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The epizootiology of avian influenza in wild birds and its risk to the UK poultry sector

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Publications

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A typical frosty start to mist netting and sample collection at Spurn.

19/11/2019.

1.1 Abstract

The understanding of avian influenza and its associated risk in the UK has changed considerably over the last five years, with new foci species being identified, and changes in our understanding of viral prevalence throughout a calendar year. Concurrent with this changing of understanding, this thesis explored and challenged the known framework of what species spread avian influenza and how can we best monitor and mitigate against overspill into the UK poultry sector using a systematic literature review alongside field sampling and use of citizen science repositories to create exposure risk models. This thesis has identified that by increasing sample sizes above detection thresholds, the vast majority of species sampled sufficiently have evidence of the viral presence of avian influenza. Anatidae have been widely sampled within the literature due to their historic association with bird flu, and their presence within datasets looking at the importance of environmental variables skew results due to their high recorded avian influenza prevalence rates. Building upon this understanding led to the development of an alternative monitoring approach to the UK's passive sampling method: active sampling via hunter-harvested waterfowl. Active sampling on a single site managed to confirm avian influenza in the UK before a national passive monitoring network, although this was discovered the following summer as testing was conducted retrospectively. The second half of the thesis focused on which species were present at two key habitats for avian influenza transmission, waterbodies, and poultry farms. Exposure risk models discovered that the family most sampled for avian influenza (Anatidae) were only recorded irregularly as a flyover to poultry holding sheds, though were universally present at Yorkshire's waterbodies. The bird species found most regularly at and near poultry farms were mostly generalist passerines. The species most common at poultry farms weren't the most common at waterbodies, but all bar one of the identified poultry farm target species were present across both key areas. This thesis presents an argument for wider sampling, a reflective look upon cost-effective monitoring techniques, and identifies the species representing the greatest exposure risk in wild bird communities at waterbodies and at Yorkshire poultry farms.

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Acronyms and common terms

Table 1 Key acronyms used across this thesis

Acroymn or term	**Description**
AIV	Avian Influenza Virus; sometimes shortened to AI (Avian Influenza) or IAV (Influenza A Virus). AIV is the chosen acronym for this thesis.
APHA	The Animal and Plant Health Agency, a department of the UK government that tackles zoonotic risk to the food industry.
ZEM	Zoonotic Exposure Model
AISES	Avian Influenza specific Exposure Score

Spurn Point, East Yorkshire. 16/08/2020

Chapter 1 : General Introduction to the Avian Influenza Virus and its context in wild and captive birds.

1.1 Background

1.1.1 Structure of the Virus

Avian Influenza virus, or bird flu virus is a group of influenza A viruses found predominantly in birds. AIV consist of eight negative-stranded RNA, which code for 11 proteins. Two glycoproteins, hemagglutinin and neuraminidase, are expressed on the surface of the virus and used to classify all influenza viruses. Within avifauna, 16 hemagglutinin and 9 neuraminidase antigenic subtypes have been detected and can be found in multiple combinations known as subtypes, for example, H5N1(Fouchier *et al.*, 2005). The main function of hemagglutinin is to allow for viral entry into cells. This is accomplished by binding to sialic acid receptors on the surface of a cell. There is specificity of hemagglutinin for different sialic acids which varies between influenza A virus subtypes. This contributes to the variety in host species and pathogenicity of AIVs(Fouchier *et al.*, 2005). Hemagglutinin is one of the reasons AIVs have been able to infect not only birds but other animal species including humans, sea mammals, horses and pigs. Some AIV subtypes (such as H5 and H7) are known to cause severe disease in humans and have pandemic potential (Claas *et al.*, 1998). Hemagglutinin is also important in its immunogenicity. The human immune response to AIVs is targeted to hemagglutinin proteins and vaccines are often designed with this in mind (Ward, 1981).

Like hemagglutinin, neuraminidase plays a critical role in the pathogenesis of the virus and is important in understanding and controlling the disease. The predominant purpose of neuraminidase is to facilitate the release of viral particles from infected cells by cleaving sialic acid residues on the surface of infected cells. This allows the virus to spread to neighbouring tissues, adding to the severity of the infection. In the case of AIV, neuraminidase enables the virus to overcome the natural defences of the host, including mucosal surfaces and innate immune mechanisms. Neuraminidase also plays an important role in the development of antiviral drugs,

such as oseltamivir (Tamiflu) and zanamivir (Relenza), which target the enzyme activity of neuraminidase. By inhibiting neuraminidase, these drugs can block the release of viral particles and reduce the severity and duration of influenza symptoms (Varghese, Laver and Colman, 1983)

In addition to hemagglutinin and neuraminidase, other viral proteins detailed in Table 2 are important to an AIV and contribute to its pathogenesis, transmission, and control.

Table 2 Important viral protein (not including hemagglutinin and neuraminidase) in the structure of an AIV

Viral Protein	Description
Matrix proteins (M1 and M2)	These proteins have an important role in viral assembly and release. M1 forms a structural shell around the viral genome, while M2 is a transmembrane protein that functions as an ion channel and facilitates the release of viral particles (Swayne, 2016).
Non-structural protein (NS1)	These proteins suppress the host immune response and plays a role in the pathogenesis of an AIV. NS1 stops the production of interferons, which are important antiviral molecules produced by infected cells (Krug, 2015).
Nucleoprotein (NP)	These bind to the viral RNA genome and forms a complex with other viral proteins to package the genome into viral particles. Nucleoproteins are a target for the host immune response and are a major component of influenza vaccines (Portela and Digard, 2002).

Polymerase proteins (PB1, PB2, and PA)	Form a complex that replicates and transcribes the viral RNA genome. They are needed for the replication and transcription of the virus and are targets for antiviral drugs (Bergervoet, 2021).
PB1-F2	A pro-apoptotic protein that contributes to the pathogenicity of an AIV. It induces cell death and may enhance the severity of the infection (Zamarin, Ortigoza and Palese, 2006).

These additional proteins to haemagglutinin and neuraminidase vary by strain, adding further complexity to understanding AIVs in the laboratory and a wild natural setting. They are especially important in the development of conservation efforts for wild birds, anthropogenic responses to AIV infections in poultry and the development of antiviral medication and associated measures in the case of a human outbreak.

AIV is classified by both viral pathogenicity in chickens and the characterisation of the amino acid sequence of the viral haemagglutinin cleavage site (Alexander, 2000). Two pathogenicity's are commonly referenced in the literature, high pathogenicity avian influenza virus (or HPAI) and low pathogenicity avian influenza virus (LPAI). An LPAI becomes an HPAI with the introduction of a basic amino acid residue to the haemagglutinin and allows for systematic viral replication. HPAI samples are mostly taken from poultry, with fewer studies looking at wild birds (Alexander, 2000). HPAI has 6 subtypes known to infect humans to date: H5N1, H7N3, H7N7, H7N9, and H9N2 with the H10N8 strain host switching most recently in 2013 [(Leong *et al.*, 2008). Whilst of significant concern to wild birds due to high observed mortality rates (varying by strain), the virus has historically recorded a high death rate in humans, and as such is monitored closely. Mammal-to-mammal transition is often the big step that warrants the most concerned response from epizootiologists. Clinically, HPAI shows higher morbidity and mortality rates in gallinaceous

poultry flocks than LPAI (which can be subclinical) due to rapid cell death in visceral organs, brain and skin (Swayne and Suarez, 2000).

1.1.2 The spread of AIV

AIV transmits through excretion during exhalation and defecation. It is most prevalent in infected animals in the intact cells of the gut lining, though this may vary by strain. The virus is passed on through exposure to fomite (Whitworth et al., 2007). The most prevalent method of spread seems to be via water sources, where fomite infects the surface water (Webster et al., 1992). Transmission of AIV in the poultry industry can be divided into spread via anthropogenic trade and through wild vectors. Transmission via the poultry trade is widely regarded as the dominant reason for the spread of disease within Asia. In Europe and North America, stricter biosecurity practices are in place, which limit this risk and suggest infection via wild birds has an increased role in spread(Butler, 2006).

Bird migration sees individuals travelling to sites all over the world to breed and often condensing in large flocks in the winter. Most bird migrations see species travel to the northern reaches of the Palearctic and Nearctic to breed, flying south to tropical or subtropical locations to winter. Migration routes are thought to be driven by food availability, which is in turn driven by temperature. Bird migration has been attributed to the spread of H5N8 in 2014 from Asia to Europe and North America (Lee *et al.*, 2015). Yang et al (2023) , showed that the geographical abundance of H5 clade 2.3.4.4 was linked to bird migration across several different families, highlighting the link between bird movement ecology and genomic epidemiology.

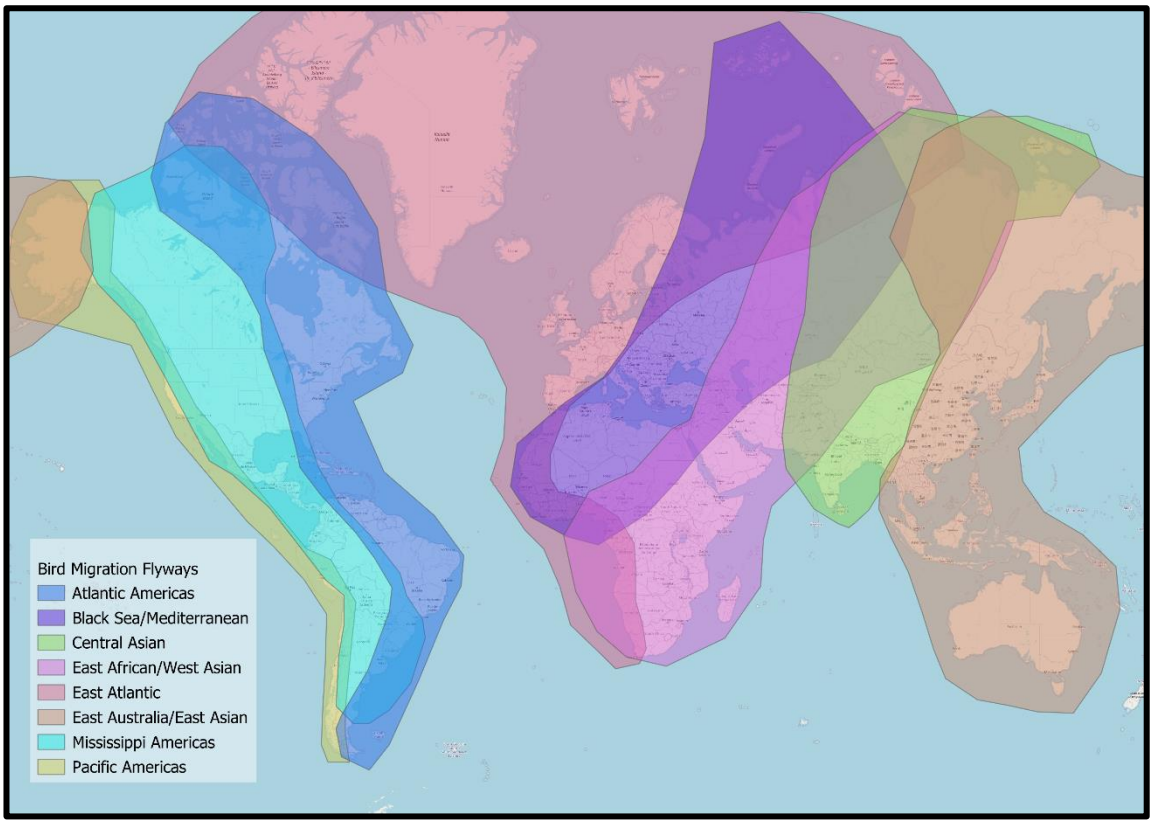

Figure 1 Map of the major bird migration flyways across the globe

Monitoring and research studies for AIV in wild birds often rely on samples from wild corpses. This strategy may create a sampling bias as larger birds such as waterfowl are often easier to find once deceased(Johnston *et al.*, 2014). Studies that sample living birds as carriers especially passerines and other smaller species are less prevalent in the literature, as AIV prevalence in living birds tends to be lower than those detected during the testing of corpses (Yasué *et al.*, 2006).

One of the first cases of HPAI in a wild migratory population not associated with the poultry industry was detected at Qinghai Lake in Western China, where over 6,000 birds (mainly Bar-headed geese, *Anser indicus*) were found dead in May 2005 (Chen *et al.*, 2005). This strain was isolated and tested on lab mice and chickens. All chickens died within 20 hours and 7 out of 8 mice within 72 hours. This study provided a evidence that wild bird populations spread AIV on a global scale and linked to AIV spread in the poultry industry (Liu *et al.*, 2005).

Wild bird migration success with pathogenic infection is poorly studied. A bird infected with a pathogen will likely expend more energy on an immune response, which may hamper migration

[24]. This might mean mortality on the route or shorter distances being covered. Waterfowl tend to make frequent stops at shorter distances along migration routes to feed up and retain body condition. This could lead to spread in more locations than nonstop migration (Weber and Stilianakis, 2007, Figuerola and Green, 2000). A 6-year study was carried out in Georgia (a country with 3 overlapping migration flyways) where over 30,000 samples were taken from various ducks and gulls to investigate the prevalence of AIVs over breeding, staging (migration rest spots) and moulting sites. 18 LPAIV strains were isolated as well as 2 HPAIV strains. Though varied across the strains, several strains were closely related to strains isolated in Eurasia and Africa, leading to the hypothesis that these birds are a facilitator in the AIV movement (Venkatesh *et al.*, 2018). The prevalence of AIV in this study varied between years in both ducks and gulls. Seasonality was also linked with autumn migration showing peak prevalence. In gulls, the detection was lower in seasons where breeding was lower, possibly linking juveniles to spread (Venkatesh *et al.*, 2018).

The age of an individual correlates with the strength of its immune response. A study on Mute Swans found that older birds were better equipped immunologically to AIV (H5N1) than juveniles (Hill *et al.*, 2016). Immunologically naïve juveniles could be key in the spread of the infection and hence years with bumper breeding seasons, could lead to higher risks of spread from wild birds. It is worth noting, however, that these could also be due to the fact more birds mean bigger flocks, higher densities and greater chances of distance movements.

1.1.3 AIV in the 2020's

Characteristically before 2020, when AIV outbreaks occurred in wild bird populations, they tended to last several weeks and although they can affect many birds in an outbreak, the events were sporadic anddid not cause extirpation of populations, only individuals. (Thomas, Hunter and Atkinson, 2007). In 2020, a significant global outbreak of AIV occurred causing widespread infection, detected across all continents with excepting to Antarctica (as of June 2023). In the UK, AIV outbreaks were widely regarded as a winter occurrence, but summer infection was detected in 2021 following the most widespread winter outbreak on record. In 2022, the winter outbreak season was worse than the prior and the following breeding season was heavily impacted, with

major seabird populations having significant AIV die-off events in globally important species(Cunningham *et al.*, 2022). Summer 2023 has seen reduced mortality in seabirds thus far, but outbreaks are now being seen to cause inflated mortality in Black-headed Gull (*Chroicocephalus ridibundus*) colonies, demonstrating viral evolution from the prior summer (Adlhoch *et al.*, 2023).

1.1.4 History of Infection

AIV was first discovered in 1880 (Perroncito, 1878) in Italy where it was known as 'fowl plague'. This description matches that of a HPAI outbreak, and further cases were discovered in 1894 and 1901. The 1901 case spread to multiple other European countries through the poultry trade. 'Fowl plague' or 'fowl cholera' became an epidemic in Italy and Central Europe until it seemingly faded out in the mid-1930s. By the mid-1900s the falsely named fowl plague had been registered in most of Europe, Russia, North America, South America, the Middle East, Africa and Asia(Stubbs, 1926,Wilkinson and Waterson, 1975).

LPAI was first recorded (albeit misidentified again) in 1949 in Germany when a deceased chicken was found with what turned out to be H10N7 (Dinter. Z, 1949, Dinter. Z, 1964,Rott and Schafer, 1960). Several other LPAI viruses were discovered globally in the years that followed demonstrating the complexity of the virus(Walker and Bannister, 1953, Koppel et al., 1956, Roberts, 1964, Tsimokh, 1961).It was originally considered that all H5 and H7 strains were HPAI, but this was debunked during the 1960s, with instances of LPAI H5 and H7 in turkeys, chickens, ducks, quails, pheasants and partridges(Easterday and Tumova, 1972, Smithies et al., 1969 , Beard and Helfer, 1972).

Studies looking at the roles of wild animals in human influenza pandemics came to the interest of the World Health Organisation and studies investigating avian influenza in wild birds started to become more common from as early as 1958 (Alexander, 1986). 10 years later serological studies of wild birds found avian influenza in the USA, Australia and Russia(Dasen and Laver, 1970, Slepuskin et al., 1972, Easterday et al., 1968, Winkler, Trainer and Easterday, 1972, Zakstel'skaja et

al., 1972, Lupiani and Reddy, 2009). The first wild outbreak of HPAI was recorded in 1961 in wild Common Terns (Sterna hirundo), where 1300 birds died as a result of H5N3 (Becker, 1966).

Since the Italian outbreak in the late 19th century, other outbreaks of HPAI have been recorded worldwide. The first officially recognized outbreak of HPAI was recorded in Scotland in 1959(Becker and Uys, 1966). During 1983 an LPAI strain of H5N2 circulated within chickens in Pennsylvania which late in the year mutated to an HPAI form. To bring this outbreak under control 17 million birds were eventually culled at an economic cost of $62 million (Eckoade and Silverman-Bachin, 1986, Fichtner, 1986). Other instances whereby LPAI outbreaks have mutated into HPAI outbreaks have occurred in Mexico (H5N2, Horimoto et al., 1995)), Italy (H7N1,Capua et al., 2002)) and Chile (H7N3,Rojas et al., 2002)). Another outbreak in Canada (H7N3,Bowes et al., 2004,Hirst et al., 2004) went from LPAI and HPAI in a matter of days after discovery. In the examples from Italy, Canada and Chile, culling was used to eradicate the virus, and in Italy, this was followed by vaccinations(Rojas et al., 2002,Capua et al., 2003). Contrastingly, in Mexico, depopulation was not used, only vaccination. Because of this, H5N2 in an LPAI form is still thought to circulate within Central America and now shows resistance to the original vaccine (Horimoto *et al.*, 1995). Further outbreaks of HPAI have been detected in Pakistan (H7N3, Naheem and Hussein, 1995and the Netherlands (H7N7, (Velkers et al., 2006, Gerritzen et al., 2006, Thomas et al., 2005,(Segeman et al., 2004)). Once again depopulation eradicated the virus in the Netherlands and surrounding countries and vaccination used in Pakistan led to associated strains being detected after the outbreak had officially ended.

