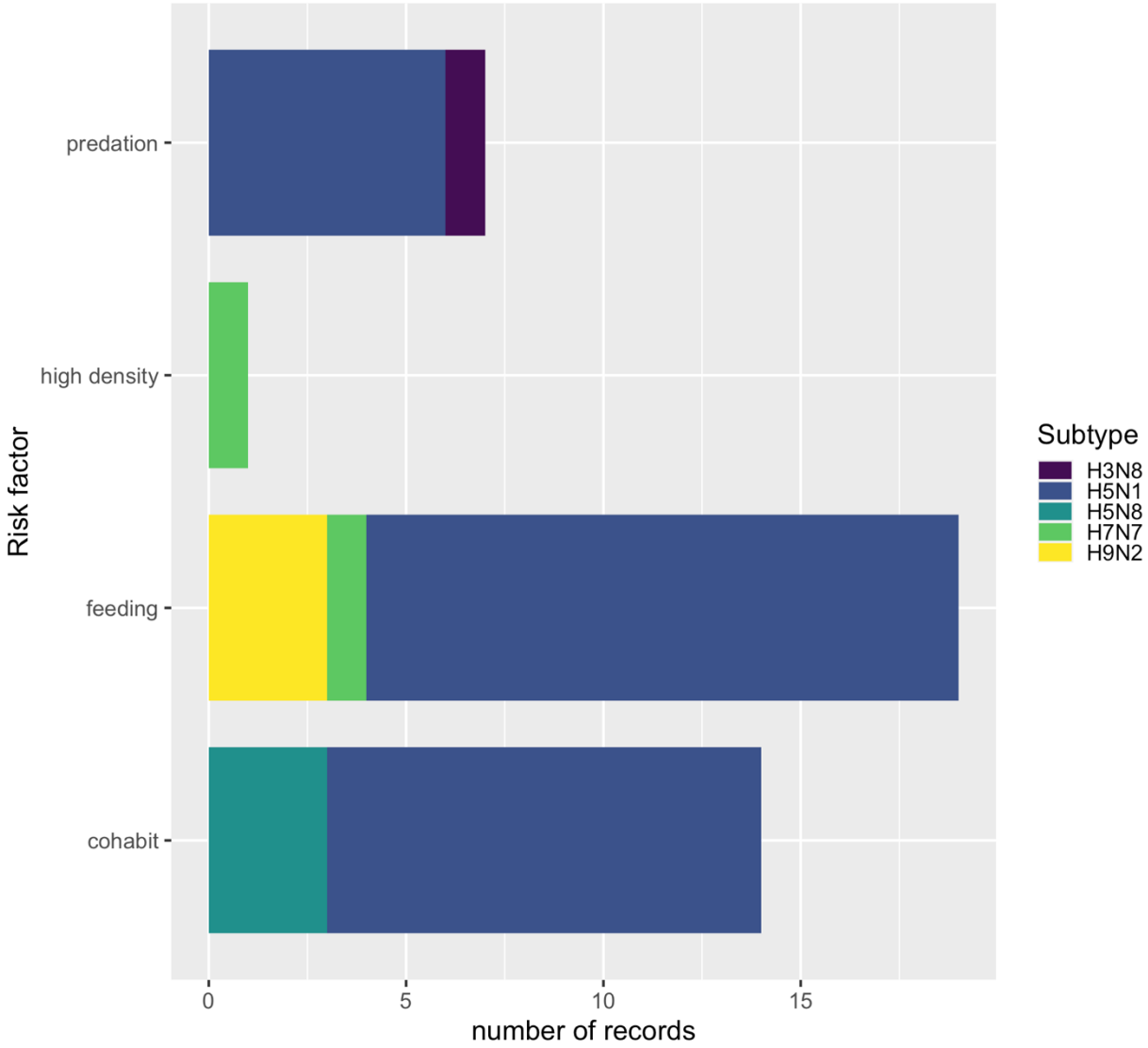

Figure B1: Risk factors identified in the systematic literature review for the cases of AI infections in wild mammals (predation differs from feeding as feeding includes scavenging and feeding in captive conditions).

