A systematic review of laboratory investigations into the pathogenesis of avian influenza viruses in wild avifauna of North America

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ABSTRACT

The lack of consolidated information regarding response of wild bird species to infection with avian influenza virus (AIV) is a challenge to both conservation managers and researchers alike, with related sectors also impacted, such as public health and commercial poultry. Using two independent searches, we reviewed published literature for studies describing wild bird species experimentally infected with avian influenza to assess host species' relative susceptibility to AIVs. Additionally, we summarize broad scale parameters for elements such as shedding duration and minimum infectious dose that can be used in transmission modeling efforts. Our synthesis shows that waterfowl (i.e., Anatidae) comprise the vast majority of published AIV pathobiology studies, whereas gulls and passerines were less represented in research despite evidence that they also are susceptible and contribute to highly pathogenic avian influenza disease dynamics. This study represents the first comprehensive effort to compile available literature regarding the pathobiology of AIV's in all wild birds in over a decade. This database can now serve as a tool to all researchers, providing generalized estimates of pathobiology parameters for a variety of wild avian families and an opportunity to critically examine and assess what is known and identify where further insight is needed.

Keywords: avian influenza, challenge study, pathogenicity, wild bird

INTRODUCTION

While low pathogenic avian influenza (LPAI) virus circulates naturally with minimal clinical signs in Anseriformes and Charadriiformes waterbirds [1,2], highly pathogenic avian influenza (HPAI) virus has historically been limited to gallinaceous poultry [3] where it would emerge following LPAI exposure by wild waterfowl (typically Anatidae). The recently circulating and expanding HPAI H5 viruses of the Goose/Guangdong/1/1996 (Gs/GD) lineage are unusual because they have adapted to be carried by certain wild waterfowl species, thereby expanding

exposure to poultry, wild birds, and more recently some mammals [4,5]. The original H5 Gs/GD virus was isolated in 1996 in southern China [6] and by 2013 had evolved into clade 2.3.4.4 with notably improved ability to infect wild waterfowl including species which are generally not carriers of LPAI virus [7,8]. In October 2020, the global reach of HPAI was again highlighted when clade 2.3.4.4b viruses spread beyond a regional level, eventually reaching over 80 countries [5]. This current outbreak has been especially notable for reaching new hemispheres as it moved into North America in 2021 [9], South America in late 2022 [10] and the Antarctic region in 2023 [11], causing extreme impact on numerous populations of wild birds such as the Peruvian Pelican (*Pelecanus thagus*), Sandwich Tern (*Thalasseus sandvicensis*), and Common Crane (*Grus grus*) [4].

During this recent epornitic of HPAI, the conservation community was challenged with trying to anticipate how diverse species might respond when exposed to novel viral strains. For instance, there has been great concern of potential impacts on penguin colonies and condor populations should AIV be introduced [12] and recent outbreaks in Antarctica and South America indicate the vulnerability of these species [11,12]. However, an extensive body of work has been produced by researchers focused on challenge studies with wild bird species in a laboratory setting with both LPAI and HPAI to determine factors including infectiousness, severity of clinical signs, and recovery potential [13,14]. However, given the limited resources available, such work has not encompassed the broad spectrum of potential species and viral strains [15]. This has left a substantial knowledge gap as AIVs continue to evolve and present risks for an ever-growing list of species [16]. Furthermore, the literature that is available is diffuse with no comprehensive review for wild species collated in nearly a decade [14,17].

The lack of consolidated information on the response of wild bird species to infection with AIV is an identified challenge to both conservation managers and researchers alike [16], with additional impacts on related sectors such as public health and commercial poultry [18,19]. For wildlife managers it is important to understand the possible implications of an HPAI outbreak, especially for sensitive populations [4]. For instance, knowing if a population is at-risk to exposure and resilient to infection may play an important role in how that species is prioritized in allocation of resources for habitat protection, captive breeding programs, and other programs. Similarly, the diffuse knowledge base makes efforts to identify data-informed parameters for transmission risk models more difficult [16,18]. While we can infer some information from wild surveillance and mortality data, such as which species have tested positive and which are most likely associated with mortality events, we cannot determine important metrics like length of infection or infectivity from these databases.

METHODS

Literature Search

Using two independent searches, we reviewed the published literature for studies in which wild bird species were experimentally infected with avian influenza to assess host species' relative susceptibility to AIVs and ability to shed virus. To provide reasonable bounds on which species we included in our search, we created a list of species from those reported to the U.S.

Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) database [20] and the U.S. National Institute of Allergy and Infectious Diseases (NIAID)-sponsored Bioinformatics Resource Center Influenza Research Database (IRD) [21,22] as of Oct 16, 2023. We then supplemented this initial list with species which were both a high priority conservation concern and at high risk of exposure to HPAI due to either shared habitat with or a close genetic relationship to known susceptible species. We conducted an initial search using Google Scholar during October and November of 2023 using the search criteria ‘"avian influenza" AND "[species name]" AND "challenge study" OR "experimental infection"’. The search was conducted twice for each species of interest, where [species name] was replaced with either the common or scientific name. This initial search was intended to produce a representative, but not exhaustive, sample of research available for use in modeling avian influenza disease dynamics. As some species produced a disproportionately large number of papers (+1000) compared to the average (0–50), we limited our search to the first 5 pages of Google Scholar results to balance search time and capturing relevant information for all species.

After completion of this initial search, we identified a number of relevant studies not identified by Google Scholar, but which could be impactful for modeling purposes; providing information on unrepresented HPAI lineages from the first search procedure. While limiting search efforts likely contributed to missing papers, there are also concerns that Google Scholar is not an appropriate method for reproducible reviews of scientific literature [23,24]. To produce a more comprehensive review of currently available HPAI challenge research and fill information gaps in our original search, we conducted a second systematic review using Web of Science. We expanded our search criteria as using the same search terms as the original review proved too restrictive when applied to Web of Science. We chose to simplify phrasing, remove quotations, and include additional language previously used in reference to challenge studies, resulting in a search criteria of ‘(TS=("avian influenza" AND "[species name]") AND TS=(challenge OR experiment OR characterization OR pathogenesis))’. We conducted the search as initially described for our first review, repeating the search for each common and scientific name for birds on our search list.