As previously mentioned, in 2003, H5N1 emerged as a global threat. Initially spread through internal trade in the poultry industry, the first case of large-scale mortality was witnessed in wild migratory bird species in western China. This infection of migratory species such as the bar-headed goose (*Anser indicus*), great black-headed gull (*Larus ichthyaetus*), and brown-headed gull (*Larus brunnicephalus*) indicated that the disease could spread large distances via these migratory vectors(Liu *et al.*, 2005).

In the UK, infection events in 2017 from the poultry industry have been attributed to waterbodies and contact with resident Mallards. Infections from Lancashire and North Norfolk in May and June 2017 were assessed to be caused by direct interaction between the stock poultry and wild birds (APHA, 2017) with one outbreak location having farmyard geese sharing a pond with wild Mallards. These geese then shared roosting quarters with chickens. It is thought that the Mallards bred locally, but the disease was introduced by roaming males using nearby estuarine systems with more wide-ranging migrant waterfowl species. This report did not have pathological evidence from the wild birds but concludes it as the likely source, coupled with known instances of deceased wild birds found in the local area (DEFRA, 2017). In August 2018, beyond UK borders, AIV is being found across Europe, with outbreaks along the East Atlantic Flyway in poultry farms in Russia and a single case in Denmark. Autumn bird migration into the UK begins in late August and the risk of spread increases as birds flock to the UK's extensive estuarine habitats for the winter period (DEFRA, 2018).

1.1.5 Poultry farming in the UK

Poultry farming in the UK can be split into categories. Commercial methods can either be considered free-ranging, where individuals have a large interaction with the outside world or housed farming where individuals are kept indoors and separate from the natural environment(Wang *et al.*, 2014). It's logical to surmise that wild birds interact more regularly with poultry where poultry are kept in outdoor paddocks without barriers in place to reduce space sharing between both parties. It should be noted that poultry sheds, whilst reducing the contact between wild birds and poultry, may not strictly eliminate this risk. The UK follows good practice protocols which are used as an advisory measure(DEFRA and APHA, 2023). This involves the reduction of movement of people and equipment to areas where they are exposed to slurry or manure, sites where AIV can congregate. Where direct contact with livestock occurs, vehicles and protective clothing are advised to be disinfected after contact, often upon exits and entry to enclosures. It is recommended that food and water be provided inside to avoid contact with wild birds who might introduce AIV into the farm. Other measures such as rodent eradication are also advised (DEFRA, 2018).

Poultry housing orders are put in place at either national or localised scales when risk is measured as high to poultry from AIV. Monitoring and response to AIV is led by the UK's Animal and Plant Health Agency (APHA). During a confirmed AIV outbreak, a coordinated response is led by the APHA with landowners to cull infected and exposed poultry and investigate the source of the infection.

1.1.6 Which species are infected with AIV?

Wildfowl (Anseriformes; swans, geese and ducks) and shorebirds (Charadriiformes; gulls, terns and waders) are often labelled as the natural reservoir for AIVs (Webster et al., 1992) for several reasons. Firstly, on a behavioural level wildfowl and waders form tight wintering and moulting flocks outside of the breeding season allowing for proximity, which is linked to high levels of AIV transmission and exposure. Proximity is also found during the breeding season in colonially nesting gulls and terns. Furthermore, these families consist predominantly of migratory species and hence have the potential for spreading AIV over long distances. AIVs can persist in water sources, which are associated with high-risk habitats for the spread of AIV through the environmental persistence of the virus. Waterbodies form a major habitat requirement for many of the species in these 'high-risk' families. AIVs remain infectious for 4 days at 22C and 30 days at 0ºC meaning once an infectious bird has been at a water source, the water may act as a virus reservoir in the immediate future. AIVs have been found in high concentrations in bird faeces, so even flyover events could potentially spread disease(Webster *et al.*, 1978).

Migration and proximity social behaviours are often the identified behavioural traits responsible for AIV spread. Waterfowl and shorebirds are not the only bird groups that exhibit both these characteristics. In the UK, annual migration occurs yearly, with large numbers of non-waterfowl species that breed here wintering in Sub-Saharan Africa to the Mediterranean. Gulls are highlighted as a colonial breeder, but likewise, seabirds are famous for their colonial breeding habits, with the UK exhibiting internationally important populations (Lloyd, 1984). Similarly, some species will roost in large flocks outside of the breeding season such as Barn Swallows (*Hirundo rustica*) and White Wagtails (*Motacilla alba*). AIV detection in waterfowl and gulls has also led to the realisation

that these species though thoroughly representing some subtypes, are not adequately representing others. This leads to the belief further research is needed on the role of species outside these families in the spread of AIV (Krauss and Webster, 2010).

With over 10,000 species of birds recognized on an international level and 198 potential combinations of HA and NA, each of which might affect an individual differently due to several factors both intra and interspecifically, science will never have a complete understanding of AIV host dynamics. AIV research needs to be focussed on critical areas, such as species of conservation concern, and on wild species that interact with captive species and humans for both food supply biosecurity and human pandemic prevention.

1.1.7 Bird immigration into the UK and spatial monitoring

Waterfowl migrating into the UK come from a multitude of different locations. This even varies within species, with one of the most studied waterfowl concerning AIV, Mallard (*Anas platyrhynchos)* breeding within the UK but also has a boosted wintering population from breeding areas in northern Europe (Wallensten *et al.*, 2007). The world's migratory birds tend to broadly follow migratory flyways across the globe. The UK is part of the East Atlantic Flyway, which encompasses Arctic Canada across to Arctic Russia and down into Sub-Saharan Africa. In each of these three corners, many major flyways overlap leaving the scope of spread to the UK large, but hard to quantify for both the abundance of virus and for the introduction of new strains and clades (Guillemain *et al.*, 2017). Looking at foreign ringing recovery records from the British Trust for Ornithology (Robinson , Leech and Clark , 2018), it is possible to understand general trends in the direction of migration for different species and groups of species. It also highlights for some species, that different populations within the same species might use the UK at different times of the year and hence spread the virus widely purely at a species level. Most of the UK's wintering waterfowl and waders breed in the arctic and tundra regions of the northern hemisphere (mostly Eurasia, but also some in Iceland, Greenland and North America). With Anseriformes known to be key factors in AIV spread it would be logical to consider species with an overlap in habitat use as being other vectors in AIV spread for both sedentary and migratory species.

Many migratory species that breed in the UK, have a wintering range in Africa and southern Europe, with varying patterns of migration across the Mediterranean Sea. Most of these breeders are not species in traditionally associated high-risk families. However, the southern hemisphere area of the East Atlantic Flyway does have species from high-risk families that winter and then move through the UK on the way to arctic breeding groups including waterfowl and waders.

It is currently thought that AIV infection peaks occur for water birds just before autumn migration where immunologically naïve birds are gathered in large densities. In shorebirds, the peak is thought to occur in the period before spring migration (Krauss *et al.*, 2004). Largely speaking, outbreak periods tend to vary by species and their associated social behaviours. It is important and challenging to establish which species can be infected by which strains of AIVs and how these are associated with their behavioural and spatial ecology. Many challenges are present in the ability to study the movements of individual birds on an international scale. Not only are many species small in size and usually indistinguishable from other individuals of the same species, but they are also often short-lived and can live in extreme conditions. This leaves conundrums surrounding how best to fill the knowledge gaps in AIV occurrence in wild birds.

The earliest and still most widely used method of tracking birds scientifically involves marking and recapture. The technique is commonly referred to as bird ringing or banding and has been in use since the beginning of the 20th century. Bird ringing involves capturing live individual birds and attaching a uniquely coded metal ring to the leg. The bird is then released and hopefully recaptured elsewhere to provide a point-to-point trajectory. This is the cheapest form of data collection about mark/recapture in birds, but it has setbacks. The return rates for ringed birds are mostly low but vary by species, and some species are easier to capture than others. Geographical bias exists towards areas with higher human populations to register recaptures whether that be through reports from ringers, hunters or people who simply find dead birds. The long-term datasets are satisfactory for many species, but these tend to be for larger or more common species in the UK.

Gaps still exist and these longer trends may not be reflective of yearly changes for some species. However other techniques now exist to assist in collecting more data (Redfern and Clark, 2001).

In addition to the addition of a metal ring to the bird's leg, a colour ring combination or uniquely coded plastic ('darvic') ring can be added to the bird's leg. These unique combinations allow a higher incidence of recapture through re-sighting, adding ease to the data collection (Rock, 1999). This is a technique commonly used for waders and waterfowl whose legs are often easiest to re-sight. Another take on this is wing tagging birds with coloured and numbered tags and nasal saddles which apply to the beak. Though this increases re-sighting, there are still the issues of monitoring under-watched and remote areas.

Radio wave emitting devices can also be used to track birds without the need for visual resighting or recapture. These devices can be tracked with a radio wave (VHF) receiver to location. This technology was effective for the study of birds that return to the same sites year after year but, migration often covers distances too large to effectively be monitored outside of a single or a selection of sites via radio tracking and hence lacks full migration route detail. It was however the first step in the use of tracking technology in the study of bird migration. As technology progresses VHF tags now weigh less than 1g in some instances and can be used on much smaller species of birds (a general rule is a tag mustn't weigh more than 3% of a bird's weight. (Fuller et al., 2005, Whitworth. D et al., 2007). Satellite tracking is still a developing field in bird migration ecology, but as technology gets smaller and cheaper, the ability to study smaller and smaller species has increased. Satellite tracking devices differ in that some tags must be retrieved to download data and others can relay data directly to the scientist via satellite. These techniques can provide huge quantities of data for little-studied species. Platform Terminal Transmitter (or PTT for short) and Global Positioning System (or GPS) tags are now available for mostly larger bird species, but they are by far the most expensive method of tracking. They do however reveal a huge amount of data for each tagged individual. Transmitters tend to have a battery life or device life if they are solar powered, but in a few years, it can be possible to gather data on not only the migration direction

but stop-off points, length of stopover and habitat use analysis. Each of these could be key to detecting where AIV might originate from as it enters the UK (Whitworth. D *et al.*, 2007).

Ringing data for Common Cuckoos (*Cuculus canorus*) had previously shed little light on where British birds wintered on the African continent. In 2011, satellite trackers were fitted to Cuckoos to discover the whereabouts of the species wintering grounds. In that winter it was discovered that Cuckoos winter in sub-Saharan Africa, but mostly spending time in the Congo Basin. Not only this, it showed that different individuals took different migration strategies to cross the Mediterranean and Sahara, with most birds crossing the Mediterranean at the straights of Gibraltar but birds also taking direct routes from France and some passing through Italy. After collecting data for 3 years, the BTO were able to find that male birds taking the eastern route suffered higher mortality than those passing through the western route. Furthermore, these mortality rates are linked to declines in the local population (Hewson *et al.*, 2016). Satellite data can in just a few years, reveal a lot more data than the ringing scheme collected in nearly 80 years. The limitation at this point is that trackers are not small enough to be fitted to the smallest migratory birds, limiting our knowledge of migrations role in their long-term declines outside of the UK.

Using a similar technique, European Nightjars (*Caprimulgus europaeus*) were tracked into Africa and found to share similar stop-over routes to other European migrants in Northern, Central and Western Africa before wintering in small areas of the Democratic Republic of Congo (Evens *et al.*, 2017). Being able to isolate such specific sites on the species migration route helps us not only to conserve species but in looking at AIV, to isolate sites where species from different areas encounter one another and potentially spread the virus via migration.

A similar technique was the use of geolocators, another forefront of bird tracking data collection. These, instead of using satellites, store the data which can be downloaded when a bird is recaptured. This technique is used for birds with breeding or wintering site fidelity. In 2009, a study on European Nightingales (*Luscinia megarhynchos*) in the UK, fitted 20 birds with geolocators of which 7 were recaptured. From this, only 1 individual's device had worked but even in this we

could find out that the bird migrated through Spain and into Africa, down the Atlantic coast and wintered until mid-December in Guinea, the tag then failed. This shows the value of a single dataset as before this, only one or two ringing recoveries had been made along the highlighted area of the species migratory route (BTO, 2010). A satellite tracking study where bar-headed geese (*Anser indicus*) and ruddy shelduck (*Tadorna ferruginea*) were both tracked via satellite tags from capture at moulting sites in India to sites where AIV outbreaks in poultry and wild birds had been recorded in the past in southeast and central Asia (Gilbert *et al.*, 2011). Using ringing data, a French project investigated the spread of bird flu by Eurasian Teal (Anas crecca). They found that the spread of H5N1 AIV was unlikely to be efficiently spread by Teal along their migration flyway and that persistence in water bodies represents an important factor in contamination risk (Lebarbenchon *et al.*, 2009). The likely link between waterbodies and high bird density can begin to help isolate locations which may act as transmission sites. Within the UK, estuarine systems are often hotspots for bird density and diversity, especially for waterfowl and shorebirds, both linked with AIV spread in other studies.

A broader spatial scale discipline taken from the geochemistry field was that of Stable Isotope Analysis or SIA. With this method a feather or other biological sample can be taken from an individual and analysis can be performed on one of many elements' isotopic ratios. These ratios allow for studies focussing on individual life history such as studies on diet or the location of growth of a feather sample depending on the element used. Hydrogen 2 isotope ratio is unique to the location of the sample, the element sourced from the local ground water. This doesn't produce the same level of spatial resolution as satellite telemetry (metres vs. hundreds of kilometres in some instances), but it is a much cheaper method of collecting migration data (Whitworth. D *et al.*, 2007). Hobson and Wassenaar (1996) used 140 samples from 6 species of neotropical passerine breeding in North America to show isotopic ratios of δD in feathers were strongly correlated to that found in precipitation of the growing season. 64 feather samples of 5 migratory passerines gave δD results within the known breeding range of the species sampled linking them from the site of capture (in Guatemala) accurately to their breeding ranges.

Mapping is dependent on geographically specific differences in the isoscapes, allowing for differentiation between different sites of interest. Data affirmed baseline maps are available (from IAEA/WMO, 2011) for areas in the Northern Hemisphere but are often lacking for those in the Southern Hemisphere (i.e., Africa, (Seifert et al., 2018,Hobson et al., 2012)).

Using multiple elements, it may be possible to create more accurate isoscapes (Seifert *et al.*, 2018). Carbon 13 can be used to establish a diet as it varies between plants using C3 and C4 photosynthetic pathways. Due to the diverse nature of floral landscapes, you can map areas by floral diversity within the isoscape (Ehleringer, 1989, Still and Powell, 2010). Nitrogen 15 ratios are found in plants (and soils) and vary between xeric and/or cultivated regions which are typically rich in N15 and mesic or uncultivated areas which are typically low in N15(Pardo and Nadelhoffer, 2010, Craine et al., 2009).

It is important to understand the biology of the species sampled. Moult is the complex strategy by which birds replace their feathers. These strategies vary in each species. Juveniles may retain feathers grown at the site of birth and adults may moult feathers near their breeding sites (Jenni and Winkler, 1994). With these strategies, it may be possible to establish these sites for infected individuals (Wunder, 2010). We know that both these occasions can lead to high densities of waterfowl which are a known carrier of AIV and considered the reservoir for the virus.

Over the past couple of decades, inter-country collaboration with the use of radar data has made it possible to use the platform to study bird migration. At this stage, it is not possible to study individuals, but it may be possible to track the biomass of migrating species into and out of countries. Bird migration happens over such a large scale, that this field is reliant on collaborative approaches to be of use to ornithologists (Kelly and Horton, 2016, Chilson, Stepanian and Kelly, 2017).

Concerning AIV, radar data may be able to identify flocks or individuals to a size level(Mirkovic *et al.*, 2016) and hence it may be possible to detect important families such as Anatidae. It may further

be possible to infer species by collaborating radar data with citizen science data from eBird or Birdtrack for these areas. It may also be possible to use visual observers or audio captures to help the proofing process. A combined approach looking at migrants caught and sampled, then stable isotope analysis plus radar data, could potentially fill in gaps along migration pathways.

In summary, a dynamic approach using multiple tracking methods is likely the best method to trace an interesting individual or species. As technology becomes more small-scale and accessible, it will be possible to know more about species movements and detections within landscapes at both a relatively small scale and over international borders. In the meantime, a mix of budget and low returns on investment (i.e., few birds have AIV, and at present there isn't a test that can tell if a species is AIV positive quick enough to place a tracking device on a known positive individual) it is likely to leave a reliance on cheaper methods to increase sample size, as project budgets are likely to be taken up by costly AIV testing protocols.

1.1.8 AIV Field sampling methods

Field sampling for AIV will vary depending on the purpose of sampling, the bird species or families being sampled, and the specific requirements of the laboratory protocols. Cloacal swabs involve collecting swabs for live virus detection from the cloaca (the opening through which birds excrete waste) of live or dead birds. This is a common method for surveillance of avian influenza in domestic poultry (Das *et al.*, 2008). The other most common forms of sampling focus on the other end of the digestive system. Oropharyngeal swabs involve collecting samples from the back of the throat of live birds. Studies conducting sampling via cloacal and oropharyngeal swabbing report variance in detection between both methods (even in swabs taken from the same individual). This is due to variance in how an infection manifests in individual birds, with the virus congregating in different organs and systems (Nuradji *et al.*, 2015). Tracheal swabs involve collecting samples from the trachea of live birds. Unlike oropharyngeal methods, this technique is more invasive to the sampled bird as the swab must be inserted deeper into a bird's throat (Cattoli *et al.*, 2004). From corpses, it is possible to detect AIV by testing tissue samples obtained from cadavers (Mo *et al.*, 1997). The final method of detecting live AIV in a bird is through faecal sampling from deposited

fomite which is a non-invasive method (Hood *et al.*, 2020). It is possible to detect evidence of infection, both present and historical, of an individual by testing samples for the presence of antibodies for AIV. Antibodies are present in blood samples which can be obtained via venepuncture of live birds, or collection from corpses (Joannis *et al.*, 2018).

Authorisation associated with sampling for AIV varies by country, with specialist training and licensing required to take samples that are considered invasive (cloacal, oropharyngeal, tracheal and blood) from live birds. In the UK, rules associated with this are defined by the Animals Scientific Procedures Act (or ASPA, 1986).

1.1.9 Laboratory Analysis

Once a sample has been collected, it is usually analysed in one of the following laboratory protocol methods (see Table 3 below).

Table 3 Laboratory analysis methods used in the detection and identification of AIV from collected samples.