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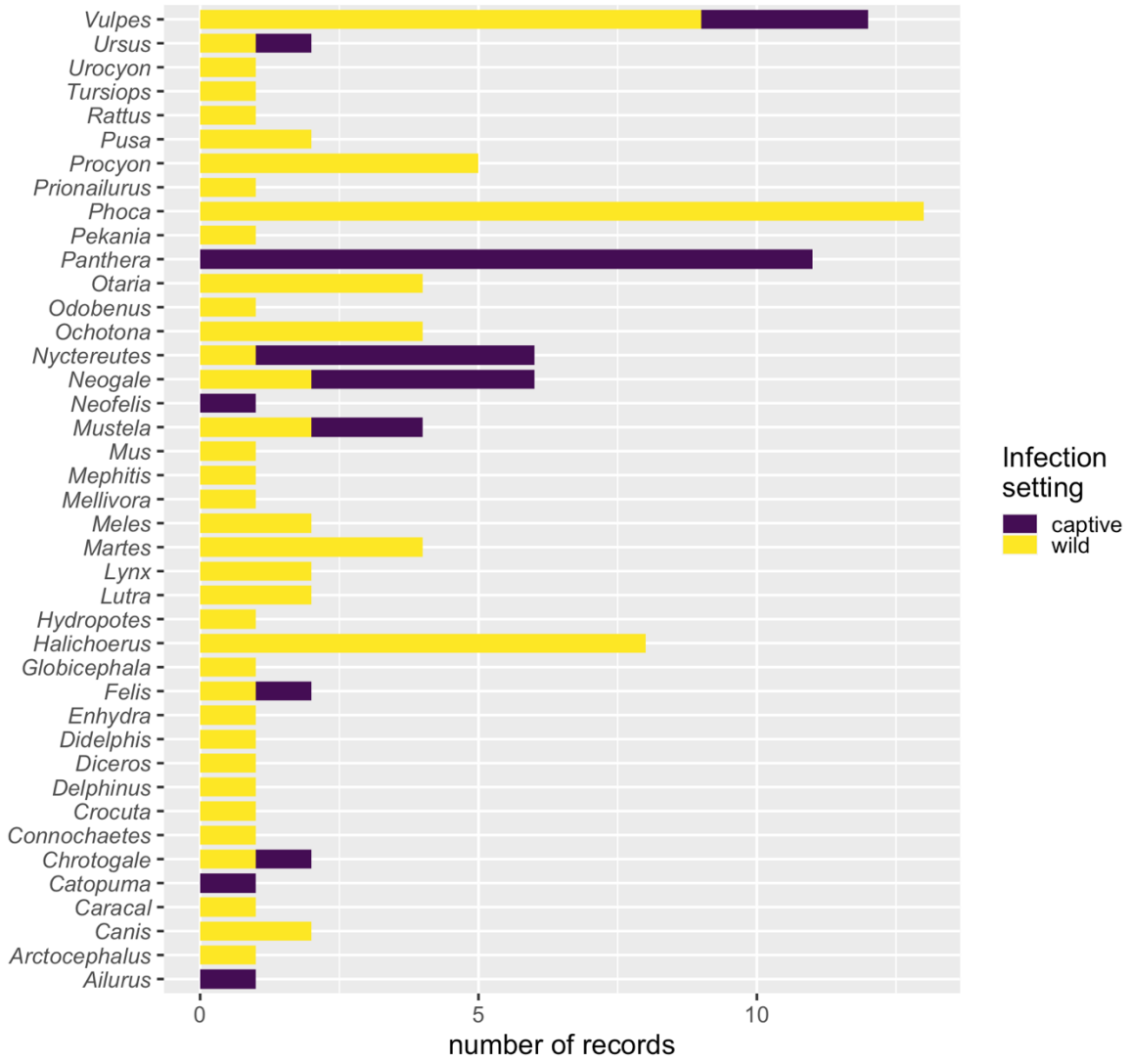

Figure B2: Setting of infection (wild vs captive) for the mammal genera recorded in the systematic literature review.

Table B1: List of species in which more than one unique subtype was recorded from the systematic literature review.

Species	**Number of subtypes recorded**
Phoca vitulina	6
Halichoerus grypus	4
Vulpes vulpes	4
Neogale vison	3
Ochotona curzoniae	3
Martes foina	2
Meles meles	2
Mustela putorius	2
Nyctereutes procyonoides	2
Otaria flavescens	2
Procyon lotor	2

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Table B2: List of species where mammal mutations were identified in AI viruses with relative type of mutation and number of records.

Species	Type of mutation	Number of records
Halichoerus grypus	*HA*	1
	Y9C [signal peptide]	
	T64A [48]	
	I81V [65]	
	S111G [95]	
	possible A154T (but might be same RCT → ACT (T), or GCT (A)) [138]	
	A/T176S [160]	
	S235Y [219]	
	MP	
	None	
	NA	
	N/S41D	
	T383A	
	NP	
	V104M	
	NS	
	None	
	PA	
	V91I	
	D/G101N	
	N/S321I	
	PB1	
	I517V	
	S678N	
	PB2	
	T/A105K	
	D161N	
	A/S395T	
	V667I	
	R/T676I	
	D701N	
	possible R753T	
Lutra lutra	PB2-E627K	2
Lynx lynx	PB2-D701N	1
Martes foina	NA	1
Meles meles	NA	1
Mus musculus	PA (genotype A) and PB2 (genotypes C, D, and E) genes of Go/Gd-like	1
Mustela putorius	T271A substitution	1
Mustela putorius	PB2-E627K	1
Neogale vison	PB2-D701N	1
Nyctereutes procyonoides	P13	1
Nyctereutes procyonoides	PB1 undefined mutation	1
Panthera tigris	PB2-E627K	1
Phoca vitulina	PB2-D701N	1
Procyon lotor	PB2-E627K	1
Vulpes vulpes	PB2-E627K	4
Vulpes vulpes	PB2-D701N	1
Vulpes vulpes	PB1 undefined mutation	1