Identifying Relevant Studies

Articles reviewed herein were considered for further review if the title, abstract, or methods discussed AIV challenge studies for wild bird species that range within North America. We excluded information for non-avian influenza viruses (i.e., mammal-derived strains of AIV) as well as information related to domestic and hybrid host bird species. There were multiple instances when a study challenged an individual with multiple viral strains in succession. In such cases, we recorded the viral subtype of the previous infection but otherwise record this information as usual, as many wild animals are likely to have previous AIV infections. However, several studies examined the impact of vaccines or viral inhibitors on host birds or local environments (i.e., water treatments), in which case we recorded these studies in the database but did not include them in any summaries.

For studies that met our above criteria, we recorded a unique entry for each combination of host bird species, virus subtype, and challenge method (e.g., direct inoculation, contact, meat fed)

used within a study. For each entry, we recorded information regarding the host animal species, family, and age at inoculation, as well as the viral subtype, origin, whether the virus was a HPAI or LPAI pathotype, and HA genetic lineage. AIV lineages for subtypes other than goose/Guangdong/1996 H5 were described based on the HA gene of the most closely related grouping classified by naming conventions utilized in previous reports or general geographic and species origin from which similar isolates have been collected. Subtype H5 isolates were classified by clade if from the goose/Guangdong/1996 lineage [25].

To describe the experimental design of each study, we recorded inoculation method, inoculation dose, and swabbing schedule, with any further details which may differentiate experimental groups within a study provided in a notes column. For each entry, we recorded the number of individuals in the study, the number of individuals identified as infected with AIV (i.e., virus identified in swabs, blood, or organs), and number of mortalities. We separately counted direct mortalities from illness versus euthanasia due to symptoms deemed indicative of imminent mortality. In some studies, individuals were euthanized (hereafter "culled") at predetermined time points throughout the study to assess viral titers in organs and tissue. We recorded these individuals separately from the prior two mortality categories to adjust estimates of mortality rate due to AIV infection. Information on mortality and culling did not include individuals culled at the end of the study (i.e., survivors). If provided, or if it could be calculated from the provided mortality data, we recorded the mean death time in days.

Summarizing Viral Response

Identification of virus in swab samples can be performed using virus isolation or RT-PCR methods[26], which are reported as at least one of multiple metrics that are not all comparable. Virus titer levels in isolates are commonly quantified and reported as the 50% $\log_{10}$ egg infective dose per milliliter (EID_{50}/mL); however, alternative mediums can be used that produce estimates that are not comparable between methods [27]. Real-time RT-PCR results are readily reported as a cycle threshold (Ct) value, but additional steps may be conducted to produce a comparable equivalent to the EID_{50}/mL of viral isolation [28]. As such, we only used information measured as EID_{50}/mL from viral isolation or, if not available, EID_{50}/mL equivalents from RT-PCR in our summaries. For simplicity we hereafter use "shedding" to refer to the greatest daily-average viral titers identified in swabs. Information on shedding could be collected via swabbing the oropharyngeal (OP) and/or the cloacal (CL) routes, which are important prerequisites of animal-to-animal and environmental HPAI transmission respectively [13]. As shedding patterns between these pathways may have important but different implications for virus transmission, we recorded this information separately. We recorded information on viral shedding by the host animal, however this information is assessed and summarized in multiple formats across studies which are not directly comparable. For example, viral shedding quantities and durations were reported using a broad range of summary statistics, including mean, median, and maximum values for a given experimental group as well as by individual, sometimes for multiple timepoints over the course of an infection. Thus, to describe the average capacity a host animal has for spreading the virus, we report the greatest mean daily shedding quantities for a given experimental group over the course of a study (Figure S1).

For all entries, we recorded the mean daily shedding quantities for a given experimental group and mean bird infectious dose (units in $\log_{10}$ 50% bird infectious doses per milliliter; BID_{50}) if estimated. We also recorded shedding duration (days that an animal shed the virus), either as a single duration estimate where provided, or as a series of time points describing when the virus was first detected (D_1; Figure S1), which day the greatest mean shedding rate occurred (D_{Max}), the last day that the virus was detected in swabs (D_L), and the next day animals were swabbed and virus was not detected following completion of shedding (D_{Neg}). From this information and information on when swabbing occurred, we can calculate a shedding duration as the $(D_L + D_{Neg})/2 - D_1$. In multiple instances, we encountered studies where shedding information was presented only in plot form. To interpret information within these papers, we used a web-based figure reader (PlotDigitizer; plotdigitizer.com) which is a free option for such data mining from scatter and line plots [29] and recorded its use.

Tables including entries for all inoculation doses, inoculation methods, and prior infection statuses were generated. To produce a final set of directly comparable metrics describing a species' viral response, we subset our dataset to entries where animals were directly inoculated with a Gs/GD lineage HPAI of dose greater than 4 $\log_{10} EID_{50}$, which is a cutoff that consistently resulted in infection in most studies that estimated infectious dose (Table 2).

Comparison to Incidence of Avian Influenza in Wild Birds

To identify potential knowledge gaps in AIV research, we compared our review of the published research to the observed incidence of avian influenza in wild birds. Information on wild bird infections was obtained via the USDA-APHIS database [20] and accessed Nov 16, 2023. The database provided information on encounter location, encounter date, viral subtype, host species, and how the animal was reported, and was summarized to number of positives per species.