Laboratory method	Description
Real-time polymerase chain reaction (RT-PCR)	A highly sensitive and specific molecular test that detects the genetic material of the virus in a sample. It is the most common test used for the detection of avian influenza in clinical samples, such as swabs or tissues (Das *et al.*, 2008).
Virus isolation	This involves the growth of the virus in cell cultures or embryonated eggs. It can help identify the specific strain of the avian influenza virus and is often used for further characterization of the virus (Woolcock, 2008).
Hemagglutination inhibition (HI) assay	This test measures the level of antibodies against the virus in a blood sample. It is used

	to determine if a bird has been exposed to the virus or has been vaccinated against it (Killian, 2008).
Enzyme-linked immunosorbent assay (ELISA)	This test measures the level of antibodies against the virus in a blood sample. It is used to determine if a bird has been exposed to the virus or has been vaccinated against it(Song *et al.*, 2009) .
DNA/RNA Sequencing	This is used to determine the genetic sequence of the virus. It can help identify the specific subtype and strain of the virus and can be used for further research and surveillance (Pasick, 2008).

1.1.10 Modelling and Epidemiological Analysis

Once viral presence is acknowledged and levels of prevalence established, it is important for those researching AIV to understand the outbreak and its potential impacts on a local, national, or international scale. Simulation modelling using statistical equations to simulate the spread of AIV in a given population, is used to help predict the potential impacts of an outbreak and evaluate the effectiveness of different control strategies. There is limitations in the number of factors that can be accounted for using these methods(Marzinek, Huber and Bond, 2020). Simulation models can be constructed with a combination of other data to help refine the scenarios being modelled. It is possible by analysing the distribution of AIV outbreaks and identifying geographic patterns of transmission to identify high-risk areas and inform targeted control and mitigation measures (Zhang *et al.*, 2015).

Phylogenetic analysis involves comparing the genetic sequences of AIVs to determine their evolutionary relationships. This helps to identify the origin of outbreaks by identifying the

relationships between different strains, tracking the spread of the AIV and identifying genetic changes that may affect the virus's virulence or transmissibility (Karamendin *et al.*, 2011).

Using a combination of the methods detailed, it is possible to conduct risk factor analysis. This can involve mapping and identifying factors that increase the risk of AIV transmission or spread, such as contact with infected birds, locations of phylogenetically related samples or exposure to contaminated environments. Risk modelling helps inform and guide development of targeted control measures and risk communication strategies (Mounts *et al.*, 1999).

1.1.11 Risk Assessment and Management

Monitoring for AIVs in wild birds is crucial for early detection and surveillance of potential outbreaks. Ongoing surveillance of collected samples of both captive and wild birds is used to monitor the incidence and distribution of AIV cases. The UK currently has a monitoring strategy with flexible thresholds for when the collection of corpses for wild birds for testing is carried out. This varies by family or species depending on their perceived risk to the poultry industry or there known AIV prevalence estimates at a population level. Surveillance monitoring can help identify trends in the spread of the virus and inform control measures on a close to real-time scale as is possible with current techniques (Hansbro *et al.*, 2010). At present the monitoring of wild birds is reliant upon citizen call-ins upon finding wild bird carcasses and the APHA responds where appropriate to collect the corpse for AIV testing.

Additionally, several international protocols and programmes exist to monitor AI in wild bird populations (see Table 4).

Table 4 Major international protocols regarding the monitoring and response to AIV

Governance and Protocol bodies	**Description**
OIE Terrestrial Animal Health Code	The World Organization for Animal Health (OIE) has guidelines which provide a framework for member countries to establish surveillance programs and reporting mechanisms. These guidelines include recommendations on sampling methods, laboratory testing, and reporting (World Organisation for Animal Health (OIE), 2023).
FAO/OIE/WHO Tripartite	The Food and Agriculture Organization (FAO), OIE, and the World Health Organization (WHO) collaborate on a global level to tackle zoonotic diseases, including AIV. They have developed joint recommendations for the monitoring and surveillance of AIV in captive and wild bird populations. These recommendations emphasize the need for coordination between animal and public health sectors (World Health Organization (WHO), 2019).
European Union (EU) Wild Bird AI Monitoring	The EU has established a monitoring program for avian influenza in wild birds to enhance early detection and control of the disease. The program involves regular surveillance of targeted wild bird populations, including waterbirds, gulls, and raptors. It emphasizes the sampling of both live and dead birds, with

	a focus on high-risk species and locations (Aznar *et al.*, 2021).
Surveillance Plan for High Pathogenic Avian Influenza in Wild Migratory Birds in the United States	The Surveillance Plan for High Pathogenic Avian Influenza in Wild Migratory Birds in the United States is a scheme to monitor and manage waterfowl populations, including those that migrate across international borders. The system includes monitoring for avian influenza in waterfowl through sampling of live birds, hunter-harvested birds, and environmental samples (United States Interagency Working Group, 2017).

While these protocols share the common goal of monitoring AIV in wild birds, there are some differences in their approaches, target species, and geographical scopes. The OIE Terrestrial Animal Health Code and the FAO/OIE/WHO Tripartite recommendations provide more comprehensive and global guidelines, whilst, the EU, and the North American Waterfowl Flyway System focus on specific regions or bird populations. The protocols also differ in their emphasis on live bird sampling versus sampling of dead birds or environmental samples.

1.1.12 AIV outbreak response in the UK

The threats to humans from AIV are assessed based on several factors, including the prevalence and distribution of the virus in domestic and wild bird populations, the risk of transmission to humans, and the potential impact on animal welfare and the economy.

With some levels of variance due to the individuality of AIV outbreaks in poultry, the following protocol tends to be followed to deal with an outbreak in the UK (APHA, 2023d).

1. Detection and Confirmation:

The outbreak of Avian Influenza in a poultry farm is typically detected through routine surveillance, farmer reporting, or clinical signs observed by the farm personnel or veterinarians.

The farm owner or manager should report any suspicion or confirmation of AIV to the Animal and Plant Health Agency (APHA) or the local veterinary authority.

2. Biosecurity Measures:

Upon detection, strict biosecurity measures are implemented to prevent the spread of the disease. This includes limiting access to the infected premises, establishing restriction zones, and implementing movement restrictions on poultry and poultry products.

Enhanced biosecurity measures are enforced on surrounding farms to minimize the risk of disease transmission.

3. Veterinary Investigation and Diagnosis:

Veterinary authorities investigate to determine the severity and strain of the AIV. This involves collecting samples from affected birds and sending them to a laboratory for testing and confirmation.

4. Culling and Disposal:

If the presence of AIV is confirmed, a culling process is initiated. The culling can involve depopulation of the infected premises and, in some cases, culling of birds in neighbouring farms to prevent the spread of the disease.

The carcasses of the culled birds are disposed of by biosecurity and environmental regulations. Common methods include incineration, landfill, or rendering.

5. Movement Restrictions and Surveillance:

Movement restrictions are imposed on poultry, eggs, and other potentially contaminated materials within specified control zones. This helps prevent the further spread of the disease.
Surveillance activities are intensified in the surrounding areas to monitor the extent of the outbreak and identify any additional infected premises.

6. Cleaning and Disinfection:

Following culling and disposal, the infected premises and any associated equipment are thoroughly cleaned and disinfected according to established protocols. The process aims to eliminate the virus and ensure that the premises are safe for future use.

7. Risk Communication and Public Awareness:

The relevant authorities, such as DEFRA and the APHA, provide regular updates and information to the public, farmers, and stakeholders regarding the outbreak, control measures, and any changes in the situation.

Public awareness campaigns are conducted to educate poultry farmers, workers, and the general public about the importance of biosecurity measures and early reporting of suspected cases.

Costs associated with an outbreak are largely taken up by the government, though poultry farmers are invariably impacted by loss of trade and will incur costs associated with the outbreak, though compensation is widely available. Insurance premiums are likely to increase as the AIV outbreak becomes more prevalent at poultry holdings.

During high alert periods, poultry owners are legally instructed to house all free-roaming birds to reduce the risk of AIV infection, these rules can be put in place for long periods, impacting bird welfare, and removing the free-range status' of poultry produce (*Animal Health Act* , 1981).

1.2 Scope and Significance

1.2.1 Why is this research important?

AIVs, as of September 2023, are one of the most important avenues of research when considering zoonotic pandemic potential. In the aftermath of the COVID-19 pandemic, AIVs have begun to overspill into humans from their bird reservoirs(Wille and Barr, 2022). Human-to-human transmission with death rates historically high (Yang *et al.*, 2007) would have major impacts on both world health and the economy with a likely reduction in quality of life worldwide including lockdowns and a vaccination race against a quickly evolving virus.

This is without forgetting that at present, AIV is causing significant die-offs in internationally important bird populations (Falchieri *et al.*, 2022), especially noting seabirds, which are already under the pressure of overfishing, climate change and their associated impacts such as fish net entanglement and phenological mismatch (Gibson *et al.*, 2022).

1.2.2 What will this research contribute to the field?

The following research chapters will look to produce an international dataset of AIV samples, that can be utilised in meta-analysis and systematic reviews to answer questions, some of which are investigated in Chapter 2 of this thesis. The dataset will include lists of species and families and the infection rates within the sampled populations. It will also look at the trends and associations represented within the dataset for factors such as habitat choice, inter and intra-familial variance and bird behaviours. Previous systematic reviews tended to take an approach of analysis at an order or family level, but it is hoped that analysis at a species level will expand upon the complexities of AIVs in wild birds. The literature review presented here has identified several issues with sampling bias in wild species which requires addressing systematically. Chapter 2 acts as the international

scale context for AIV in wild birds, identifying sampling and AIV prevalence trends in wild species and assisting the following chapters by suggesting focus families and species that require further research interest.

Chapter 3 begins the UK focus of the thesis and looks at the immigration of AIVs into the UK via wild bird migration during the autumn period. Whilst sampling to investigate which species are present during migration and their associated direction of immigration, it may be possible to determine infection in less studied species. The chapter's main findings also include a comparison of sampling methods, with an interest in cost-effective methods of monitoring AIV within UK shores. Sampling results help to add data and commentary to the findings of the systematic review in Chapter 2, demonstrating both agreed and contrasting findings.

Chapter 4 takes a backwards first approach to AIV spread to poultry from wild birds, by investigating which species of wild bird are present at poultry sites and where within these sites do each species use. By doing this and factoring in data collected from the systematic review in Chapter 2, basic and more complex models can be compared to help indicate which species present the greatest exposure risk to poultry farms in the UK. The risk modelling approach factoring in the data from chapter 2, investigates whether at this stage, more simplistic models are most relevant for use, or whether enough information on AIV prevalence estimates exists for present species to be able to factor in this important variable.

The final research chapter (5) takes the data from the models in Chapter 4 and the systematic review in Chapter 2 and adds in citizen science data (from eBird) to investigate if the utility of citizen science can be widely applicable to show habitat sharing habits between high-risk species and families such as Anatidae, with identified species from chapter 4 that present the greatest exposure risk at the UK's poultry sheds.

1.3 Outline of Thesis

The overall aim of this thesis is to identify avenues to reduce risk to the UK's poultry sector from wild birds in a way that is sustainable and reflective of the conservation status of the wild bird

species involved. Techniques used include literature review, virus sampling, citizen science and a variety of statistical models.

It is hoped this thesis will add an ecological context to known virological science and question how much we know about wild birds when it comes to disease movement outside a laboratory environment. The following chapters, research questions and objectives have been identified to form the research elements of this thesis (see Figure 2):

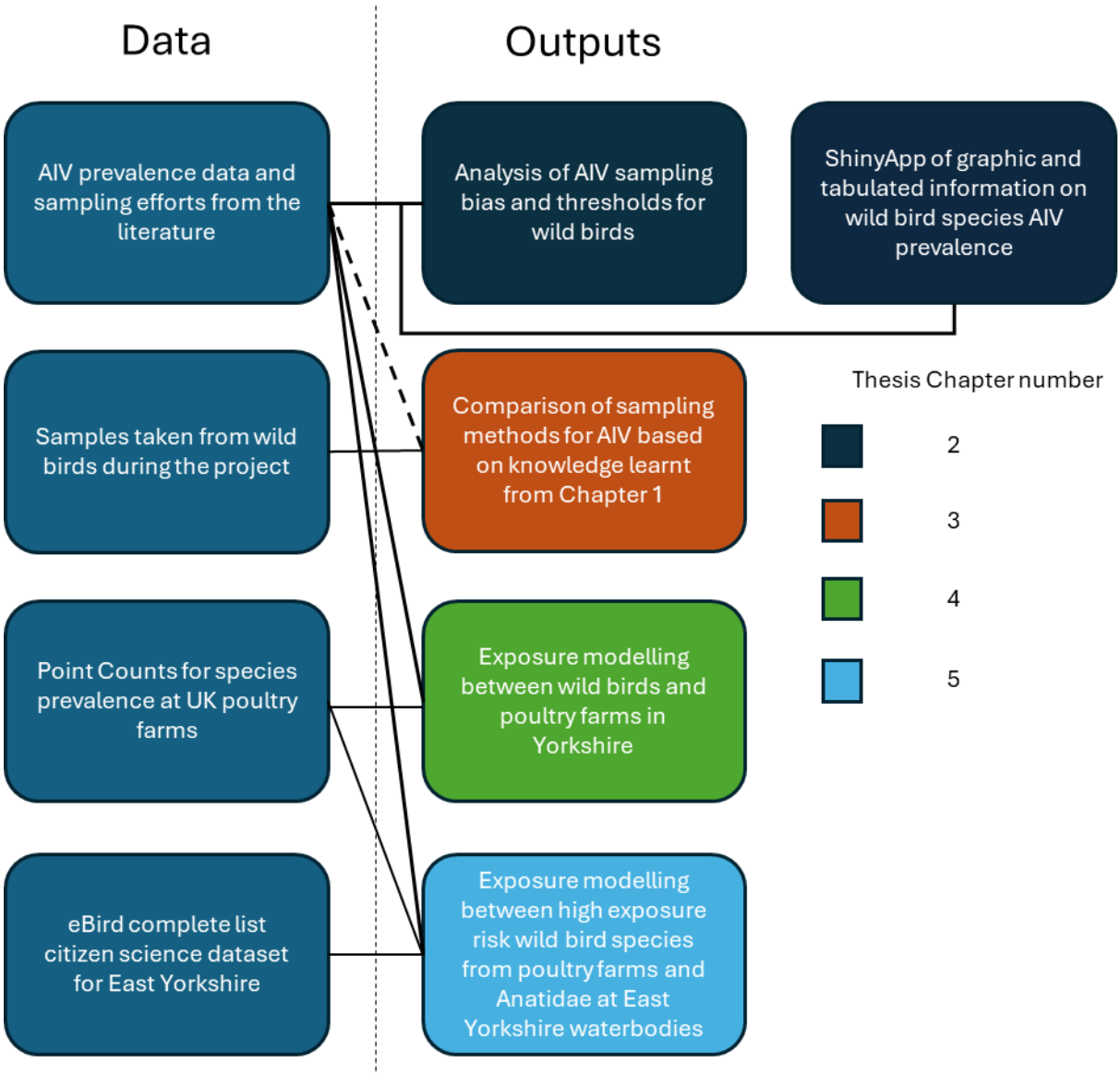

Figure 2: Conceptual Model of Data Inputs and proposed outputs for this thesis. Dashed Line for structural contributions, undashed line for data contributions

Each of the following research chapters looks to address the following questions:

Chapter 2: 'Systematic bias in wild bird sampling for influenza A virus limits understanding of avian influenza epidemiology'.

This chapter was designed as a systematic literature review to answer the following questions:

1. Identify bias in sampling effort for AIV sampling in wild birds, focussing on inter and intra-familial variation, behaviour, and sampling location.
2. Are any sampling biases warranted by their prevalence of positive AIV samples?
3. What are the effects of sample size and subsequent thresholds for inclusion in analysis on the significance of variables associated with sampling bias and AIV prevalence?
4. Is it possible to identify any species or families that could warrant future research focus relating to their AIV prevalence levels?

Chapter 3: 'Bird migration and AIV. High pathogenicity avian influenza: Targeted active surveillance of wild birds to enable early detection of emerging disease threats.'

By sampling for AIV in wild birds, this chapter aims to investigate the following question:

1. Which species and families have detectable levels of live AIV virus in their samples during autumn migration in East Yorkshire?
2. Do these species match the species and families highlighted in the systematic review (Chapter 2)?
3. How do active sampling methods compare to passive sampling methods currently used by the UK government?

Chapter 4: 'Wild birds at poultry farms: are species assemblages reflective of current AIV understanding?'

A wild bird point count methodology was conducted at poultry farms across Yorkshire and modelled to answer the following questions:

1. Identify which species are most prevalent at poultry farms and assess their potential for AIV exposure to captive poultry.
2. Identify if it is possible to attribute risk based on species presence and AIV prevalence estimates.
3. Is it possible to identify species for future AIV sampling efforts and movement studies in the context of how AIV moves amongst the landscape through avian vectors?
4. Can using qualitative observations add value to quantitative risk model estimates?

Chapter 5: 'Assessing the utility of citizen science in modelling avian influenza transmission networks at wetland sites'.

Using a mixture of data obtained across the prior three chapters and using the eBird citizen science repository for East Yorkshire, modelling was performed to calculate the following:

1. Are identified poultry exposure species (identified in Chapter 4) present at waterbodies?
2. Are poultry exposure species present at waterbodies at the same time as different Anatidae species?
3. Are any priority species co-occurrences particularly notable as potential links between wetlands and poultry farms?

Chapter 2 : Systematic bias in wild bird sampling for influenza A virus limits understanding of avian influenza epidemiology

2.1 Introduction

As zoonotic viruses become an increasing focus of research efforts due to the emergence of severe acute respiratory syndrome coronavirus 1 and 2, a diverse range of scientific foci has shifted reactionarily to research how to tackle outbreaks and prepare for the future effectively. Influenza A viruses (herein AIV) are another zoonotic pathogen of note, with a history of detection in humans and livestock in anthropocentric food chains. Since 2020, AIV has become increasingly prevalent worldwide being detected year-round in wild and captive birds (Michelle Wille and Barr, 2022).

AIVs are viruses found within the family Orthomyxoviridae. They are subsequently characterized into subtypes based on their haemagglutinin (HA; 16 types identified) and neuraminidase antigens (NA; 9 types identified (Fouchier et al., 2005, Olsen et al., 2006, Webster et al., 1992)). AIVs are categorized into two classes based on the symptoms of infection in gallinaceous poultry, high pathogenicity avian influenza (HPAI) and low pathogenicity avian influenza (LPAI). Whilst LPAIs demonstrate typically low-impact symptoms varying from subclinical infection to a reduction in egg production and low levels of mortality, HPAIs can cause high levels of morbidity and mortality within a population (Gonzales and Elbers, 2018). The definitions of HPAI and LPAI are not uniform to the symptoms demonstrated by different bird species, with each demonstrating varying clinical reactions to infection (Perkins, E and Swayne, 2003).