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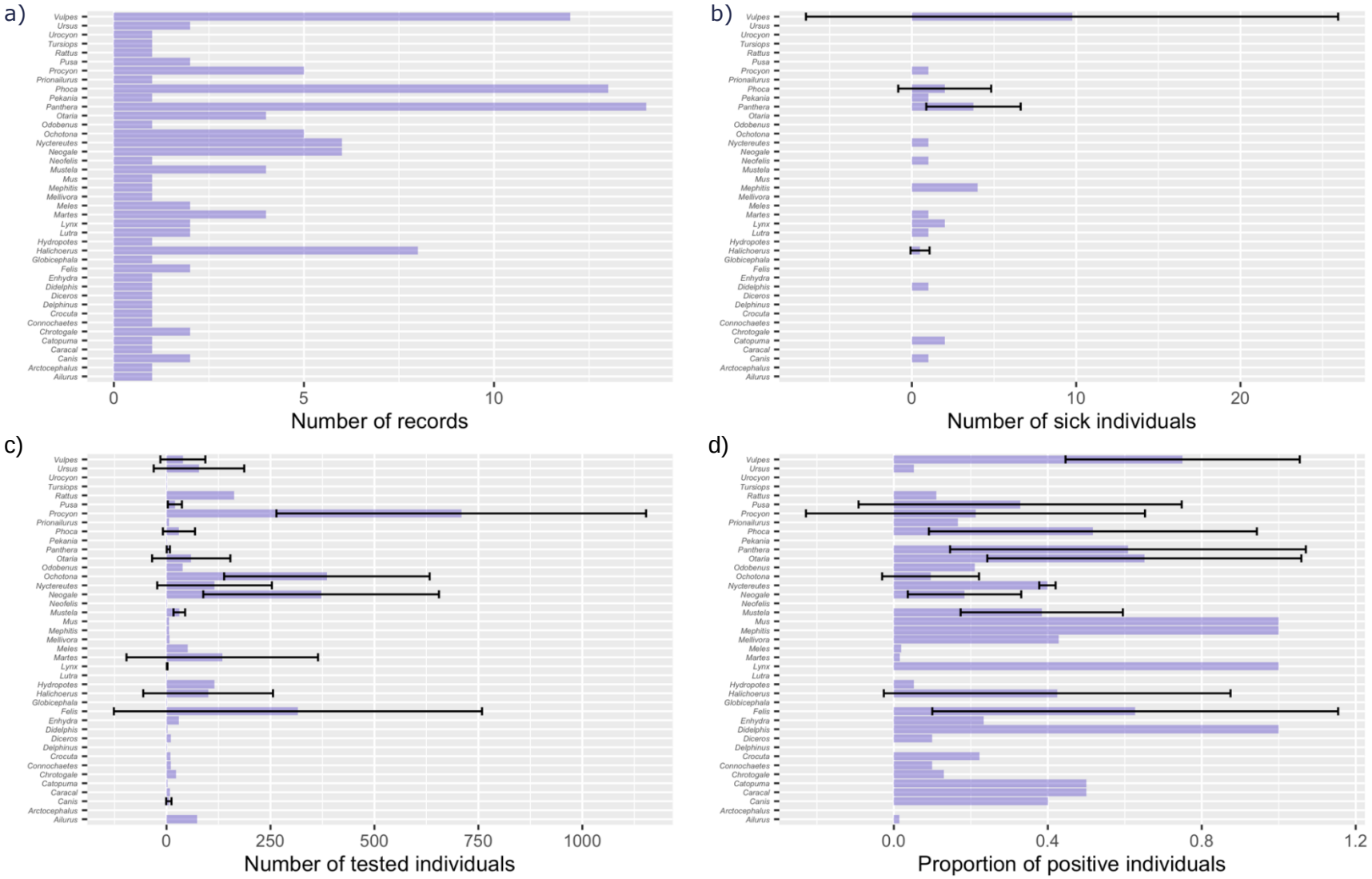

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Figure B3: Quantitative data available in the collected records aggregated by Genus: a) number of records; b) average number of sick individuals reported; c) average number of individuals tested; d) proportion positive. Values are average across records; error bars represent standard deviation.

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