RESULTS

Summary of Relevant Studies

Through this review, 132 unique challenge studies for AIV in wild bird species were identified, and of these, 104 had at least one focal species from our list (Supplemental 1). Of these 104 studies, Anatidae was the most well represented family identified (78 studies), followed by Laridae (11), Passeridae (8), Columbidae (6), and Scolopacidae (4; Table S1). Within Anatidae (Table S2), challenge studies were distributed across 18 species including mallard (*Anas platyrhynchos*; 54 studies), Muscovy duck (*Cairina moschata*; 11), Canada goose (*Branta candadensis*; 5), northern pintail (*Anas acuta*; 4), and wood duck (*Aix sponsa*; 4). Outside of Anatidae, house sparrow (*Passer domesticus*; 8), laughing gull (*Leucophaeus atricilla*; 7), and rock pigeon (*Columba livia*; 6) were the most studied. Of note, 6 of the top 10 families with the most reported AIV infections had no studies identified within our review, including Accipitridae (727 reported infections), Cathartidae (691), Strigidae (248), Pelecanidae (119), Corvidae (118), and Phalacrocoracidae (39). 43 studies challenged animals with LPAI subtypes and 74 with HPAI subtypes, of which 66 included viruses of the Gs/GD HPAI lineage (Figure 1). Only 5 of the 1282 animals challenged with LPAI in the identified studies perished over the course of the infection. Animals challenged with Gs/GD perished with greater frequency (67%, N = 1755)

compared to those challenged with other lineages of HPAI (6%, N = 300). Information on viral susceptibility and shedding was only available from one species for 6 of the 10 families identified in the review, and for 3 of these families only 1 study was found (Table 2, 3, S2, S3). One of these, Ardeidae, had challenge data for only 3 individuals, of which 1 was infected and died.

Summarizing Viral Response

Articles were subset according to challenge type (i.e., directly inoculated seronegative animals with >4 $\log_{10}$ EID_{50}/mL of a Gs/GD HPAI) then summarized (Table 1, Table 2). The resulting dataset contained information on only 24 species, of which 13 are from the Anatidae family. Within Anatidae, American black duck (*Anas rubripes*), blue-winged teal (*Spatula discors*), lesser scaup (*Aythya affinis*), Northern pintail, and redhead (*Aythya americana*) all exhibited 0% mortality when challenged with Gs/GD whereas Canada goose, mute swan (*Cygnus olor*), and trumpeter swan (*Cygnus buccinator*) all experienced 100% mortality. For waterfowl, oral shedding (measured by RNA presence from OP swabs) rates averaged 4.54 $\log_{10}$ EID_{50} and ranged from 1.1 $\log_{10}$ EID_{50} to 7.7 EID_{50} while cloacal shedding (measured by RNA presence from cloacal swabs (CL)) rates averaged 3.28 $\log_{10}$ EID_{50} and ranged from 0.9 $\log_{10}$ EID_{50} to 6.8 $\log_{10}$ EID_{50}. Shedding durations averaged 5.72 and 5.21 days for oral and cloacal routes respectively. Trumpeter and mute swans exhibited the highest average oral (6.14 $\log_{10}$ EID_{50}) and cloacal (4.46 $\log_{10}$ EID_{50}) shedding rates respectively. The top eight oral shedders and seven of the top eight cloacal shedders were all from the Anatidae family. A number of these species, such as mallards and surf scoter (*Melanitta perspicillata*), also exhibit relatively low mortality rates (31% and 22 %) when challenged with HPAI. Mallards exhibited higher than average shedding rates (4.78 $\log_{10}$ EID_{50} via OP, 3.52 $\log_{10}$ EID_{50} via CL) and durations (6.42 days via OP, 5.83 days via CL) across studies.

We identified only a limited number of comparable studies for non-waterfowl species. For example, within Laridae, we identified herring gull (*Larus argentatus*) and laughing gull as the only species with data available. Herring gull exhibited high infection rates (100%) and moderate mortality rates (67%) when challenged and shed more via the OP route (3.82 $\log_{10}$ EID_{50} for 5.27 days) than the cloaca (2.01 $\log_{10}$ EID_{50} for 2.93 days). Comparatively, laughing gull had lower infection (64%) and mortality (29%) rates, longer shedding durations (9 days and 6.5 days for OP and CL), but similar shedding rates (3.48 $\log_{10}$ EID_{50} and 1.89 $\log_{10}$ EID_{50}). Considering passerines, house sparrows had relatively high infection (78%) and mortality (58%) rates and shed moderately via both the OP and CL routes (3.28 $\log_{10}$ EID_{50} via OP, 2.7 $\log_{10}$ EID_{50} via CL) for approximately 4.8 days. Limited information was also available for American robins (*Turdus migratorius*), which were readily infected (92%) but showed no indication of negative impacts to survival (0% mortality). Rock pigeons, a similarly broadly abundant bird species, were much less readily infected (30%) and had similarly low mortality rates (7%). Within Falconidae, two studies challenged a total of 22 American kestrels (*Falco sparverius*), of which all perished.

When the dataset was expanded to include any animal challenged with a Gs/GD HPAI (Table S3), we found Canada goose exhibited the greatest average viral titers in cloacal swabs (5.75

$\log_{10}$ EID_{50}/mL, range 3.8-7.3) and trumpeter swan in OP swabs (6.14 $\log_{10}$ EID_{50}/mL). Laughing gulls had the longest observed shedding duration via the OP route (9 days post challenge, range 8-10), whereas surf scoters shed the longest via the CL route (8 days post challenge).

DISCUSSION

This study represents the first comprehensive effort to compile available literature regarding the pathobiology of AIV's in all wild birds in over a decade [13]. The database produced through this effort can serve as a tool to all researchers, providing generalized estimates of pathobiology parameters such as mortality and viral shedding for a variety of wild avian families. However, this database also provides the opportunity to critically examine previous research to assess what has been learned from these efforts as well as to identify where additional targeted efforts can provide further insight.

Our synthesis shows that waterfowl (i.e., Anatidae) comprise the vast majority of published AIV pathobiology studies. Such an emphasis is warranted given the widely recognized role of these species in the spread of AIVs [18,30]. Indeed, previous efforts have demonstrated that *Anatidae* are competent vectors for avian influenza due to their high shedding rates [31,32], long duration of infection [33,34], and comparatively high prevalence in the wild [35,36]. However, while previous studies have provided us with valuable information on the general response to infection for Anseriformes, the importance of these species to the transmission of AIVs both across space [30,37] and across the wild waterfowl – domestic poultry interface [18,38] suggests that future work with these species could aim to develop better estimates of parameters needed for transmission risk modeling [39] such as minimum infectious dose which is currently only rarely included [40,41]. Similarly, the continued high representation of Anatidae in mortality events and surveillance sampling [20] emphasizes the importance of studies focused on including emerging viral strains to inform possible outcomes prior to outbreak events and to maintain current data.