AIVs are transmitted via virions excreted via faeces, exhaled breath, and sputum by infected birds to which other individuals become exposed, and the cycle of transmission continues. An infected bird can emit virions over a period of 6-10 days in high quantities (Webster et al., 1992,Stallknect et al., 1990). There has been a particular focus on the persistence of AIV in waterbodies as Anseriformes (ducks, swans, and geese) and Charadriiformes (waders and gulls), both water-associated groups are notably affected by AIV. AIVs can persist in the environment and modelling attempts have shown that environmental persistence may play a significant role in the spread and maintenance of AIVs in an ecosystem (Stallknect et al., 1990, Brown et al., 2009). Within

waterbodies, AIV has been shown to persist for 100 days at 28 ºC and 200 days at 17 ºC (Stallknect *et al.*, 1990).

Within the majority of the literature, AIVs have been closely associated with Anseriformes, with the highest rates of infection and number of strains detected in ducks, geese, and swans (Stallknecht and Shane, 1988, Torrontegi et al., 2019). Charadriiformes (waders and gulls) are considered the second most likely candidates for transmission and maintenance due to the high level of spatiotemporal overlap with Anseriformes and the diversity of detected viruses (Olsen *et al.*, 2006). However, infection prevalence in Charadriiformes has been comparable to that found in many other bird families not traditionally associated with AIV infection in previous studies (Caron, Cappelle and Gaidet, 2017).Hanson et al., (2008) demonstrated that in North America, there was variation in the prevalence of AIVs in wading birds between different species and different sites, with Ruddy Turnstone (*Arenaria interpres*) at Delaware Bay, showing a significantly higher rate of infection than other waders at other sites. Delaware Bay represents a key location in Ruddy Turnstone (and other waterbird) migration in North America, but similar sites exist across the world including WTP waste-stabilization ponds (a RAMSAR site) in Australia (Ferenzci *et al.*, 2016), which with a comparatively high prevalence of AIV have been detected for Australasia, this is still low by comparison to Delaware Bay.

Spatial patterns in avian influenza prevalence are not solely found in Charadriiformes and many projects have demonstrated similar in other families and orders. Traditionally, AIV peaks in the northern hemisphere during autumnal bird migration (Xu *et al.*, 2016). From September to December, large numbers of immunologically naïve juvenile birds travel across traditional flyways between breeding to non-breeding sites (Hoye, Fouchier and Klaassen, 2012). Van Dijk et al., (2014)[showed that juvenile birds demonstrated higher levels of AIV than adults and that timings in Mallard (*Anas platyrhynchos*) migration were linked to increases in prevalence of viral detection. Research has varied on how often AIV is found during the summer months in the temperate northern hemisphere, but evidence of summer persistence has been found worldwide (Gronesova et al, 2008, Hénaux et al., 2012). In 2022, a global summer outbreak of H5N1 AIV was witnessed, with seabirds (namely northern gannet, *Morus bassanus*, great skua, *Stercorarius skua*, and tern species)

being particularly impacted to the point of causing conservation concern (Cunningham *et al.*, 2022). AIV has been detected on every continent, but traditionally the higher rates of detection are found in wild birds on flyways associated with the northern hemisphere across Europe, North America and Asia (Caron, Cappelle and Gaidet, 2017).

Although there is a clear focus towards Anseriformes in the literature, research has been carried out on non-Anatidae, and non-Charadriiform families. AIV sampling has been carried out on every continent, and on hundreds of different families to increase our understanding of avian influenza epidemiology, but with the great diversity of the class Aves, full taxonomic coverage has yet to be achieved (Machalaba *et al.*, 2015).

Migratory species have been linked to AIV spread for a long time, with the first wild outbreak detected in Common Terns (*Sterna hirundo*) in South Africa in the 1960s (Becker, 1966) and subsequent large wild die-off events in China during the early 2000s attributed to migratory Bar-headed Geese (*Anser indicus*) (Chen *et al.*, 2005). In addition to migration, certain behaviours such as communal nesting and roosting also increase exposure risk (Van Dijk *et al.*, 2014).

Birds from particular taxonomies may be more prone to AIV due to behavioural or ecological similarities. As Anatidae are associated to wetlands during their lifecycles, attention has subsequently often focused on birds that associate with waterbodies. However, species from other families (for example Alcidae species) are known to nest in dense colonies of thousands of individuals, increasing their relative exposure risk, and many non-Anatidae species demonstrate cross-continental migration but are not associated with waterbodies and wetlands (Billerman S *et al.*, 2020).

Research on Bewick's swans (*Cygnus colombianus bewickii*) in Asia has found that infected individuals make more regular stops during migration than those uninfected(van Gils *et al.*, 2007). This increase in stopovers leads to a potential increase in environmental contamination with AIV across a migration flyway.

Reliable detection of AIV among wild birds can require large sample sizes, as prevalence rates have tended to be low, although they have also varied between families and between studies . Caron,

Cappelle and Gaidet, (2017) used a minimum sample size of >300, designed to detect 1 positive sample at an estimated >1% prevalence in the sampled population. In this study as well as another similar study by Alexander, (2000) birds were categorized by order rather than by family or species. Sampling thresholds are important across epizootiology, as under-sampling can lead to both underestimation and overestimation of infection prevalence. Small sample sizes usually lead to low levels of confidence and increases the chance of Type 2 errors. Determination of an adequate sample size can be investigated via a sensitivity analysis; whereby different thresholds are tested on the same dataset and compared(Rothman, Greenland and Lash, 2008).

In this chapter, a systematic literature review was conducted to investigate the following questions::

1. Which species demonstrate high and low avian influenza prevalence and is this supported by a sufficient sample size?
2. What variables are significantly associated with trends in avian influenza prevalence in wild birds?
3. When sampled over a calculated threshold sample size, do certain species and their representative families demonstrate zero prevalence (and hence low risk with high confidence)?
4. When investigating avian influenza prevalence, which research gaps are evident? How might they be filled?
5. In a UK context, can this global study inform which species to focus research effort upon?

2.2 Materials and Methods

2.2.1 Systematic Review

A systematic review following a PRISMA structure (Moher *et al.*, 2009) of the published literature from January 2014 to February 2019 was conducted using the broad search term 'avian influenza' in two scientific journal online databases, JSTOR and ISI Web of Science. This period included two seasons of national AIV outbreaks in UK poultry and is later than two previous reviews on this subject [25, 2], although it does include surveillance data also reviewed by Caron, Cappelle and Gaidet (2017).

Articles with titles that were taken as implying the content of data on wild bird sampling for AIV were retained and others were discarded. Retained articles were read to confirm data availability; those reporting complete data on samples testing positive and negative for AIV or antibodies to AIV were retained and those that did not were discarded. Articles that reported testing for AIV regardless of strain were retained and those testing for specific strains were discarded

2.2.2 Data extraction

Data were extracted from articles and their supplementary material where possible, and authors were contacted if data were not automatically available. When an author responded with relevant data the article was included in the analysis, if not then the article was discarded. The final articles were filtered for duplicates collected from both journal directories.

Individual wild bird sample data were extracted from articles. Data were collected for the publication details of the paper, the period in which the sample was collected, the family and species of bird from which the sample was obtained, the type of sample, the method used to test the sample and if the sample tested positive or negative for AIV. If a strain was identified, this was also noted. The country, continent and where possible, the longitude and latitude of each sample were also recorded. For birds where samples were independently tested for viral presence and for serological studies, two rows of data were recorded for one individual (one per sample).

Three additional binary variables were extracted for species sampled over 100 times from Billerman et al. (2020) asking if a species was a colonial nester, a species that regularly resides at waterbodies, and if a species is oceanic.

For analysis, the data was split into two datasets, one for viral presence and one for antibody presence, this avoided positive or negative results from the same bird being counted twice within an analysis.

2.2.3 Sample size sensitivity analysis

The following formula, taken from Rothman et al (2008), and Canon and Row (1982), was used to help construct a sample size estimate to test against those used in Alexander, (2000) and Caron, Cappelle and Gaidet, (2017).

$$n = \frac{Z^2 \cdot P \cdot (1 - P)}{E^2}$$

Where:

- n = required sample size
- Z = Z-score (the number of standard deviations from the mean), corresponding to the desired confidence level (e.g., 1.96 for 95% confidence)
- P = expected prevalence (expressed as a decimal)
- E = margin of error (precision), expressed as a decimal

2.2.4 Statistical Analysis

Statistical analysis was undertaken using RStudio1.2.5 (Team R, 2008). Linear Models (performed using the package lme4) were constructed on data with a normal distribution after logarithmic transformation. Analysis of the antibody presence dataset was done via a Bayesian method; using zero-inflated beta regressions from priors defined by a beta distribution ((Cribari-Neto and Zeileis, 2010)). Model validation for linear models was conducted by plotting fitted vs. residual plots and looking at Cooks' distance. For Bayesian models, the Rhat statistic (also known as the "potential scale reduction factor") was used to test for model convergence. Testing against the null hypothesis was also conducted differently for both models, with brief descriptions below:

- p in frequentist statistics (p-value) quantifies evidence against a null hypothesis based on the likelihood of the observed data under that null hypothesis.

- P(D) in Bayesian statistics (probability of the data) quantifies the likelihood of observing the data under a specific model or hypothesis, considering prior information and updating beliefs based on the observed data.

Table 5: Summary of collected data variables and their sources.

Variable	Values	Data Source
Article	Number allocated to paper	JSTOR, ISI Web of Science
Sample collection period	Date	Articles
Family	Name	Articles
Genus	Name	Articles
Species	Name	Articles
Sample type	Categorical	Articles
AIV Diagnostic test	Categorical	Articles
Test Result	Positive or Negative	Articles
Longitude	Coordinates	Articles or Google Maps
Latitude	Coordinates	Articles or Google Maps
Country	Location	Articles
Continent	Location	Articles
Sampling Habitat	Categorical	Articles
Migratory behaviour (species)	Categorical	Billerman et al, 2020
Colonial nesting (species)	Categorical	Billerman et al, 2020
Waterbody Centricity (species)	Categorical	Billerman et al, 2020

2.3 Results

2.3.1 Systematic Review

A total of 1314 article titles were collated into a dataset using the search term 'avian influenza'. From these 756 were discarded for not containing wild bird sampling data or being published outside of the study period. Of the remaining 427 papers, 220 were deemed not relevant to the study due to not containing individual sample data, 15 were repeats across the two journal article repositories and 6 could not be located due to lack of a presented DOI. Data extraction was achieved from 116 articles (see appendix 1). In order to test the dispersion of paper selection between authors a Kappa Assessment (Moher *et al.*, 2009) of the first 100 papers identified by the ISI Web of Science search was conducted which showed statistically significant variance between observers (one post-doc, two PhD candidates).

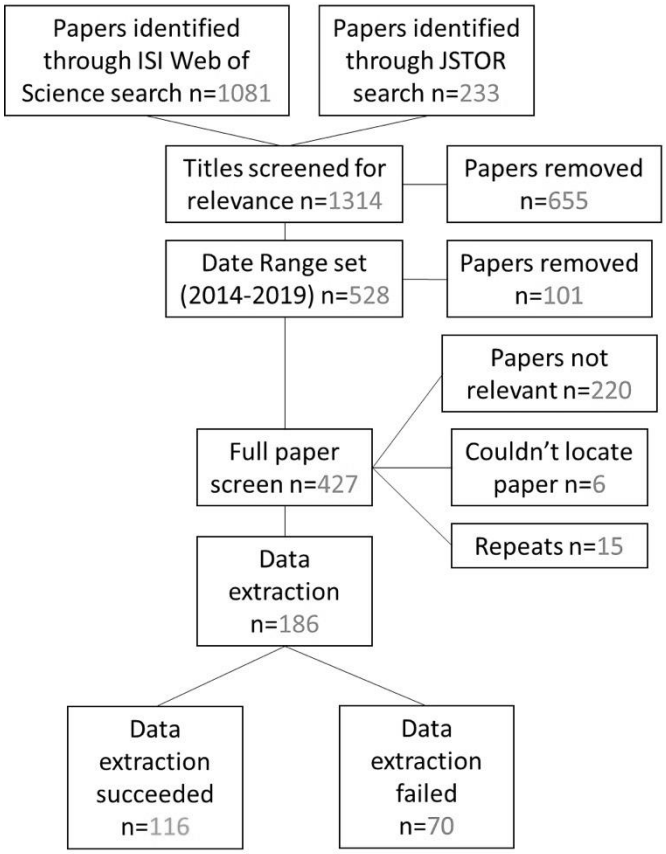

Figure 3: Flowchart of article inclusion assessment as part of the systematic review.

In total, data was collated for 893,993 samples. Among these, 371,007 were tested for AIV presence and 25,846 were tested for AIV antibodies. The nature of 497,140 samples (i.e., virus or antibody) was not specified.

2.3.2 Analysis of AIV presence

2.3.2.1 Geographical variation

Sampling for AIV was reported for all continents, with most viral presence samples originating from North America (57.51%) and Europe (25.22%). The highest prevalence rates mirrored this with 11.23% AIV-positive samples in North America and 4.60% in Europe.

Table 6: Provenance of AIV samples by continent

	% of total collated samples (n)	% provenance of AIV+ samples from each continent (n)
Continent		
Africa	3.53 (13093)	2.43 (318)
Antarctica	0.33 (1223)	0.74 (9)
Asia	6.61 (24506)	3.8 (931)
Europe	25.22 (93552)	4.6 (4305)
North America	57.51 (213379)	11.23 (23971)
Oceania	3.08 (11420)	0.33 (38)
South America	2.46 (9139)	1.43 (131)
Not defined	0.4 (1500)	0 (0)
Total	100 (371007)	8.01 (29703)

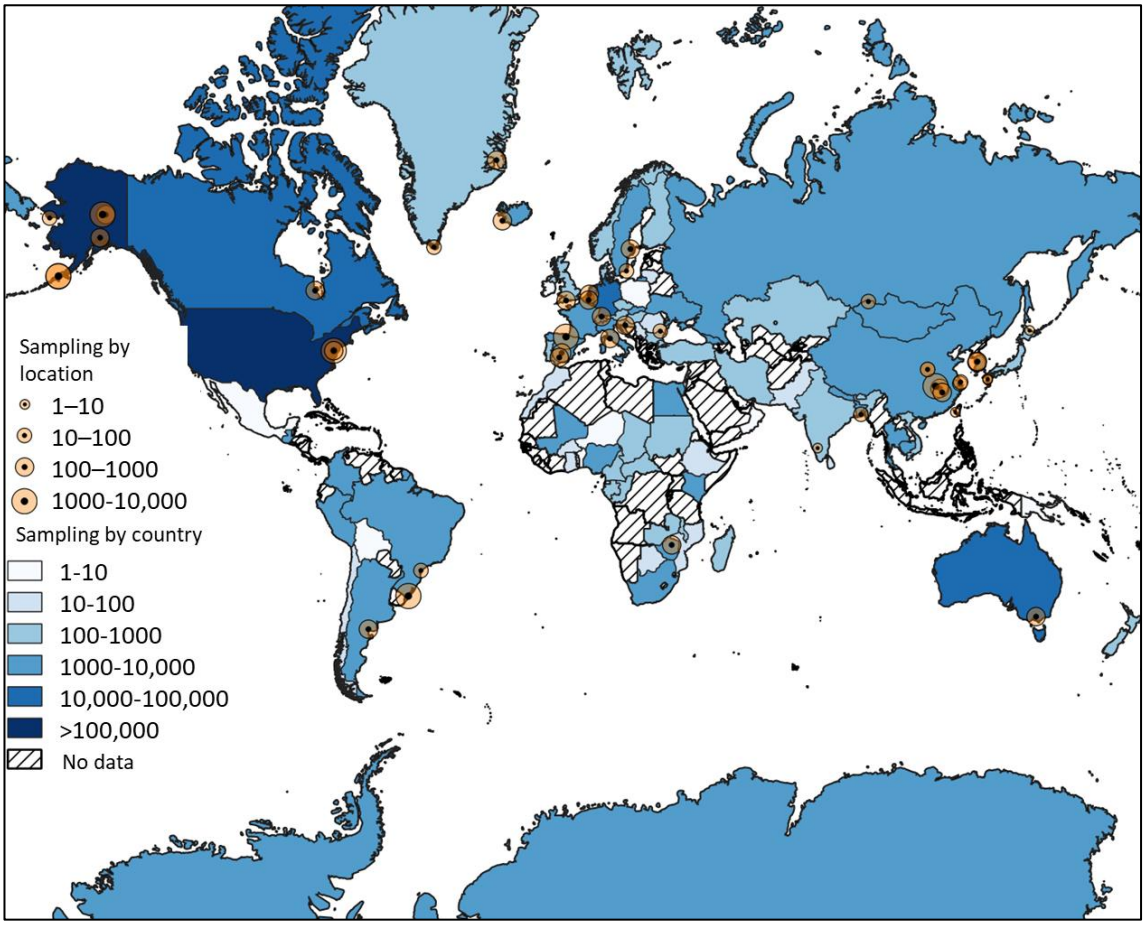

Figure 4: Heatmap of sampling effort by recorded country, with locations of sites and their sample sizes provided where given in article.

The distribution of study sites (see table 7) was skewed, with the top three most sampled countries being the USA (27 sites), China (11) and Canada (10).

Table 7: Distribution of included articles by country.

Country (or area) of sampling	Number of articles providing data on.			Total number of articles
	Virus prevalence	Seroprevalence	Virus and seroprevalence	
Antarctica	1	1	0	2
Argentina	1	0	0	1
Australia	4	0	1	5
Bangladesh	1	0	1	2
Belgium	2	0	0	2
Brazil	4	0	1	5
Bulgaria	0	1	0	1
Canada	7	0	3	10
Chile	0	0	1	1
China	6	3	0	11
Croatia	0	0	1	1
European Union	0	0	0	1
Finland	1	0	0	1
Germany	1	3	1	5
Greenland	1	1	1	3
Guadeloupe	1	0	0	1
Guatemala	2	0	0	2
Hong Kong	0	0	0	1
Iceland	1	0	2	3
India	1	0	0	1
Italy	1	0	1	2
Japan	3	0	0	3
Latin America	0	0	1	1

Lebanon	1	0	0	1
Mongolia	1	0	0	1
Multiple areas	2	0	0	2
Nepal	1	0	0	1
Netherlands	2	0	5	7
New Zealand	0	0	1	1
Norway	1	0	0	1
Oman	0	0	0	1
Pakistan	0	0	1	1
Georgia	0	0	1	1
Russia	1	0	1	2
Serbia	1	0	0	1
Slovenia	0	0	1	1
South Korea	1	1	1	3
Spain	3	2	0	5
Sweden	2	0	0	2
Switzerland	1	1	0	2
Taiwan	1	0	0	1
Thailand	1	1	1	3
Ukraine	1	0	1	2
United Kingdom	0	1	0	1
USA	14	3	8	27
Former Yugoslavia*	0	0	1	1
Zimbabwe	2	0	0	2

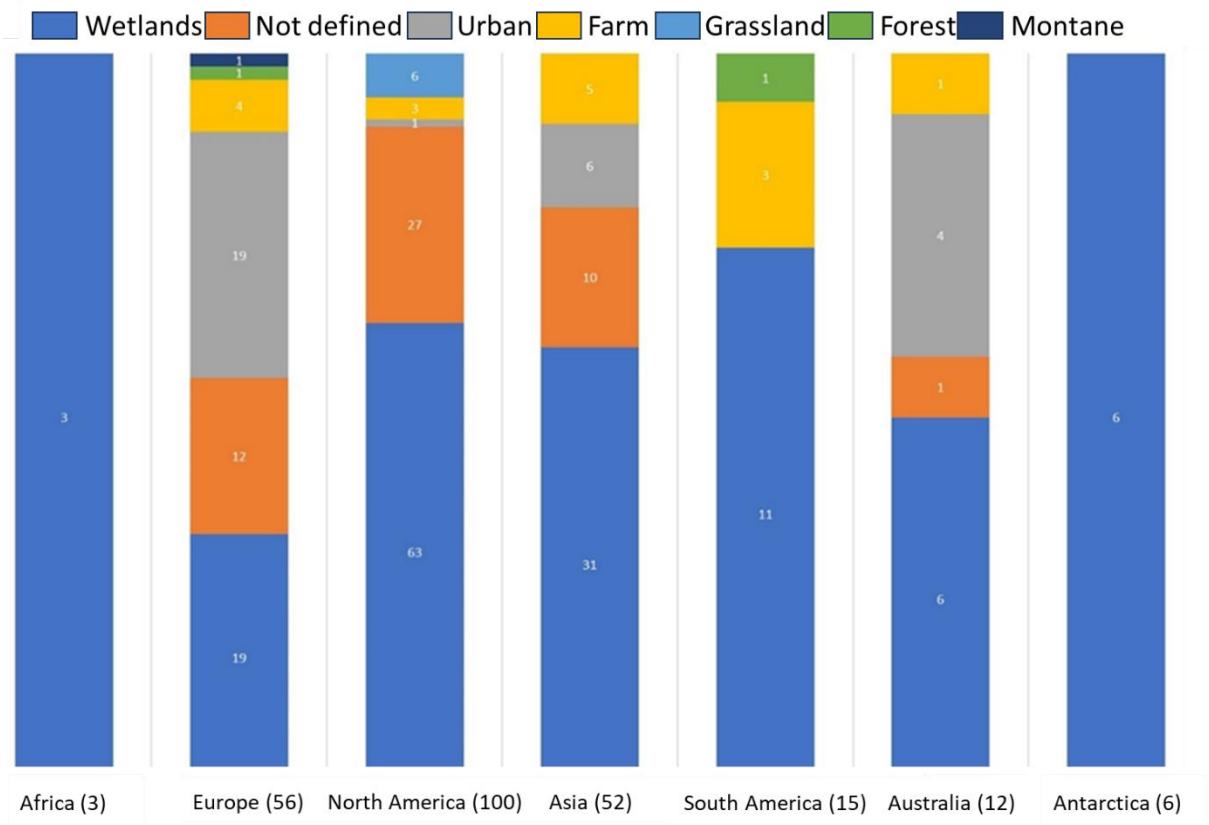

Figure 5: Number of identified sampling locations from selected articles categorized by continent and ecotype.

Sampling locations were consistently focused on wetland sites (including coastal) in at least 50% of cases on each continent, the only exception being Europe (33.93%). Only three of the 244 (1.23%) sampling locations were focused away from anthropocentric sources (Farms and Urban areas) or wetlands.

2.3.2.2 Phylogenetic variation

The Anatidae family constituted a substantial percentage of samples (62.25%), particularly in North America (84.82%). Anatidae samples revealed a higher prevalence of AIV (11.83%) than non-Anatidae (2.08%) (Table 8).

Table 8: Provenance of AIV samples by continent and whether they are collected from Anatidae or not.

	% of total collated samples taken from Anatidae (n)	% of AIV+ samples for Anatidae (n)	% of AIV+ samples for non-Anatidae (n)
Continent			
Africa	0 (0)	0 (0)	2.43 (318)
Antarctica	0 (0)	0 (0)	0.76 (9)
Asia	15.19 (3723)	7.87 (293)	3.07 (638)
Europe	41.23 (38567)	8.32 (3208)	2.00 (1097)
North America	84.82 (184987)	12.89 (23231)	2.24 (740)
Oceania	3.08 (3690)	0.79 (28)	0.13 (10)
South America	0.01(1)	0 (0)	1.43 (131)
Total	62.25 (230968)	11.83 (26760)	2.08 (2943)

The most sampled countries were the USA (189,948), Netherlands (28,625), Canada (20,013), Australia (17,343) and Belgium (15,481) (see full list in appendices).

2.3.2.3 Sample size thresholds

An estimated prevalence rate (P) for AIV across families in the study was calculated to be 2.08% from the extracted dataset. Anatidae was removed from this calculation due to its significant

influence on the mean AIV prevalence estimate. Z was set to 1.96 to use a 95% confidence level and E was set at 1% for a population size of >100,000 (Canon and Row, 1982).

Using Rothman et al's formula a sample size of >738 was calculated to be the minimum sample size to be able to provide a robust estimate of prevalence rates of AIV in wild birds. This contrasts from the >300 sample size threshold that is used by Alexander (2000) and Caron et al (2017).

2.3.2.4 Interfamilial variation

Twelve families had higher than average (2.08%) AIV prevalence estimates. Anatidae is frequently described in the literature as the main cause of international spread of AIVs and Anatidae revealed the highest AIV prevalence (11.83%). 38 of 39 other families with sample sizes >738 also revealed AIV-positive samples (Figure 6).

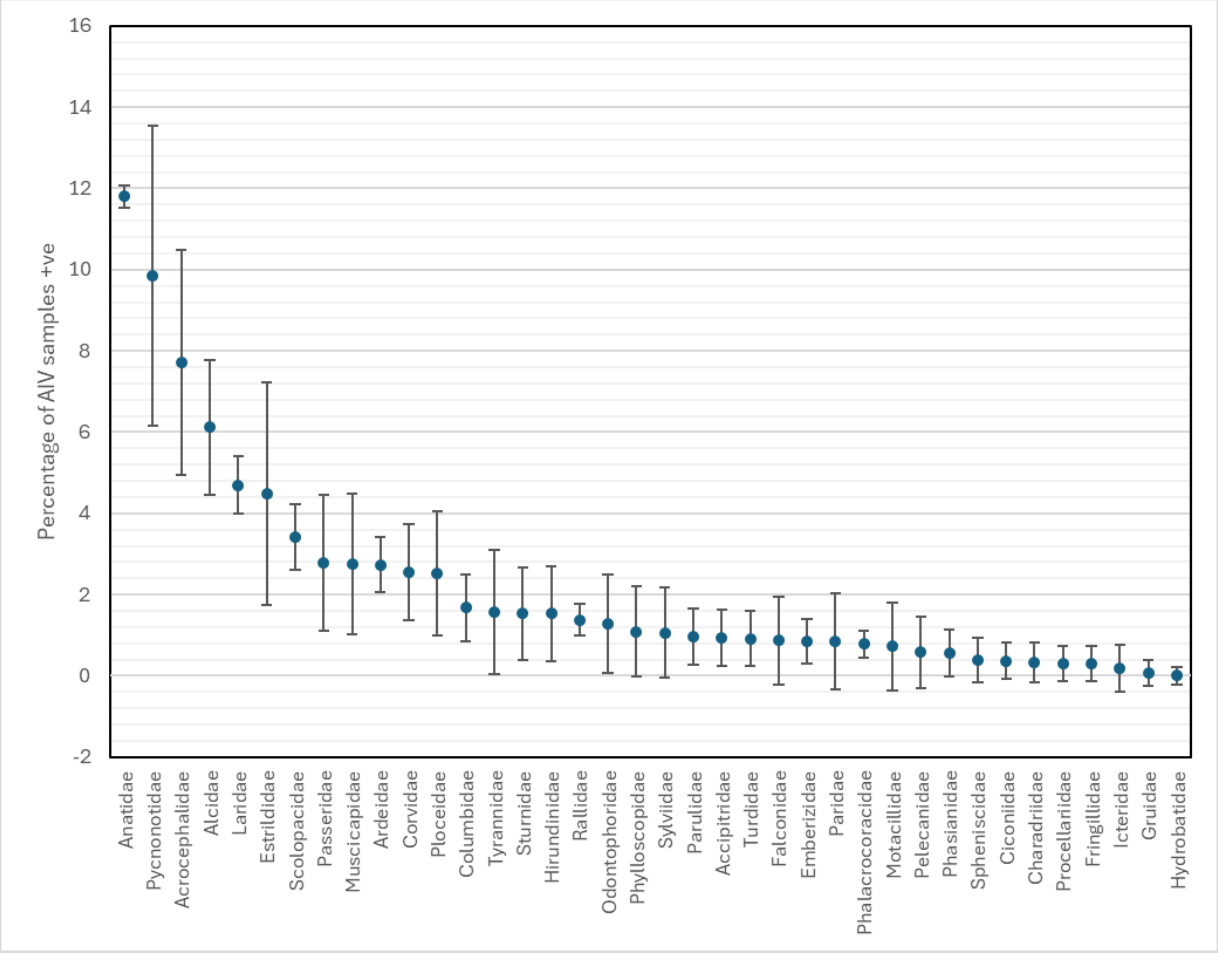

Figure 6: Box Whisker plot of the provenance of AIV-positive samples for bird families sampled >1000.

2.3.2.5 Intrafamilial variation

When considering variation within a family by looking at AIV prevalence at a species level, of the twenty families sampled over 738 times, only five had more than one representative species. When including species sampled over 300 times (like sampling thresholds used by Caron et al (2017) and Alexander (2000)), seventeen of those families were represented by multiple species.

Table 9: Provenance of AIV prevalence amongst families when calculated by mean amongst species within a given family.

Family	Mean AIV prevalence (%) amongst distinct species when n>738 (number of species)	Mean AIV prevalence (%) amongst distinct species when n>300 (number of species)	Difference between mean AIV prevalence (%) amongst distinct species when n>738 and n>300
Scolopacidae	14.05 (1)	3.97 (8)	-10.08
Alcidae	10.05 (1)	3.60 (3)	-6.45
Anatidae	8.09 (21)	6.73 (30)	-1.36
Ardeidae	6.09 (3)	3.64 (6)	-2.45
Laridae	4.83 (4)	3.38 (8)	-1.45
Hirundinidae	2.65 (1)	1.09 (3)	-1.56
Columbidae	2.04 (2)	0.68 (3)	-1.36
Corvidae	1.61 (1)	1.61 (1)	0
Rallidae	1.09 (4)	1.39 (5)	0.30
Charadriidae	0.71 (1)	0.36 (2)	-0.35
Phalacrocoracidae	0.68 (2)	0.41 (4)	-0.27
Sturnidae	0.61 (1)	0.39 (2)	-0.22
Passeridae	0.60 (1)	5.68 (2)	5.08
Procellaridae	0.37 (1)	0.23 (3)	-0.14
Phasianidae	0.37 (1)	0.91 (2)	0.54
Ciconiidae	0.22 (1)	0.59 (2)	0.37
Spheniscidae	0 (1)	0.61 (2)	0.61
Phylloscopidae	0 (1)	1.69 (2)	1.69
Hydrobatidae	0 (1)	0 (1)	0

Forty-eight species were sampled >738 times for AIV viral presence, and 121 species were sampled >300 times. Intra-familial sampling demonstrated variation between species for both sampling thresholds, though the level of variation differs.

2.3.2.6 Intra-familial Sampling Dataset

Graphs and tables for the 1709 species of 151 families sampled for viral presence in the dataset are presented through ShinyApp (Chang *et al.*, 2017), the files and code to run the ShinyApp through RStudio (RStudio Team, 2019) are available in appendix 2.

The application has been constructed to simplify the visual interrogation of an extensive dataset, designed to be used as a tool for researchers wishing to access both table and graphical representations of sampling effort and AIV viral presence in samples across the entirety of the sampled species in the dataset, at the species level. These graphs accumulate the sampling efforts of multiple long-term and short-term studies at both site and country levels.

The use of this ShinyApp is demonstrated here (Figure 7) for the Accipitridae family, a family sampled regularly as an assumed bio-accumulator of AIVs as both avian predators and carrion consumers.

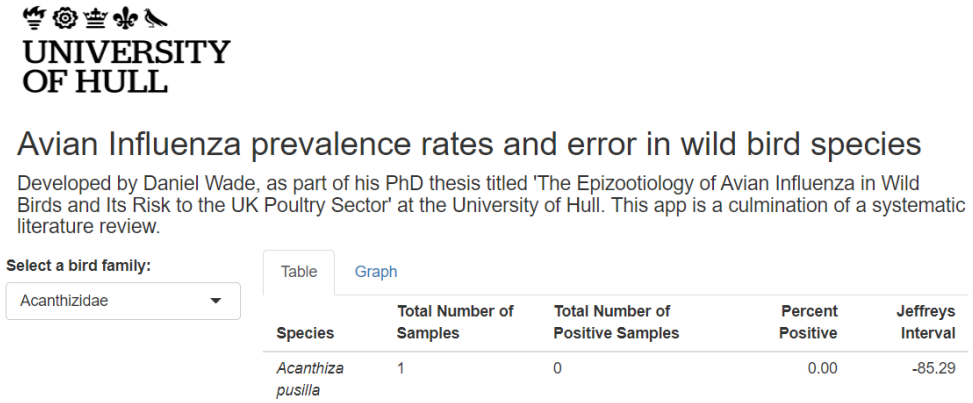

Figure 7 Screenshot of Avian Influenza prevalence rates in wild bird species ShinyApp demonstrating user interface.

The interface (Figure 8) shows the option to select different bird families from a dropdown of all families sampled in the dataset with a tabbed option for either graphical or tabulated data visualization.

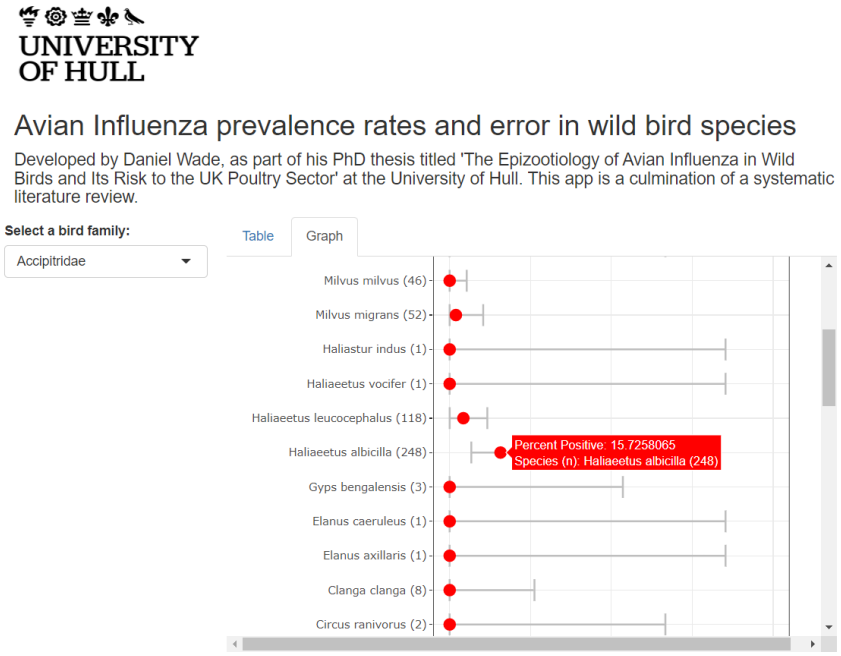

Figure 8 demonstrating the graphical interface for the AIV prevalence data for wild birds. The user can hover the cursor over the percent positive data point to see more information.

2.3.2.7 Effects of sampling threshold : Sensitivity Analysis

Models were run to investigate the differences between these sampling thresholds. >738 per the Rothman et al derived method, >300 as conducted by Alexander and Caron et al and a further sampling threshold of >100 also being run to look at the extremes of an analysis using an under-sampled population.

General linear models were constructed to investigate the relationships between two independent variables, sample size and AIV prevalence per species and two dependent variables, nesting behaviour and waterbody habitat preference.

The goodness-of-fit of the model was assessed using deviance and Pearson chi-square statistics. Additionally, overdispersion was checked by comparing the residual deviance to the degrees of freedom. Model validation was performed through cross-validation techniques and examining

residual plots to ensure the assumptions were met. Model assumption tests are found in the appendix.

A sampling bias was identified for wetland-centric species, which were sampled over species that are not frequent at wetland sites (Billerman S *et al.*, 2020) in all scenarios ($p<0.001$ for >100 and >300, and $p<0.05$ for >739). Colonial nesting species were sampled less than non-colonial nesting species (>738: $p<0.05$), with semi-colonial nesters (n=1) sampled more than colonial nesters (>300, $p>0.01$).

The proportion of presence of AIV in sampled species was higher in wetland-centric species; but only for those sampled >300 ($p<0.001$) and over >100 times ($p<0.01$).

Anatidae were sampled more than other families (>62%) so a further Poisson regression model was constructed for non-Anatidae species sampled over 738 times to investigate differences in estimates. Whilst wetland-centric species were sampled more frequently than species that do not frequent wetland habitats ($p<0.001$), no trends were identified in the prevalence of AI in the species sampled.

Table 10: Results from linear regressions on logarithmically transformed sample size and AIV positive proportions under different sampling thresholds.