Unlike data for Anseriformes, which are well represented in the literature, we found only 10 studies across 4 species of Laridae. This paucity of information is noteworthy given the recognized importance of Laridae in the inter-continental spread and persistence of AIV [42,43]. Given the apparent susceptibility of these species in the current HPAI outbreak, their large geographic distribution and long-distance migrations, as well as frequent overlap with agriculture, Laridae seem to be prime candidates for continued pathobiology research [15]. For similar reasons, other sea birds, such as skuas [44], have been implicated as possible long-distance carriers of the virus, but a lack of available research limits our ability to infer potential dynamics.

While identified as having a role in the spatial movement of AIVs [45], predatory and scavenging species appear especially susceptible to infection and mortality based on data from the current outbreak [20], as well as limited pathobiology studies with species outside North America [46]. In fact, Accipitridae, Cathartidae, and Strigidae were three of the top four families with the most reported infections to USDA yet had no studies identified by our review. However,

Falconidae were among the highest infectious rate and mortality rate (100% for each), though this finding was based on a single species across two studies (the American Kestrel). Given the dramatic impacts of HPAI on these species there is a clear need to improve the understanding of all aspects of pathobiology of AIV in raptors and other predatory avian species. Additionally, efforts to model broader ecological transmission and persistence could benefit from studies that explore transmission through meat/carcass consumption instead of traditional inoculation [47].

Of course, many other species outside of those specifically mentioned above have been impacted by the ongoing outbreak of HPAI. Our summary demonstrates that seven families had over 100 reported HPAI infections to USDA [20], but only two of these families have been studied (Anatidae and Laridae). Of the 10 families identified in our review, only three reported challenge data from more than 1 species, and 1 of these only included three individuals total. It should also be noted that these metrics rely on data from infections reported to USDA [20], so species which are under-sampled due to low detectability of sick or dead birds will also be underrepresented in this effort. Therefore, the real number of species which lack sufficient pathobiology data is likely larger than demonstrated here. This is partly because challenge studies with HPAI require BSL-3 laboratories, which are limited and often have overriding study priorities. Additionally, it can be difficult to obtain and care for wild birds or wild bird eggs needed for conducting these experiments. Working to close this large data gap is a notable potential avenue for future research, even for species that are not suffering from large scale mass mortality events and that do not have recognized roles in AIV spread.

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Literature Cited

1. Kuiken, T. Is Low Pathogenic Avian Influenza Virus Virulent for Wild Waterbirds? *Proceedings of the Royal Society B: Biological Sciences* **2013**, *280*, 20130990, doi:10.1098/rspb.2013.0990.
2. Fouchier, R.A.M.; Munster, V.J. Epidemiology of Low Pathogenic Avian Influenza Viruses in Wild Birds. *Rev. sci. tech. int. Epiz.* **2009**, *28*, 49–58.
3. Sonnberg, S.; Webby, R.J.; Webster, R.G. Natural History of Highly Pathogenic Avian Influenza H5N1. *Virus Res* **2013**, *178*, 63–77.
4. Wille, M.; Waldenström, J. Weathering the Storm of High Pathogenicity Avian Influenza in Waterbirds. *Waterbirds* **2023**, *46*, doi:10.1675/063.046.0113.
5. Harvey, J.A.; Mullinax, J.M.; Runge, M.C.; Prosser, D.J. The Changing Dynamics of Highly Pathogenic Avian Influenza H5N1: Next Steps for Management & Science in North America. *Biol Conserv* **2023**, *282*, 110041, doi:10.1016/j.biocon.2023.110041.
6. Xu, X.; Subbarao, K.; Cox, N.J.; Guo, Y. Genetic Characterization of the Pathogenic Influenza A/Goose/Guangdong/1/96 (H5N1) Virus: Similarity of Its Hemagglutinin Gene to Those of H5N1 Viruses from the 1997 Outbreaks in Hong Kong. *Virology* **1999**, *261*, 15–19, doi:10.1006/viro.1999.9820.