Species sampled over...	Total Number of Samples				Proportion Present			
738 times	>738	Estimate	Standard Error	t p	>738	Estimate	Standard Error	t p
	Intercept	3.131	0.179	17.517***	Intercept	0.699	0.374	1.868.
	Wetland Centric	0.469	0.188	2.493*	Wetland Centric	1.162	0.394	2.951**
	Semi Colonial Nester	0.737	0.428	1.723.	Semi Colonial Nester	-0.719	0.896	-0.802
	Colonial Nester	-0.375	0.157	-2.393*	Colonial Nester	-0.437	0.328	-1.332
	Oceanic Centric	0.058	0.191	0.304	Oceanic Centric	-0.69	0.4	-1.724.
300 times	>300	Estimate	Standard Error	t p	>300	Estimate	Standard Error	t p
	Intercept	2.762	0.078	35.218***	Intercept	0.686	0.157	4.555***
	Wetland Centric	0.345	0.096	3.591***	Wetland Centric	0.651	0.185	3.526***
	Semi Colonial Nester	1.229	0.446	2.758**	Semi Colonial Nester	-0.195	0.856	-0.228
	Colonial Nester	-0.203	0.105	-1.928.	Colonial Nester	-0.293	0.203	-1.448
	Oceanic Centric	0.043	0.132	0.324	Oceanic Centric	-0.404	0.253	-1.599
100 times	>100	Estimate	Standard Error	t p	>100	Estimate	Standard Error	t p
	Intercept	0.686	0.151	4.555***	Intercept	0.729	0.084	8.728***
	Wetland Centric	0.651	0.184	3.526***	Wetland Centric	0.361	0.117	3.079**
	Semi Colonial Nester	-0.195	0.856	-0.228	Semi Colonial Nester	-0.614	0.436	-1.408
	Colonial Nester	-0.293	0.203	-1.448	Colonial Nester	-0.244	0.134	-1.815.
	Oceanic Centric	-0.405	0.253	-1.599	Oceanic Centric	-0.264	0.165	-1.601
738 times (and Non-Anatidae)	Non-Anatidae	Estimate	Standard Error	t p	Non-Anatidae	Estimate	Standard Error	t p
	Intercept	3.027	0.122	24.82***	Intercept	0.473	0.374	1.263
	Wetland Centric	0.233	0.233	1.584	Wetland Centric	0.734	0.451	1.627
	Colonial Nester	-0.065	-0.065	-0.454	Colonial Nester	0.242	0.437	0.554
	Oceanic Centric	0.059	0.139	0.425	Oceanic Centric	-0.675	0.426	-1.585

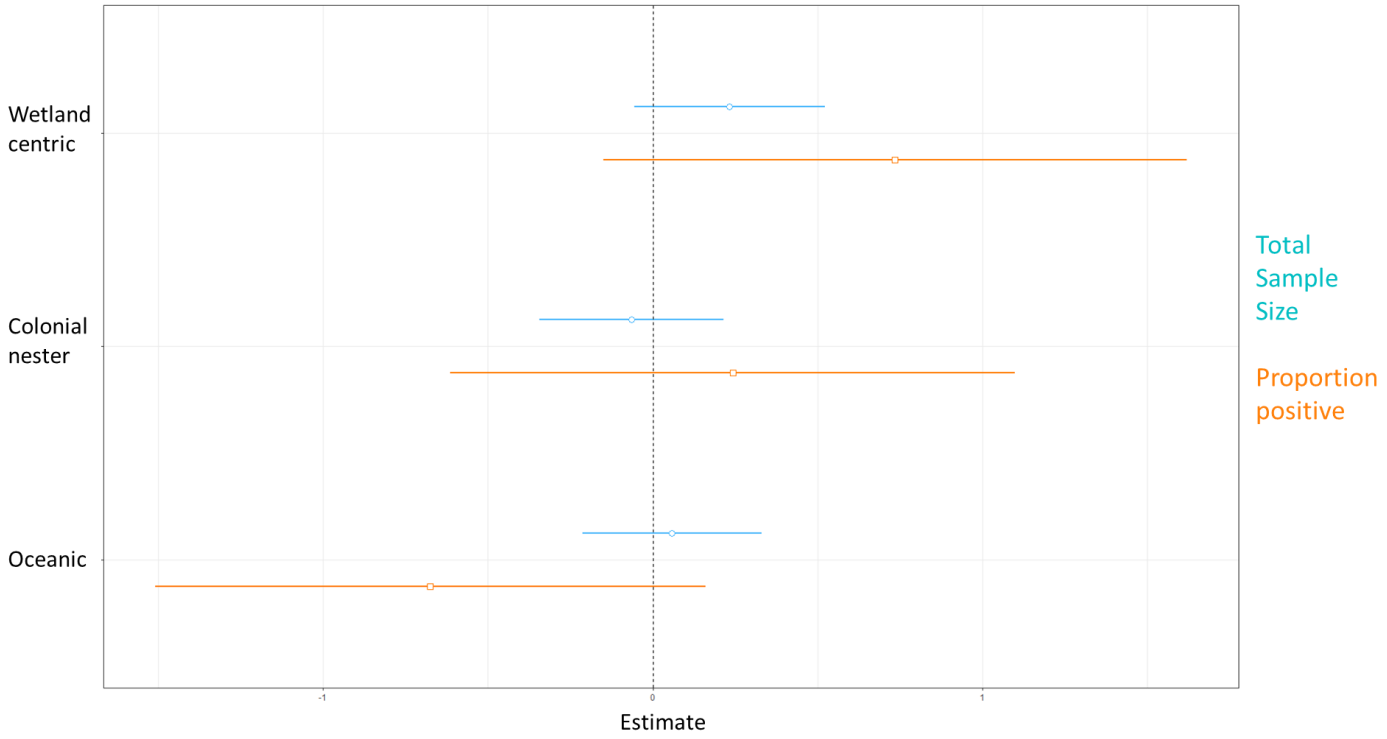

Figure 9: General Linear Model estimates for non-Anatidae models modelling behavioral attributes against total sample size and the proportion of positive samples per species sampled over 738 times.

2.3.3 Analysis of AIV Antibody Samples

2.3.3.1 Geographical variation

Similarly, to data on viral presence, AIV antibody samples were collected in North America (27.16%) which also had the highest mean antibody prevalence (32.14%). Whilst Oceania had the second highest sample size for antibody prevalence (23.33% of total sample, 9.02% AIV antibody prevalence), both Asia and Europe demonstrated higher antibody prevalence (18.19% and 14.63%).

Table 11: Antibody sampling and provenance of positive AIV samples per continent.

	% of samples (n)	% of AIV+ per continent (n)
Continent		
Africa	0 (0)	0 (0)
Antarctica	1.63 (422)	0 (0)
Asia	8.73 (2256)	14.63 (330)
Europe	19.14 (4948)	18.19 (900)
North America	47.16 (12189)	32.14 (3917)
Oceania	23.33 (6031)	9.02 (544)
South America	0 (0)	0 (0)
Na	0 (0)	0 (0)
Total	100 (25846)	22.01 (5691)

2.3.3.2 AIV antibody sampling

In comparison to the general linear models used previously, for AIV antibody sample analysis a Bayesian approach was used to create zero-inflated beta models using non-informative priors. The posterior inference was calculated using Markov Chain Monte Carlo simulations.

Sampling bias for AIV antibody samples was identified towards species in the Anatidae family (again, split by Anatidae vs non-Anatidae, p(D)=1). Communal nesting and waterbody centric

species both have uncertain probabilities of direction (estimate=0.073, p(D)=0.56 and estimate=-0.391, p(D)=0.84), with a bias toward terrestrial centric species identified.

Table 12: Results of Bayesian zero-inflated model for behavioural attributes by number of antibody samples per species sampled over 738 times.

	Estimate	U 95% Credible Interval	L 95% Credible Interval	Rhat	Probability of Direction p(D)
Intercept	6.059	5.361	6.869	0.999	1.00
Anatidae	+1.181	0.576	1.784	1.000	1.00
Communal Nester	+0.073	-0.831	1.053	0.999	0.562
Waterbody Centric	-0.392	-1.322	0.402	1.000	0.838

Bayes R_2=0.249 (0.066-0.479)

The proportion of positive AIV antibody samples was weakly associated with non-communal nesting species (estimate=-0.858, p(D)=0.89), but the difference was negligible between waterbody centric species and those not associated with waterbodies (estimate=0.034, p(D)=0.52). The proportion of positive samples increased with the number of samples collected (estimate=0.704, p(D)=0.95, see Table 12).

Table 13: Results of Bayesian zero-inflated model for behavioural attributes by proportion of positive AIV antibody samples per species sampled over 738 times.

	Estimate	U 95% Credible Interval	L 95% Credible Interval	Rhat	Probability of Direction p(D)
Intercept	-3.342	-5.661	-0.841	1.00	0.996
Waterbody Centric	0.034	-1.132	1.284	1.00	0.523
Communal Nester	-0.858	-2.420	0.515	1.00	0.889
Log10(Number of Samples)	0.704	-0.126	1.548	1.00	0.946

Bayes R_2=0.249 (0.066-0.479)

2.3.3.3 H5 positive species.

The sample size for species providing positive samples for the H5 AIV virus demonstrated a sampling bias towards species within the Anatidae family (36 of 92) and among wetland-centric species (64 of 92). There was also a trend towards avian consumers (both predators and carrion-feeders) in the remaining non-wetland-centric species (18 of 28). Lists of species with H5 strain AIV positive samples are found in appendix 3 and 4.

2.4 Discussion

The results of this study indicate most species of birds can become infected with AIV, and importantly, at similar infection prevalence to Anatidae. Despite this, a sampling bias exists at a familial level towards Anatidae. Whilst Anatidae does demonstrate one of the highest prevalence

rates for AIV among families sampled, other families such as Acrocephalidae were sampled much less but had similar estimated AIV prevalence, though with increased Jeffreys Confidence Intervals. AIV prevalence estimates may be biased by uneven sampling efforts.

Sampling bias is present at an intrafamilial level with variation in margins of error (Jeffreys intervals) showing high levels of uncertainty for most species due to small sample sizes. As such it is very difficult to accurately calculate if any given species has a higher AIV prevalence estimate than another. As with at an interfamilial level, more uniform AIV sampling amongst all species will aid in expanding understanding and reducing uncertainty on which species within which families demonstrate the highest AIV prevalence. The focused research on Anatidae has led to a better understanding of AIV dynamics within the family compared with all others.

The ShinyApp produced from the dataset collected from the literature as part of this chapter is designed to be an updateable and easy-to-use tool for researchers to search for AIV prevalence rates and confidence in species of interest. The dataset behind the application is sizeable but demonstrates that in the vast majority of cases, more sampling is required to make robust estimates of AIV prevalence and help identify species concern in need of further risk modelling. By publishing the app, alongside a data entry sheet to allow for updates, it would be possible to create a dynamic tool for researchers and modellers.

A broad search term was used to try and accommodate as much data as possible into the review. Whilst this was successful, a Kappa assessment (Moher *et al.*, 2009) did show a dispersion of selection choices between observers on a sample of the data (n=3). The consequences of this would be variation in the samples included in an analysis by different observers. It is determined that the selection criteria being broad to include a large sample size with the capacity to demonstrate increasingly accurate data trends validate the use of a broad search term, and whilst there may be variance between final results if the survey was conducted by multiple researchers, the large sample size should reduce this variance.

A minimum sample size of n>300 compared to n>738 led to a narrower credible interval, but the Bayes R2 value was lower, indicating that n>300 explains less of the variation in the dataset. It is

important to be cautious when deciding sample sizes for analysis, as a more stringent sampling threshold led to more uncertainty in our variable's effects on prevalence estimates but increased our explained variation, whereas a more relaxed sampling threshold increases our sample size and decreases the credible intervals (increasing certainty) but explained less of the variation in the data.

Using different sample size thresholds leads to variance in families and species being considered by the different models. At current, reported estimates of AIV prevalence at species and family level in the literature may have inaccuracies which would be solved by increased sampling efforts across less sampled groups. Once this data is available against an appropriate sampling threshold, it will be more accurate to compare AIV prevalence estimates.

Geographically, sampling for avian influenza is concentrated towards North America and Europe, Sampling was conducted the least in Antarctica and generally in the southern hemisphere likely due to smaller or less well-reported impacts of AIV on the poultry industry (Wille and Barr, 2022)Geography and economics both likely lead to bias in sampling effort, for example, Antarctica is much more isolated and hard to visit for studies than the UK, and whilst there might be more AIV in South-East Asia, the effects of outbreaks are weighed against other socio-economic pressures and hence resource for studies may have to come from external interests, such as higher economically developed countries where AIV can have drastic effects on the food industry.

Wetland habitats were sampled from the most across the locations defined in the reviewed articles, . The varying habitat preferences of species sampled, including wetland-associated generalists, contribute to uncertainty in understanding the epidemiological role of species utilizing different habitat types. Research suggests that the role of waterbodies is significant in the spread of AIV(Ann Kathrin Ahrens *et al.*, 2022). As waterbodies are a core habitat for Anatidae, known to have high AIV prevalence estimates, logic suggests that birds that share these spaces with wild Anatidae have greater exposure risk to AIV and hence are more likely to form the next link in the spread from wild birds to poultry(Blagodatski *et al.*, 2021). With the results adding uncertainty on how prevalent AIV is in other families outside of Anatidae, with some AIV prevalence rates potentially being higher, the role of water bodies may be less logical an avenue of focused research than has been

previously thought. However, until a time when there is more certainty in AIV prevalence estimates from other species and families outside of Anatidae, it remains uncertain if this is the case.

When sampling for evidence of avian influenza in wild birds, more studies have sought to isolate the virus rather than antibodies to the virus. Viral samples can demonstrate if a bird has an active infection but reveals very little about infection or exposure history(Nagy *et al.*, 2021). The presence of antibodies indicates exposure to the virus at some point in the past but reveals very little about the current infection status. Sampling bias between samples of live AIV virus and antibodies can be dictated by laws surrounding animal rights. In the UK, ASPA(Animals (Scientific Procedures) Act 1986) requires strict licencing of invasive sampling of live animals, and as such the collection of cloacal, oropharyngeal and blood samples is more complex than the collection of deposited faeces and corpses. Both viral and antibody prevalence estimates are consistent with the consensus that ducks, geese and swans tend to demonstrate higher rates of AIV infection than most other families, with the caveat that smaller sample sizes have led to statistical overlap with other families that require more research to identify if Anatidae stands as a significant prevalence outlier.

Models varied depending on the sampling threshold used; the Canon and Row sampling calculator (Canon and Row, 1982) derived thresholds matched for wetland-centric species (i.e., species sampled the most demonstrated higher AIV prevalence rates), but an Anatidae removed model following the same threshold showed that neither sampling effort nor AIV prevalence was related to the wetland-centricity of a species. Anatidae's high sample effort is warranted to an extent, but care must be taken to analyse the roles of other species and families, with only one family of the top 20 sampled (Hydrobatidae) returning no positive samples.

H5-specific sampling was levied towards 3 target categories: Anatidae, wetland-centric species, and avian consumers. The learnings from generic AIV prevalence suggest that bias towards Anatidae in sampling effort might be warranted. It is hard to define the roles of other families and if infection might be short term, and hence AIV movement risk be potentially low, or that other families and species may have the potential for subclinical infection (H5 and other strains) which allows them to be vectors for long-distance spread (Wade *et al.*, 2022).

2.5 Conclusions and direction of thesis.

The systematic literature review in Chapter 2 has shown that we have a biased understanding of AIV in wild birds, with prevalence estimates much better understood for Anatidae than any other family, with species from waterbody-centric families also being better understood than those whose ecological niches are more terrestrially focused.

Relating these to the defined aims of Chapter 2, for families sampled over a calculated sampling threshold (>783), almost all families contained a positive sample for AIV, with prevalence rates varying. This could imply that with a sufficiently comprehensive sampling of all species, positive AIV samples could be discovered, or conversely, it could indicate that existing research has accurately identified the appropriate species for assessing AIV prevalence. Most AIV research focuses on Anatidae; whilst passerines and allies are relatively neglected. The smaller songbirds represent the most abundant wild birds in most arable landscapes, where most poultry farms are located. Future sampling would be beneficial to quantify the role of passerines and allies in short and long-distance transmission of AIV in the landscape, through direct infection and through fomite transfer between sites, particularly those where high congregations of known high AIV prevalence species and families occur.

Within a UK context, sampling during autumn migration would help to define which wild birds are responsible for AIV outbreaks within the UK, with further focus on sampling around poultry farms and waterbody sites to begin to define which species act as carriers in the local landscape. Achieving an understanding of AIV prevalence in UK wild birds at a landscape scale will aid in developing better monitoring and response mechanisms aimed at reducing outbreaks at poultry holdings reducing risk to the UK's poultry sector.

Chapter 3 : Bird migration and AI. High pathogenicity avian influenza: Targeted active surveillance of wild birds to enable early detection of emerging disease threats

3.1 Abstract

Avian influenza (AIV) is an important disease that has significant implications for animal and human health. High pathogenicity AIV (HPAI) has emerged in consecutive seasons within the UK to cause the largest outbreaks recorded. Statutory measures to control outbreaks of AIV virus (AIV) at poultry farms involves disposal of all birds on infected premises. Understanding of the timing of incursions into the UK could facilitate decisions on improved responses. During the autumnal migration and wintering period (autumn 2019- spring 2020), three active sampling approaches were trialled for wild bird species considered likely to be involved in captive AI outbreaks with retrospective laboratory testing undertaken to define the presence of AIV.

Faecal sampling of birds (n=594) caught during routine and responsive mist net sampling failed to detect AIV. Cloacal sampling of hunter-harvested waterfowl (n=146) detected seven positive samples from three species with the earliest detection on the 17th October 2020. Statutory sampling first detected AIV in wild and captive birds on 3rd November 2020. We conclude that hunter sourced sampling of waterfowl presents an opportunity to detect AI

within the UK in advance of outbreaks on poultry farms and allow for early intervention measures to protect the national poultry flock.

3.2 Introduction

Avian Influenza (AI) is caused by a zoonotic viral pathogen (Influenza A virus, AIV) hosted predominantly by wild birds but with the ability to jump to other taxonomic groups (Herfst et al, 2014) including humans. AIV is divided, based on infection in poultry into high pathogenicity (HPAIV) and low pathogenicity (LPAIV) with outcomes resulting from infection with the former being associated with high mortality whilst LPAIV infection is invariably asymptomatic in poultry (Bucko and Geiger, 2019). Further, within the UK, the detection of H5 and H7 virus subtypes is legally notifiable and impacts both on national AIV status and international trade. Globally, the annual number of HPAIV cases on poultry farms and among wild birds caused by H5Nx viruses has increased in recent years, with substantial increases observed during 2020/21 and 2021/22 (Miller, 2022). Between 2017 and the end of 2019, only 40 AIV - positive wild birds were detected in Great Britain (GB). In each of the following autumnal migration and wintering periods, the incidence of wild bird detections increased substantially (317 in 2020/2021 and as of the 21st July 2022, 1413 in 2021/2022 where multiple summer peaks were observed following the end of winter) with the trend similarly mirrored in poultry farm AI outbreaks (APHA, 2022a). The cost to the industry of statutory measures to control AI on UK poultry

farms between 2016 and 2017 was estimated to exceed £100 million, with additional cost to the government for monitoring and outbreak control (Riddler, 2017).