7. Lee, D.H.; Bertran, K.; Kwon, J.H.; Swayne, D.E. Evolution, Global Spread, and Pathogenicity of Highly Pathogenic Avian Influenza H5Nx Clade 2.3.4.4. *J Vet Sci* 2017, *18*, 269–280.
8. Kang, H.-M.; Lee, E.-K.; Song, B.-M.; Jeong, J.; Choi, J.-G.; Jeong, J.; Moon, O.-K.; Yoon, H.; Cho, Y.; Kang, Y.-M.; et al. Novel Reassortant Influenza a(H5N8) Viruses among Inoculated Domestic and Wild Ducks, South Korea, 2014. *Emerg Infect Dis* **2015**, *21*, 298–304, doi:10.3201/eid2102.141268.
9. Caliendo, V.; Lewis, N.S.; Pohlmann, A.; Baillie, S.R.; Banyard, A.C.; Beer, M.; Brown, I.H.; Fouchier, R.A.M.; Hansen, R.D.E.; Lameris, T.K.; et al. Transatlantic Spread of Highly Pathogenic Avian Influenza H5N1 by Wild Birds from Europe to North America in 2021. *Sci Rep* **2022**, *12*, doi:10.1038/s41598-022-13447-z.
10. Gamarra-Toledo, V.; Plaza, P.I.; Gutiérrez, R.; Luyo, P.; Hernani, L.; Angulo, F.; Lambertucci, S.A. Avian Flu Threatens Neotropical Birds. *Science (1979)* 2023, *379*, 246.
11. Bennison, A.; P Byrne, A.M.; Reid, S.M.; Lynton-Jenkins, J.G.; De Sliva, D.; Peers-Dent, J.; Finlayson, K.; Hall, R.; Blockley, F.; Blyth, M.; et al. Detection and Spread of High Pathogenicity Avian Influenza Virus H5N1 in the Antarctic Region. *bioRxiv* **2023**, doi:10.1101/2023.11.23.568045.
12. Leguia, M.; Garcia-Glaessner, A.; Muñoz-Saavedra, B.; Juarez, D.; Barrera, P.; Calvo-Mac, C.; Jara, J.; Silva, W.; Ploog, K.; Amaro, Lady; et al. Highly Pathogenic Avian Influenza A (H5N1) in Marine Mammals and Seabirds in Peru. *Nat Commun* **2023**, *14*, doi:10.1038/s41467-023-41182-0.
13. Hénaux, V.; Samuel, M.D. Avian Influenza Shedding Patterns in Waterfowl: Implications for Surveillance, Environmental Transmission, and Disease Spread. *J Wildl Dis* **2011**, *47*, 566–578, doi:10.7589/0090-3558-47.3.566.
14. Pantin-Jackwood, M.J.; Swayne, D.E. Pathogenesis and Pathobiology of Avian Influenza Virus Infection in Birds. *Rev. sci. tech. Off. int. Epiz* **2009**, *28*, 113–136.
15. Arnal, A.; Vittecoq, M.; Pearce-Duvet, J.; Gauthier-Clerc, M.; Boulinier, T.; Jourdain, E. Laridae: A Neglected Reservoir That Could Play a Major Role in Avian Influenza Virus Epidemiological Dynamics. *Crit Rev Microbiol* 2015, *41*, 508–519.
16. Ramey, A.M.; Hill, N.J.; Deliberto, T.J.; Gibbs, S.E.J.; Hopkins, M.C.; Lang, A.S.; Poulson, R.L.; Prosser, D.J.; Sleeman, J.M.; Stallknecht, D.E.; et al. Highly Pathogenic Avian Influenza Is an Emerging Disease Threat to Wild Birds in North America. *Journal of Wildlife Management* **2022**, *2022*, 1–21, doi:10.1002/jwmg.22171.
17. Abolnik, C. A Current Review of Avian Influenza in Pigeons and Doves (Columbidae). *Vet Microbiol* 2014, *170*, 181–196.
18. Blagodatski, A.; Trutneva, K.; Glazova, O.; Mityaeva, O.; Shevkova, L.; Kegeles, E.; Onyanov, N.; Fede, K.; Maznina, A.; Khavina, E.; et al. Avian Influenza in Wild Birds and Poultry: Dissemination Pathways, Monitoring Methods, and Virus Ecology. *Pathogens* **2021**, *10*, 1–23, doi:10.3390/pathogens10050630.
19. Philippon, D.A.M.; Wu, P.; Cowling, B.J.; Lau, E.H.Y. Avian Influenza Human Infections at the Human-Animal Interface. *Journal of Infectious Diseases* **2020**, *222*, 528–537, doi:10.1093/infdis/jiaa105.
20. USDA 2022-2023 Detections of Highly Pathogenic Avian Influenza in Wild Birds. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022/2022-hpai-wild-birds.

21. Zhang, Y.; Aevermann, B.D.; Anderson, T.K.; Burke, D.F.; Dauphin, G.; Gu, Z.; He, S.; Kumar, S.; Larsen, C.N.; Lee, A.J.; et al. Influenza Research Database: An Integrated Bioinformatics Resource for Influenza Virus Research. *Nucleic Acids Res* **2017**, *45*, D466–D474, doi:10.1093/nar/gkw857.
22. Squires, R.B.; Noronha, J.; Hunt, V.; García-Sastre, A.; Macken, C.; Baumgarth, N.; Suarez, D.; Pickett, B.E.; Zhang, Y.; Larsen, C.N.; et al. Influenza Research Database: An Integrated Bioinformatics Resource for Influenza Research and Surveillance. *Influenza Other Respir Viruses* **2012**, *6*, 404–416, doi:10.1111/j.1750-2659.2011.00331.x.
23. Haddaway, N.R.; Collins, A.M.; Coughlin, D.; Kirk, S. The Role of Google Scholar in Evidence Reviews and Its Applicability to Grey Literature Searching. *PLoS One* **2015**, *10*, doi:10.1371/journal.pone.0138237.
24. Gusenbauer, M.; Haddaway, N.R. Which Academic Search Systems Are Suitable for Systematic Reviews or Meta-Analyses? Evaluating Retrieval Qualities of Google Scholar, PubMed, and 26 Other Resources. *Res Synth Methods* **2020**, *11*, 181–217, doi:10.1002/jrsm.1378.
25. Hnevolution, W.; Group, W. Toward a Unified Nomenclature System for Highly Pathogenic Avian Influenza Virus (H5N1). *Emerg Infect Dis* **2008**, *14*, e1, doi:10.3201/eid1407.071681.
26. Van Den Brand, J.M.A.; Verhagen, J.H.; Veldhuis Kroeze, E.J.B.; Van De Bildt, M.W.G.; Bodewes, R.; Herfst, S.; Richard, M.; Lexmond, P.; Bestebroer, T.M.; Fouchier, R.A.M.; et al. Wild Ducks Excrete Highly Pathogenic Avian Influenza Virus H5N8 (2014-2015) without Clinical or Pathological Evidence of Disease Article. *Emerg Microbes Infect* **2018**, *7*, doi:10.1038/s41426-018-0070-9.
27. Krauss, S.; Walker, D.; Webster, R.G. Influenza Virus Isolation. In *Influenza virus: methods in molecular biology*; Kawaoka, Y., Neumann, G., Eds.; Humana Press, 2012; Vol. 865.
28. Lee, C.-W.; Suarez, D.L. Application of Real-Time RT-PCR for the Quantitation and Competitive Replication Study of H5 and H7 Subtype Avian Influenza Virus. *J Virol* **2004**, *119*, 151–158.
29. Aydin, O.; Yassikaya, M.Y. Validity and Reliability Analysis of the PlotDigitizer Software Program for Data Extraction from Single-Case Graphs. *Perspect Behav Sci* **2022**, *45*, 239–257, doi:10.1007/s40614-021-00284-0.
30. Gorsich, E.E.; Webb, C.T.; Merton, A.A.; Hoeting, J.A.; Miller, R.S.; Farnsworth, M.L.; Swafford, S.R.; DeLiberto, T.J.; Pedersen, K.; Franklin, A.B.; et al. Continental-Scale Dynamics of Avian Influenza in U.S. Waterfowl Are Driven by Demography, Migration, and Temperature. *Ecological Applications* **2020**, *0*, 1–12, doi:10.1002/eap.2245.
31. Spackman, E.; Pantin-Jackwood, M.J.; Lee, S.A.; Prosser, D. The Pathogenesis of a 2022 North American Highly Pathogenic Clade 2.3.4.4b H5N1 Avian Influenza Virus in Mallards (*Anas Platyrhynchos*). *Avian Pathology* **2023**, 1–10, doi:10.1080/03079457.2023.2196258.
32. Brown, J.D.; Stallknecht, D.E.; Swayne, D.E. Experimental Infection of Swans and Geese with Highly Pathogenic Avian Influenza Virus (H5N1) of Asian Lineage. *Emerg Infect Dis* **2008**, *14*, 136–142, doi:10.3201/eid1401.070740.
33. Stephens, C.B.; Prosser, D.J.; Pantin-Jackwood, M.J.; Berlin, A.M.; Spackman, E. The Pathogenesis of H7 Highly Pathogenic Avian Influenza Viruses in Lesser Scaup (*Aythya Affinis*). *Avian Dis* **2019**, *63*, 230–234.

34. Van Den Brand, J.M.A.; Verhagen, J.H.; Veldhuis Kroeze, E.J.B.; Van De Bildt, M.W.G.; Bodewes, R.; Herfst, S.; Richard, M.; Lexmond, P.; Bestebroer, T.M.; Fouchier, R.A.M.; et al. Wild Ducks Excrete Highly Pathogenic Avian Influenza Virus H5N8 (2014-2015) without Clinical or Pathological Evidence of Disease. *Emerg Microbes Infect* **2018**, *7*, 7–67, doi:10.1038/s41426-018-0070-9.
35. Kent, C.M.; Ramy, A.M.; Ackerman, J.T.; Bahl, J.; Bevins, S.N.; Bowman, A.S.; Boyce, W.; Cardona, C.; Casazza, M.L.; Cline, T.D.; et al. Spatiotemporal Changes in Influenza A Virus Prevalence among Wild Waterfowl Inhabiting the Continental United States throughout the Annual Cycle. *Sci Rep* **2022**, *12*.
36. Kent, C.M.; Bevins, S.N.; Mullinax, J.M.; Sullivan, J.D.; Prosser, D.J. Waterfowl Show Spatiotemporal Trends in Influenza A H5 and H7 Infections but Limited Taxonomic Variation. *Ecological Applications* **2023**.
37. Dusek, R.J.; Hallgrimsson, G.T.; Ip, H.S.; Jónsson, J.E.; Sreevatsan, S.; Nashold, S.W.; TeSlaa, J.L.; Enomoto, S.; Halpin, R.A.; Lin, X.; et al. North Atlantic Migratory Bird Flyways Provide Routes for Intercontinental Movement of Avian Influenza Viruses. *PLoS One* **2014**, *9*, doi:10.1371/journal.pone.0092075.
38. Humphreys, J.M.; Ramey, A.M.; Douglas, D.C.; Mullinax, J.M.; Soos, C.; Link, P.; Walther, P.; Prosser, D.J. Waterfowl Occurrence and Residence Time as Indicators of H5 and H7 Avian Influenza in North American Poultry. *Sci Rep* **2020**, *10*, 1–16, doi:10.1038/s41598-020-59077-1.
39. Kirkeby, C.; Ward, M.P. A Review of Estimated Transmission Parameters for the Spread of Avian Influenza Viruses. *Transbound Emerg Dis* **2022**, *69*, 3238–3246, doi:10.1111/tbed.14675.
40. Spackman, E.; Prosser, D.J.; Pantin-Jackwood, M.J.; Berlin, A.M.; Stephens, C.B. The Pathogenesis of Clade 2.3.4.4 H5 Highly Pathogenic Avian Influenza Viruses in Ruddy Duck (Oxyura Jamaicensis) and Lesser Scaup (Aythya Affinis). *J Wildl Dis* **2017**, *53*, 832–842, doi:10.7589/2017-01-003.
41. Spackman, E.; Prosser, D.J.; Pantin-Jackwood, M.; Stephens, C.B.; Berlin, A.M. Clade 2.3.4.4 H5 North American Highly Pathogenic Avian Influenza Viruses Infect, but Do Not Cause Clinical Signs in, American Black Ducks (Anas Rubripes). *Avian Dis* **2019**, *63*, 366–370, doi:10.1637/11950-081418-ResNote.1.
42. Wille, M.; Robertson, G.J.; Whitney, H.; Bishop, M.A.; Runstadler, J.A.; Lang, A.S. Extensive Geographic Mosaicism in Avian Influenza Viruses from Gulls in the Northern Hemisphere. *PLoS One* **2011**, *6*, 1–10, doi:10.1371/journal.pone.0020664.
43. Hall, J.S.; TeSlaa, J.L.; Nashold, S.W.; Halpin, R.A.; Stockwell, T.; Wentworth, D.E.; Dugan, V.; Ip, H.S. Evolution of a Reassortant North American Gull Influenza Virus Lineage: Drift, Shift and Stability. *Virol J* **2013**, *10*, 1–9, doi:10.1186/1743-422X-10-179.
44. Lean, F.Z.X.; Falchieri, M.; Furman, N.; Tyler, G.; Robinson, C.; Holmes, P.; Reid, S.M.; Banyard, A.C.; Brown, I.H.; Man, C.; et al. Highly Pathogenic Avian Influenza Virus H5N1 Infection in Skua and Gulls in the United Kingdom, 2022. *Vet Pathol* **2023**, doi:10.1177/03009858231217224.
45. Bodewes, R.; Kuiken, T. Changing Role of Wild Birds in the Epidemiology of Avian Influenza A Viruses. In *Advances in Virus Research*; Kielian, M., Mettenleiter, T., Roossinck, M.J., Eds.; Academic Press, 2018; pp. 279–307 ISBN 9780128152010.
46. Fujimoto, Y.; Ogaswara, K.; Isoda, N.; Hatai, H.; Okuya, K.; Watanabe, Y.; Takada, A.; Sakoda, Y.; Saito, K.; Ozawa, M. Experimental and Natural Infections of White-Tailed

Sea Eagles (Haliaeetus Albicilla) with High Pathogenicity Aviancinfluenza Virus of H5 Subtype. *Front Microbiol* **2022**, *13*.