UK poultry farmers are legally obliged to report suspicion of AIV infection within their flocks, which is followed by testing and implementation of control measures if notifiable AIV is confirmed ('Diseases of Poultry (England) Order', 2003). Detection of AIV in wild birds is passive, relying on submission of found dead bird carcasses for testing by the UK statutory agency ('The Avian Influenza (H5N1 in Wild Birds)(England) Order', 2006). Under these approaches, detection of AIV in UK poultry and wild birds has been approximately concomitant.

However, it may be advantageous to detect re-emergence of infection in the country among wild birds before the first outbreaks among poultry. In this way, enhanced biosecurity measures, and informed decisions on housing free-range poultry, could be implemented early in order to attempt to reduce the risk and frequency of AI outbreaks.

The primary wild host for both LP and HPAVI is thought to be the Anatidae, ducks, geese, and swans(Cromie and Hughes, 2006) with annual re-emergence of AI on European poultry farms following soon.

after their seasonal immigration [9]. However, many species of passerine have been found to carry AI and have also been proposed as potential candidates for direct exposure to poultry

(Burns *et al.*, 2012). Substantial variation in the prevalence of infection among passerines and other non-

Anatidae bird families has been found between studies (Račnik et al, 2008, Schnebel et al, 2005, Slusher et al, 2014, Cumming et al, 2011, Fuller et al, 2010, Gronesova et al, 2008, Peterson et al, 2008, Han et al, 2012). The mechanism by which AIV transmits from immigrational Anatidae into poultry is unclear but transfer via intermediate, bridge species, which may include passerines, has been proposed (Root, Ells and Shriner, 2021). Confirmation of the same strains of AIV infecting wild passerines and domestic poultry concomitantly in space and time could help further elucidate the mechanisms of transfer of AIV from wild to domestic birds.

Hundreds of thousands of individual passerines (and other birds) are ringed (fitted with uniquely coded rings upon the tarsus or tibia) across the UK as part of voluntary national monitoring of bird populations overseen by the British Trust for Ornithology (Robinson, Leech and Clark, 2018).

Furthermore, more than 140 wildfowling clubs with over 9000 members, lawfully shoot ducks and geese, every year for recreational purposes (The British Association for Shooting and Conservation, 2022). These two activities offer potential for AI surveillance in wild bird populations due to their temporal and spatial coverage and the potential sample sizes being very large. This study sought to evaluate whether active sampling of wild birds could result in the detection of AIV in advance of outbreaks on poultry farms. The study chose to sample birds that were temporarily (bird ringing) or permanently (shooting) removed from the wild during lawful routine activities to establish whether these activities might offer opportunities for cost-effective AI surveillance. Also

sampled were birds caught and ringed at established ringing sites at locations local to a poultry farm that had recently experienced an AI outbreak to identify whether the same strains of AIV were detectable in wild birds likely to visit those farms, and to evaluate the potential for highly targeted surveillance of AIV in wild bird populations.

3.3 Materials and Methods

Three approaches were tested to evaluate their ability to detect AIV in wild birds between Autumn 2019 and Spring 2021: 1) sampling of hunter-harvested waterfowl, 2) volunteer sampling of migrating birds and 3) responsive sampling of birds caught close to a poultry farm AI outbreak.

Cloacal swab samples were collected post-mortem from hunter-harvested waterfowl at a private site on the northern side of the outer Humber Estuary in northeast England (Lat/Long, 53.653476, 0.073939). The site was chosen because of its position on the east coast of the UK, where many migratory birds enter the country during their autumn migration (Bradaric et al, 2020).

Faeces passed by migratory birds upon capture were collected in the same region at three sites with ongoing bird banding/ringing projects during autumn 2019 and 2020 (Figure 10).

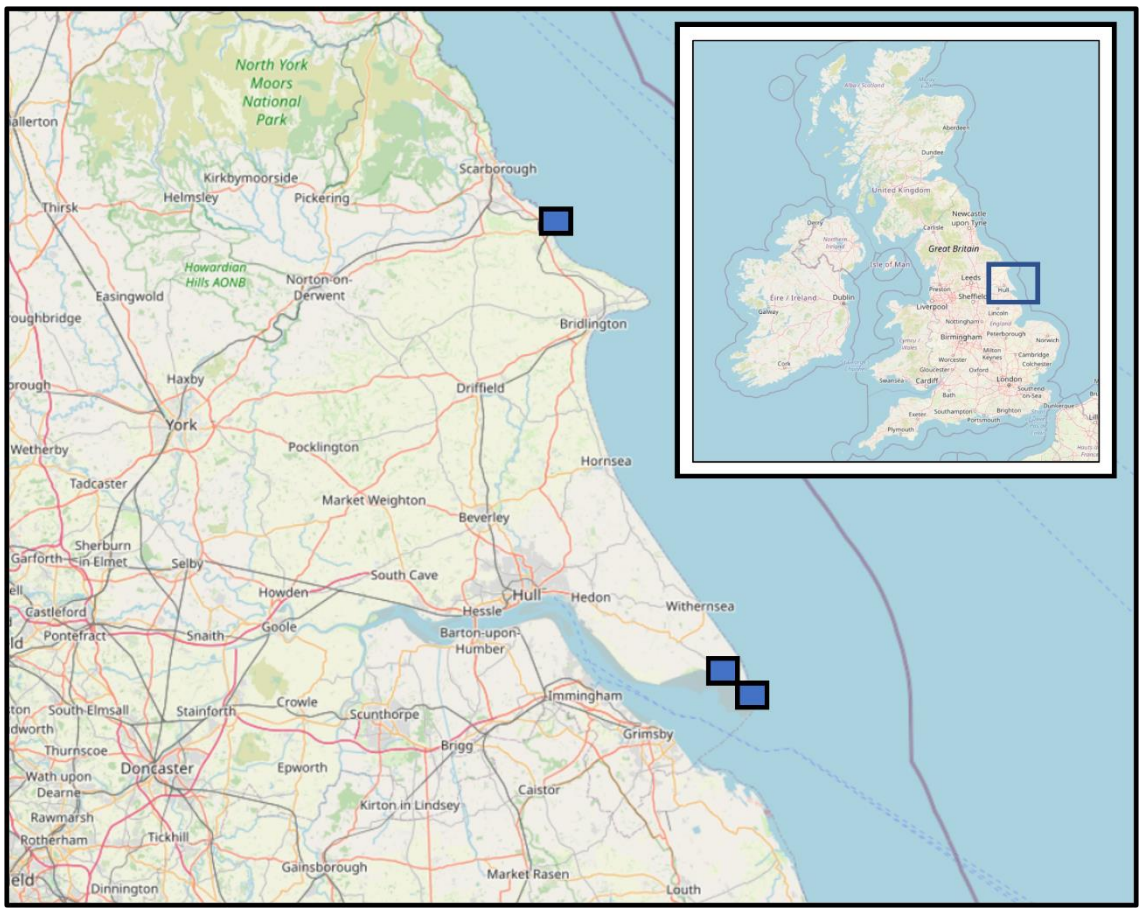

Figure 10: Locations of migratory bird sampling sites at Filey Brigg (A.), Welwick saltmarsh (B.) and along the Spurn peninsula (C.) within the UK.

Faecal samples were collected from passerines caught in mist nets from one hour before dawn until catches became minimal during daylight hours. Mist nets were used under licence from the British Trust for Ornithology (BTO) and birds were extracted and placed singly into a clean cloth bag from where faecal samples were collected into 1.5ml screw cap microcentrifuge tubes. Tubes were labelled with a unique sample code, the date, species of origin, and the number of any ring present or fitted during the bird's capture.

Each cloth bag was only used once per sampling session to avoid cross contamination of

samples, and bags were soaked in a weak bleach solution (Suarez et al, 2003) and washed at a high temperature before subsequent use to deactivate AIV and prevent its amplification by RRTPCR.

Wading birds were caught monthly in mist nets over autumn and winter 2019/2020 and 2020/2021 during nights with a waning or new moon to limit the ability of waders to see the nets. Mixed species vocalisation play backs were used to attract waders to the catching areas.

Upon capture, waders were placed into single species holding crates, which were lined with plain paper. Once all birds were processed (biometrics taken and ringed), individual faecal samples were collected from the boxes and placed into 1.5ml microcentrifuge tubes labelled by batch number (for which the ring numbers of birds in each batch were recorded), species and date. Lining paper was replaced between each batch to avoid cross contamination.

A single duck trap was placed on Kilnsea Wetlands (Spurn peninsula) and baited with grain during the winter 2020/2021. Further, dead birds found in the wider Spurn area were collected and sampled for AIV via a cloacal swab.

On 7th December 2019, low pathogenicity H5N3 was confirmed on a commercial poultry farm in mid-Suffolk as part of the UK notifiable disease investigation process. In response to this outbreak, passerines were captured at bird ringing sites nearby (see Figure 11) using mist nets, and faecal samples were collected, as described above.

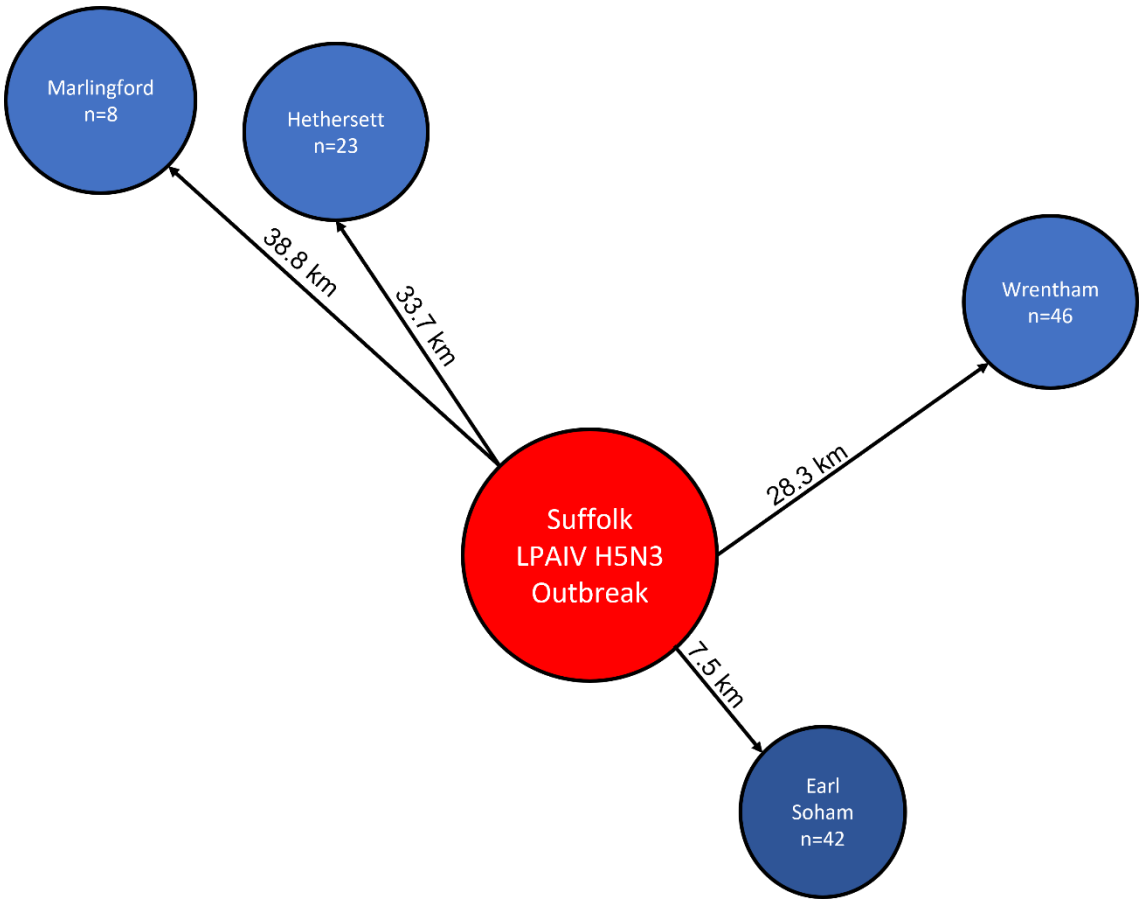

Figure 11: Faecal sampling locations in response to December 2019 LPAI H5N3 captive outbreak near Athelington, Mid-Suffolk. No sampling took place at the outbreak site due to 1km exclusion zone

The Animal and Plant Health Agency (APHA) publishes data on AIV detections in wild birds (APHA, 2022b) in addition to poultry outbreak reports (APHA, 2022a). From these, data on detection time and

species were extracted to compare with data collected during active sampling.

All swabs and faecal samples were stored dry in 35ml centrifuge tubes and placed into a chest freezer at -20 ºC within 24 hours and then stored within a -80 ºC freezer within 4 days of sample collection. Samples were transported on dry ice within 24 hours of removal from -

80 ºC storage for virological investigation at the APHA in Weybridge, Surrey. Samples were retrospectively tested with the Nagy matrix (M)-gene detection real-time reverse transcription polymerase chain reaction (RRT-PCR) for generic detection of AIV RNA (Nagy et al, 2021). Positive samples were then tested by H5-specific RRT-PCR (Slomka and Al, 2007). Samples testing positive by H5-specific RRT-PCR were further tested by a high pathogenicity H5 detection RRT-PCR (James et al, 2022) to confirm the presence of HPAIV H5 in these samples.

To compare the timing of AIV incursion detected by each of the sampling approaches with bird migration trends, data on relative abundance (% of sites in the UK where a species was present in any given week) were downloaded from eBird's Basic Dataset (EBD), a downloadable citizen science repository for bird sightings (Cornell Lab of Ornithology, 2021)

3.4 Results

3.4.1 Active sampling of hunter-harvested wildfowl.

Between 18th October 2020 and 13th January 2021, cloacal swabs were collected from 146 shot birds from 7 different species of waterfowl. A total of 7 shot birds tested positive for AIV (Table 14).

Table 14: Results from cloacal swab sampling for detection of all strains of AIV in Hunter-harvested waterfowl on the Humber Estuary, UK.

Species of waterfowl	Number of samples collected	Number of Positive Samples (% of samples)
Mallard (*Anas platyrhynchos*)	**12**	**1 (8.3%)**
Northern Shoveler (*Spatula clypeata*)	**2**	**0**
Eurasian Teal (*Anas crecca crecca*)	**101**	**4 (4.0%)**
Eurasian Wigeon (*Mareca penelope*)	**23**	**2 (8.7%)**
Greylag Goose (*Anser anser*)	**4**	**0**
Canada Goose (*Branta canadensis*)	**1**	**0**
Pink-footed Goose (*Anser brachyrhynchos*)	**3**	**0**
Total	***146***	***7 (4.8%)***

Low pathogenicity H5 was detected in Teal (1) and highly pathogenicity H5 was detected in Eurasian Teal (1) and Eurasian Wigeon (1) (Table 15).

Table 15: H5 strain and highly pathogenic H5 identification results from retrospective PCR typing from cloacal swab sampling in hunter-harvested waterfowl on the Humber Estuary, UK.

Species	Date of sample collection	H5 HA2 result [26]	HP H5 [27]
Eurasian Teal	**18th October 2020**	-	-
Eurasian Teal	**19th October 2020**	+	-
Eurasian Wigeon	**31st October 2020**	+	+
Mallard	**11th November 2020**	-	-
Eurasian Teal	**11th November 2020**	+	+
Eurasian Wigeon	**28th November 2020**	-	-
Eurasian Teal	**28th November 2020**	-	-

'+'= positive, '-'= negative.

Among teal, one shot on 19th October 2020 had been ringed on the 10th April 2017 at Nidingen, Halland, Sweden, one shot on 28th November 2020 had been ringed at Ottenby, Öland, Sweden on 4th August 2019, and another shot on 28th November 2020 had been ringed in Murmansk Oblast, Russia on 21st July 2016 (see Figure 12).

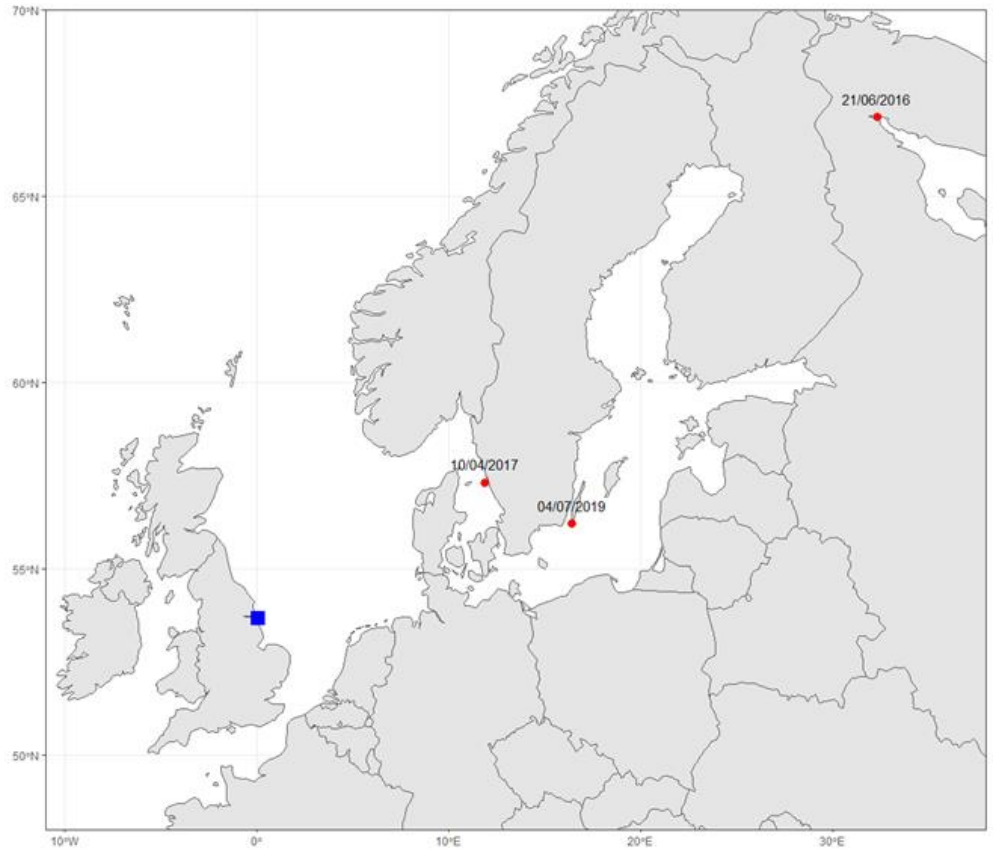

Figure 12: Locations of ringing location of already ringed Eurasian Teal shot on the Humber Estuary during winter 2020/2021.