47. Brown, J.D.; Stallknecht, D.E.; Swayne, D.E. Experimental Infections of Herring Gulls (Larus Argentatus) with H5N1 Highly Pathogenic Avian Influenza Viruses by Intranasal Inoculation of Virus and Ingestion of Virus-Infected Chicken Meat. *Avian Pathology* **2008**, *37*, 393–397, doi:10.1080/03079450802216595.

48. Gonnerman, M.G.; Leyson, C.; Sullivan, J.D.; Pantin-Jackwood, M.J.; Mullinax, J.M.; Prosser, D.J. Database Collating Previous Laboratory Investigations into the Pathogenesis of Avian Influenza Viruses in Wild Avifauna of North America: U.S. Geological Survey Data Release. *https://doi.org/10.5066/P14MOUAF* 2024.

TABLES AND FIGURES

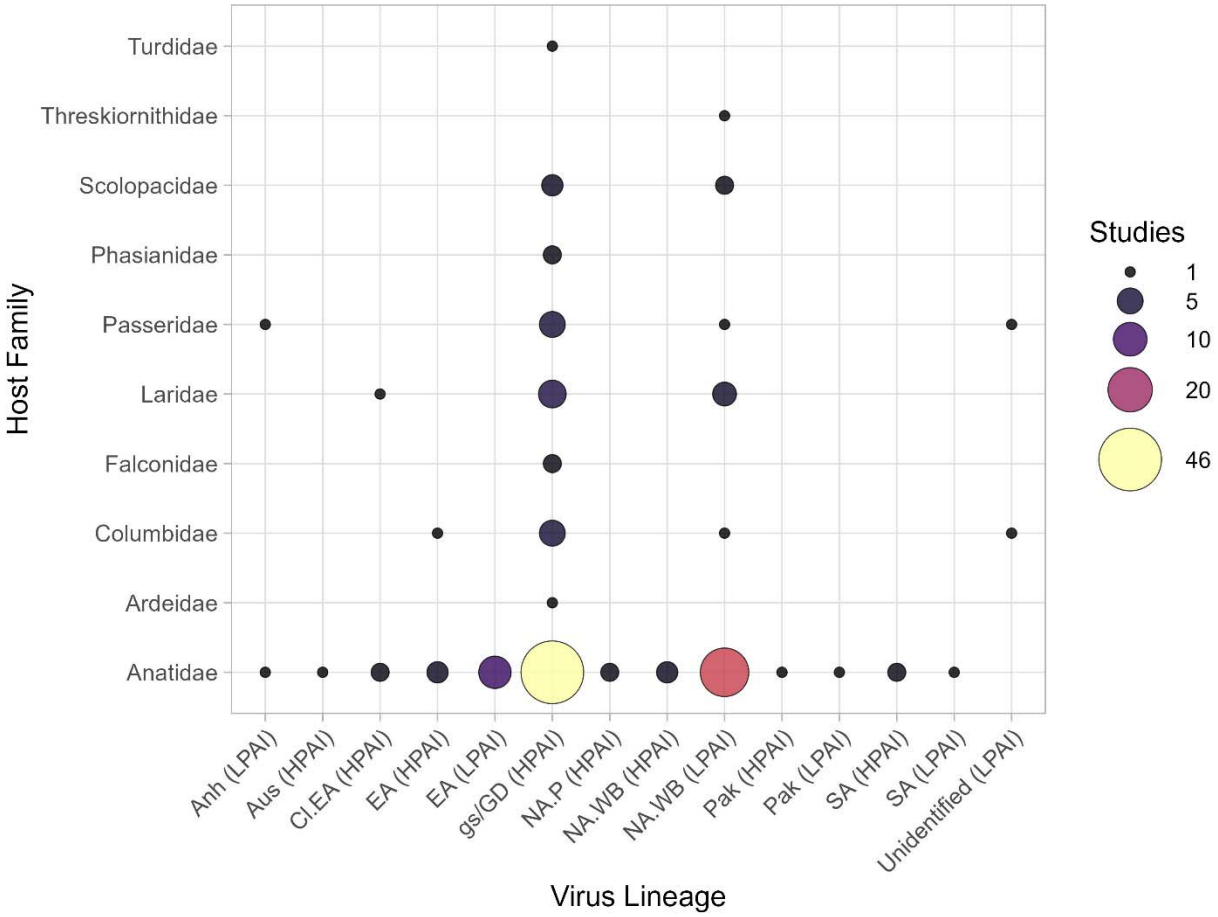

Figure 1. Bubble plot depicting the number of avian influenza virus challenge studies, grouped by host species family and viral lineage. Viral lineages identified include Anhui (Anh), Australian (Aus), Classical Eurasian (Cl.EA), Eurasian (EA), Gs/GD, North American poultry (NA.P), North American wild birds (NA.WB), Pakistan H7 (Pak), and South American (SA).

Table 1. Summary of infection rates for avian species directly inoculated with greater than 4 $\log_{10}$ EID_{50}/mL of Gs/GD HPAI virus, reported by host species. We report the number of studies identified (Studies), total number of individuals challenged (Sampled), the number removed according to study design (Culled), the total number and percentage of challenged individuals infected (Infection) and the total number of individuals that died after accounting for the number culled (Mortality).

					Infection			Mortality	
Family	Scientific	Common	Studies	Sampled	Total	Rate	Culled	Total	Rate
Anatidae	*Aix sponsa*	Wood Duck	3	41	39	0.95	0	29	0.71
Anatidae	*Anas acuta*	Northern Pintail	4	30	20	0.67	6	0	0.00
Anatidae	*Anas platyrhynchos*	Mallard	24	702	514	0.73	161	169	0.31
Anatidae	*Anas rubripes*	American Black Duck	1	16	16	1.00	0	0	0.00
Anatidae	*Aythya affinis*	Lesser Scaup	1	18	18	1.00	0	0	0.00
Anatidae	*Aythya americana*	Redhead	1	6	2	0.33	0	0	0.00
Anatidae	*Branta canadensis*	Canada Goose	1	20	20	1.00	0	20	1.00
Anatidae	*Branta hutchinsii*	Cackling Goose	1	4	3	0.75	0	3	0.75
Anatidae	*Cairina moschata*	Muscovy Duck	8	216	204	0.94	57	115	0.72
Anatidae	*Cygnus buccinator*	Trumpeter Swan	1	5	5	1.00	0	5	1.00
Anatidae	*Cygnus olor*	Mute Swan	2	7	7	1.00	0	7	1.00
Anatidae	*Melanitta perspicillata*	Surf Scoter	1	9	7	0.78	0	2	0.22
Anatidae	*Oxyura jamaicensis*	Ruddy Duck	1	15	14	0.93	0	4	0.27
Anatidae	*Spatula discors*	Blue-winged Teal	1	6	1	0.17	0	0	0.00
Ardeidae	*Nycticorax nycticorax*	Black-crowned Night Heron	1	3	1	0.33	0	1	0.33
Columbidae	*Columba livia*	Rock Pigeon	3	67	20	0.30	38	2	0.07
Falconidae	*Falco sparverius*	American Kestrel	2	8	8	1.00	0	8	1.00
Laridae	*Larus argentatus*	Herring Gull	2	42	42	1.00	0	28	0.67
Laridae	*Leucophaeus atricilla*	Laughing Gull	3	22	14	0.64	8	4	0.29
Passeridae	*Passer domesticus*	House Sparrow	4	36	28	0.78	0	21	0.58
Phasianidae	*Phasianus colchicus*	Ringed-neck pheasant	2	20	20	1.00	0	20	1.00
Scolopacidae	*Arenaria interpres*	Ruddy Turnstone	1	17	14	0.82	0	0	0.00
Scolopacidae	*Calidris alpina*	Dunlin	1	1	6	6.00	0	6	6.00
Turdidae	*Turdus migratorius*	American Robin	1	24	22	0.92	0	0	0.00

Table 2. Summary of viral shedding for avian species directly inoculated with greater than 4 $\log_{10}$ EID_{50}/mL of Gs/GD HPAI virus and reported as EID_{50}/mL or an EID_{50}/mL equivalent (eqEID). We report the number of studies identified, the number of unique species challenged, the duration in days and the viral shedding rates measured using virus isolation or RT-PCR methods. We report information separately for oropharyngeal (OP) and cloacal (CL) pathways.

				Viral Shedding (EID_{50}/mL)		Duration (Days)	
Family	Scientific	Common	Studies	Orpharyngeal	Cloacal	Oropharyngeal	Cloacal
Anatidae	*Aix sponsa*	Wood Duck	3	4.79 EID (4.43 - 5.14)	3.31 EID (2.8 - 3.8)	7.67 Days (6 - 10)	3.67 Day (3 - 5)
Anatidae	*Anas acuta*	Northern Pintail	3	2.22 EID (1.1 - 3.92)	1 EID	2 Days (0.5 - 4)	1 Days
Anatidae	*Anas platyrhynchos*	Mallard	21	4.78 EID (1.8 - 7.7)	3.52 EID (0.9 - 6.8)	6.42 Days (1 - 13)	6.03 Day (0.5 - 13)
Anatidae	*Anas rubripes*	American Black Duck	1	4.47 EID (4 - 5.1)	2.45 EID (2.3 - 2.6)	6.25 Days (3.5 - 9)	6.5 Days
Anatidae	*Aythya affinis*	Lesser Scaup	1	-	-	2 Days	2 Days
Anatidae	*Aythya americana*	Redhead	1	3.4 EID (2.8 - 4)	1.2 EID	4 Days	1 Days
Anatidae	*Branta hutchinsii*	Cackling Goose	1	5.25 EID	3.05 EID	5 Days	3 Days
Anatidae	*Cairina moschata*	Muscovy Duck	8	5.31 EID (2.5 - 6.8)	3.1 EID (2.3 - 4.6)	4.33 Days (3 - 8)	4.38 Day (3 - 7)
Anatidae	*Cygnus buccinator*	Trumpeter Swan	1	6.14 EID	3.18 EID	5 Days	4 Days
Anatidae	*Cygnus olor*	Mute Swan	1	5.58 EID	4.46 EID	5 Days	4 Days
Anatidae	*Melanitta perspicillata*	Surf Scoter	1	6.1 EID	2.9 EID	8 Days	8 Days
Anatidae	*Oxyura jamaicensis*	Ruddy Duck	1	-	-	2 Days	2 Days
Anatidae	*Spatula discors*	Blue-winged Teal	1	2.9 EID (2 - 3.8)	1 EID	2 Days	1 Days
Ardeidae	*Nycticorax nycticorax*	Black-crowned Night Heron	1	4.3 EID	2.5 EID	3.5 Days	3.5 Days
Columbidae	*Columba livia*	Rock Pigeon	2	2.15 EID	-	-	-

Laridae	*Larus argentatus*	Herring Gull	2	3.82 EID (3.74 - 3.89)	2.01 EID (1.96 - 2.07)	5.27 Days (4.3 - 6.5)	2.93 Day (2.3 - 3.5)
Laridae	*Leucophaeus atricilla*	Laughing Gull	3	3.48 EID (1.24 - 5)	1.86 EID (0.97 - 2.6)	9 Days (8 - 10)	6.5 Day (6 - 7)
Passeridae	*Passer domesticus*	House Sparrow	4	3.28 EID (2.6 - 4.7)	2.7 EID (1.3 - 4.1)	4.8 Days (4 - 6)	4.78 Day (3.95 - 6)
Phasianidae	*Phasianus colchicus*	Ringed-neck pheasant	2	-	-	6 Days (4 - 8)	6 Day (4 - 8)
Scolopacidae	*Calidris alpina*	Dunlin	1	3.54 EID	3.54 EID	4 Days	4 Days
Turdidae	*Turdus migratorius*	American Robin	1	-	-	3.83 Days (3.5 - 4.5)	-