3.4.2 Active faecal sampling of immigrant birds

A total of 475 faecal samples from 34 species were collected during autumn migration between October 2019 and November 2020. The majority (n = 382) were collected at Spurn peninsula and 66 were collected from Filey Brigg (Table 16).

Table 16: List of faecal samples collected by location, year, and species during active migration sampling for AI.

Species	Filey	Spurn		Total
	2019	2019	2020	
Blackbird (*Turdus merula*)	3	20	1	24
Blackcap (*Sylvia atricapilla*)	3	6	16	22
Blue Tit (*Cyanistes caeruleus*)	13	0	1	14
Brambling (*Fringilla montifringilla*)	1	0	0	1
Bullfinch (*Pyrhulla pyrhulla*)	1	0	0	1
Chaffinch (*Fringilla coelebs*)	2	3	3	8
Chiffchaff (*Phylloscopus collybita*)	0	0	4	4
Coal Tit (*Periparus ater*)	2	0	0	2
Common Whitethroat (*Curruca communis*)	0	0	1	1
Dunnock (*Prunella modularis*)	3	0	9	12
Goldcrest (*Regulus regulus*)	2	2	8	12
Goldfinch (*Carduelis carduelis*)	0	2	4	6
Great Spotted Woodpecker (*Dendrocopos major*)	0	1	0	1
Great Tit (*Parus major*)	2	2	0	4
Greenfinch (*Chloris chloris*)	3	0	0	3
House Sparrow (*Passer domesticus*)	0	4	1	5
Lesser Redpoll (*Acanthis flammea cabaret*)	0	0	55	55
Linnet (*Linaria cannabina*)	0	0	2	2
Long-tailed Tit (*Aegithalos caudatus*)	1	0	0	1
Meadow Pipit (*Anthus pratensis*)	0	0	77	77
Red-flanked Bluetail (*Tarsiger cyanurus*)	1	0	0	1
Redstart (*Phoenicurus phoenicurus*)	0	0	2	2
Redwing (*Turdus iliacus*)	3	11	2	16
Reed Bunting (*Emberiza schoeniculus*)	1	1	12	14
Reed Warbler (*Acrocephalus scirpaceus*)	0	1	0	1
Robin (*Erithacus rubecula*)	7	4	23	34
Sedge Warbler (*Acrocephalus schoenibaenus*)	0	0	1	1
Siskin (*Spinus spinus*)	1	4	1	6
Song Thrush (*Turdus philomelos*)	1	5	4	10
Starling (*Sturnus vulgaris*)	0	0	1	1
Tree Sparrow (*Passer montanus*)	6	54	30	90
Willow Warbler (*Phylloscopus trochilus*)	0	1	1	2
Wren (*Troglodytes troglodytes*)	5	1	1	7
Yellow-browed Warbler (*Phylloscopus inornatus*)	0	1	2	3
Yellowhammer (*Emberiza citrinella*)	1	0	0	1
Total	66	146	262	474

Faecal samples (n = 12) were also collected from 3 species of wader: bar-tailed godwit (*Limosa lapponica*, n = 1), redshank (*Tringa totanus*, n = 10) and knot (*Calidris canutus*, n = 11). The duck trap caught a small sample of waterfowl: mallard (n = 4) and shoveler (n= 1). Finally, four birds were sampled when discovered dead or weakened at Spurn: one each of cormorant (*Phalacrocorax major*), whooper swan (*Cygnus cygnus*), mute swan (*Cygnus olor*) and common scoter (*Melanitta nigra*). None of the active faecal migration samples tested positive for AIV.

3.4.3 Responsive faecal sampling

Faecal samples (n = 119) were collected from 16 species at 4 sites between 7.5 and 38.8km of the outbreak site (see figure 11) as an AIV-infected poultry farm (Table 17), but none tested positive for AIV.

Table 17: The number of faecal samples collected per species during outbreak responsive sampling for AI in Norfolk and Suffolk.

Species	Number of Samples
Blue Tit (*Cyanistes caerulus*)	**59**
Great Tit (*Parus major*)	**7**
Dunnock (*Prunella modularis*)	**11**
Blackbird (*Turdus merula*)	**4**
Bullfinch (*Pyrrhula pyrrhula*)	**1**
Chaffinch (*Fringilla coelebs*)	**3**
Coal Tit (*Periparus ater*)	**2**
Goldcrest (*Regulus regulus*)	**1**
Goldfinch (*Carduelis carduelis*)	**1**
House Sparrow (*Passer domesticus*)	**2**
Long-tailed Tit (*Aegithalos caudatus*)	**3**
Red-legged Partridge (*Alectoris rufa*)	**1**
Reed Bunting (*Emberiza schoeniclus*)	**3**
Robin (*Erithacus rubecula*)	**9**
Wren (*Troglodytes troglodytes*)	**2**
Yellowhammer (*Emberiza citrinella*)	**7**
Total	**119**

3.4.4 Passive sampling

During autumn/winter of 2020/2021, HPAI H5N8 AIV was first confirmed in the UK by passive surveillance of wild birds on the 9th November 2020. The birds (a greylag *Anser anser* and Canada goose *Branta canadensis*) had been found dead on the 3rd November 2020.

Over the outbreak season (November 2020- April 2021), 311 of 1345 different wild bird carcasses from 22 species tested positive for AIV from locations across the UK (Cornell Lab of Ornithology, 2021).

Waterfowl migration into the UK during autumn 2020 (Figure 13) varied by species but increases in numbers of teal began in mid-August, with the highest peak witnessed during the last week of October. Eurasian Wigeon were most abundant with the first influxes detected at the end of August and peaking during December (Cornell Lab of Ornithology, 2021).

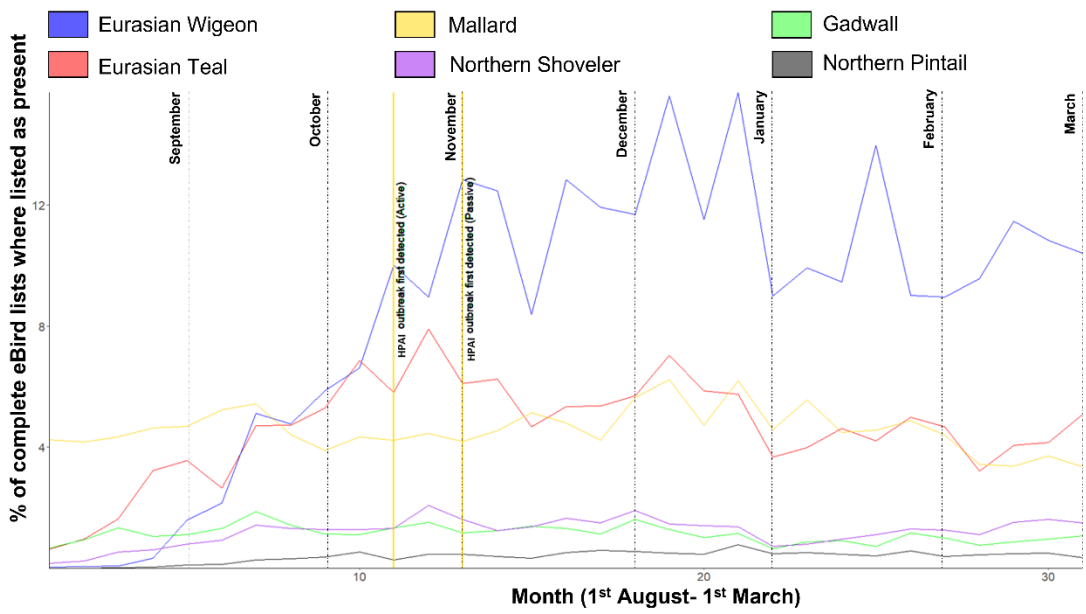

Figure 13: Relative abundance (% of complete ebird species presence checklists where present

per week) of migratory dabbling ducks in the UK from the 1st August 2020 to 1st March 2021 constructed from Ebird abundance data (Cornell Lab of Ornithology, 2021). First detection dates for both passive and active

methods are shown and labelled in vertical yellow lines. Month lines signify the week that included the first day of the month.

3.5 Discussion

During the autumn/winter of 2020/21 LPAI was retrospectively confirmed in a Eurasian teal that had been shot and sampled on 19th October, a full 11 days before LPAI was first detected as part of a statutory notifiable avian disease investigation following a non-negative result from active serological surveillance within poultry. Further, HPAI was retrospectively detected in a hunter-harvested Eurasian wigeon that was shot on 31st October, three days before HPAI was first detected on a poultry farm and three days before HPAI was detected in wild birds as part of the UK AIV passive surveillance scheme (which was confirmed 6 days later). Despite a small sample size (n = 152), 7 ducks tested positive for AIV in 2020 in comparison with no positive results obtained from 474 samples collected from passerines during 2019 and 2020. Moreover, none of 119 samples collected within the locality of an AI infected poultry farm tested positive for AIV. These results further support priority surveillance for AIV in Anseriformes. The percentage of hunter-harvested samples testing

positive for HPAIV was surprisingly consistent with the results of a study involving 4729 hunter-harvested birds in the USA during 2014-15; 1.3%, with Eurasian teal and Eurasian wigeon prevalent within the sample (Bevin et al, 2016).

Use of a walk-in duck trap was proposed to be used to contrast with the other active sampling methodologies, but this element was constrained by delays and sampling only began into the wintering period producing a small number of samples. Other sites within the UK and abroad have used this method for bird ringing of waterfowl (producing a larger sample size (Cromie and Hughes, 2006)) so,

whilst not demonstrated during the current study, live duck traps could offer an alternative active sampling method.

A similar study was conducted in wild waterfowl at two sites in northern Italy between November 2020 and January 2021 where 823 hunted and 521 live captured ducks were sampled (cloacal and oropharyngeal). Results demonstrated higher AIV prevalence than was detected on the Humber, with 6.7% of samples positive for AIV in hunter-harvested birds and 9.7% in samples from birds that were captured and released, compared with 4.8% on the Humber. Whilst AIV detection was most frequent in northern Italy during November and January, different peaks were evident between the two sets of samples. Week 49 and 50 (of the year) showed the largest number of positive samples for live captured birds and week 47

was highest for hunter harvested birds (though no birds were sampled by this method in week 49). Whilst no live captured birds tested positive after the 1st week of January, 5 hunter harvested birds tested positive in the last week of the study (week 4 of the year) indicating that a longer study period may reveal more about changing AIV prevalence in different locations (Gobbo et al, 2021).

The active sampling methods in the current study were highly spatially focussed in comparison with the UK's current passive surveillance of found-dead birds, but these approaches offered the additional advantage of sampling clinically healthy individuals as well as those that had yet to develop symptoms of disease. Asymptomatic but transmissible infections of AIV have been detected in waterfowl and other avian species during challenge experiments and it is plausible that the same findings may be seen in wild birds (Olsen *et al.*, 2006). The importance of infected asymptomatic waterfowl in AIV epidemiology has yet to be fully evaluated (Gaidet et al, 2010).

Passive monitoring did detect infection among wild birds across the UK, but no spatiotemporal pattern was discernible. Most migratory bird species enter the UK from breeding grounds to the east (Scandinavia, Central and Eastern Europe, Arctic Russia), but the first passive detection within British wild birds was recorded in Gloucestershire, in the southwest

of the UK. If Anatidae species are predominantly responsible for the seasonal re-emergence of AIV (Hansen et al, 2018), then it seems likely that the earliest detection of infection is most likely to occur at east coast locations that attract large numbers of immigrant waterfowl, such as the Humber Estuary. Early detection of AIV offers opportunities to better understand the dynamics of the disease (Bevin et al, 2016) and to advise enhanced biosecurity practices among poultry farmers. However, detection of AIV from a larger, more geographically dispersed sample size over a longer study period would be required to afford greater confidence in the ability of surveillance of hunter harvested Anatidae to reliably indicate the seasonal re-emergence of AIV within the UK. Furthermore, similar methods used along the migration pathways of these species would further aid in tracking international AIV dynamics.

Detection of the same strain of AIV among wild birds that occupy poultry farms could help identify those species that pose the greatest risks to poultry (Fuller et al, 2010). However, the responsive sampling of birds on land close to a farm experiencing an outbreak of AIV yielded no samples positive for AIV, probably due to small sample size and too great a distance from the farm. The minimum distance was a statutory limitation and could not have been overcome. At present, any sampling (wild bird or otherwise) for AIV within 5km of an outbreak in poultry can only be performed by trained APHA personnel.

Previous studies investigating AIV prevalence in passerines have mostly detected low levels or no AIV within their samples, though the numbers of studies focussing on wild passerines is heavily outweighed by those focussing on Anatidae. However, this is not universal, with Gronesova et al. (2008) detecting 16% prevalence in both oropharyngeal and cloacal swabs from summering birds at a reedbed site in Slovakia. Han et al., (2012) reported no AIV positive samples from rectum eluate from 1300 tree sparrows (Passer montanus) but 94/800 seropositive samples from the same species indicating that although 94 individuals had been immunologically challenged by AIV, none were actively excreting at the time of sampling. An extensive US study (Fuller et al, 2010) involving the collection of cloacal samples at ringing stations

found that AIV prevalence was higher in passerines than in 8 other sampled orders (n=13,046). Whilst there will likely be differences in the epidemiological network between the new and old world (different species and families), a UK or flyway-wide study of similar magnitude may be required to clarify the potential roles of passerines in AIV epidemiology in Eurasia.

Active sampling of hunter-harvested waterfowl is limited to certain species that can be lawfully harvested (see below) and by the UK open season which covers the period from the September 1st to January 31st under all devolved administrations except the Isle of Man. This

extends to February 20th in England, Wales and Scotland when hunting below the high-water mark. Legal quarry also limits what can be sampled. Gadwall (*Mareca strepera*), common goldeneye (*Bucephala clangula*), mallard, northern pintail (*Anas acuta*), common pochard (*Aythya ferina*), northern shoveler, Eurasian teal, tufted duck (*Aythya fuligula*) and Eurasian wigeon are legal quarry for ducks and Canada goose, greylag goose, pink-footed goose (*Anser brachyrhynchus*) and European white-fronted goose (*Anser albifrons*)can all be lawfully shot, but other duck and goose species and all swans cannot (The British Association for Shooting and Conservation, 2022). These are the most abundant land-based species within the family of Anatidae in the UK with the possible exception of barnacle geese (Branta leucopsis) (Frost et al, 2021).The restrictions of the hunting season, whilst clearly important from a wildlife conservation perspective, limit the ability to utilise this method as a year-round approach to AIV surveillance, and thus is most relevant to detection of autumnal influxes and overwinter fluctuations of AIV in legally huntable waterfowl. Whilst this study has assessed sampling methods for AIV, other avian zoonotic diseases of anthropocentric concern, such as Newcastle disease, could be monitored through a similar scheme.

UK autumn migration in dabbling ducks rose in mid-late August varying by species, with most wintering birds present by mid to late November (peaks for Eurasian teal in October,

Eurasian wigeon and mallard in December (Cornell Lab of Ornithology, 2021)), but the hunter-harvested active sampling

protocol was only implemented from mid-October. Consequently, AI may have been present on the Humber estuary in wild birds before the actual initial detection date. Future research to identify the earliest date of incursion of AI into the UK via wild birds should start sampling Anatidae from 1st September, obtain much larger samples size from a wider distribution of locations. A more precise assessment of new strains of AIV present in wild birds could inform the timing of enhanced and targeted biosecurity practices on poultry farms and captive flocks and hence has potential to enhance preparations for AIV incursions and subsequently reduce the impact of AI during the peak season. Poultry holdings lose their free-range status during winter periods during enforced biosecurity lockdown of free-ranging flocks, which might affect consumers' purchasing decisions.

3.6 Conclusions

Bevin et al. (2016)and Gobbo et al. (2021), have also argued that the hunter network offers a potentially cost-effective approach to AI monitoring. This study has shown with a single sampling site that it was possible to detect AIV in the UK via an active sampling approach before a nationwide passive approach did. Utilization and expansion of a hunter harvested AI surveillance network may provide the UK with an alternative to its current passive surveillance and could allow for important increases in time between AIV detection in the

wild and captive environments. This would allow for increasingly informed decisions on suitable AI mitigation and further understanding of AI dynamics during wild outbreaks.

Red-legged Partridge, mist netted and sampled in East Anglia. 19/01/2020.

Chapter 4 : Wild birds at poultry farms, are species assemblages reflective of current AIV understanding?

4.1 Abstract

In 2022, the UK recorded its highest number of avian influenza (AIV) outbreaks on poultry farms, with a wider range of AIV-positive wild species detected than ever before. However, species commonly associated with agricultural landscapes, where most poultry units are located, were mostly absent from sampling efforts for AIV monitoring purposes in wild birds. We evaluated the potential direct exposure risk posed to poultry by wild birds observed around poultry farms in Yorkshire, UK during 2019 and 2020 by point-counting the prevalence of wild birds around housed poultry units. Species within Anatidae were estimated to pose only small exposure risks to poultry whereas several species less frequently implicated in AIV epidemiology were estimated to pose greater risks. Moreover, exposure scores attributed to each species varied between zones with increasing distance from poultry houses. While there is considerable uncertainty associated with exposure risk scores, we propose a prioritised list of wild bird species that merit closer inspection of their role in AIV epidemiology to better target mitigation approaches to AIV at poultry holdings.

4.2 Introduction

Pathogens of wild animals pose significant threats to the security of livestock-derived economies worldwide (Dudley, 2006, Souris et al., 2014). Avian influenza virus (herein AIV) has a natural reservoir within wild birds, with Anatidae commonly implicated in its epidemiology (Hénaux *et al.*, 2012). AIV was first identified within the poultry industry and was referred to under different names (e.g., fowl plague) until its identification as influenza in 1955 (Schäfer, 1955) with known outbreaks among wild birds dating back to the 1950s(Adlhoch *et al.*, 2022). Throughout Europe (until 2021), AI outbreaks at poultry farms were considered winter seasonal events matching the immigration of waterfowl, with the number of outbreaks varying between years (Adlhoch and Baldinelli, 2023). In winter 2021, highly pathogenic AIV H5N1 became widespread around the world (Adlhoch and Baldinelli, 2023) and persisted across Europe during the summer for the first time, with many outbreaks among poultry farms (Adlhoch *et al.*, 2022). In 2022-2023, H5N1 AIV

has been recorded in multiple mammalian species worldwide, with high mortality rates among pinnipeds (Stokholm *et al.*, 2023) and farmed American mink (Neovison vison) (Aguero *et al.*, 2023). In late February 2023, the World Health Organisation declared concern over the death of a 12-year-old girl in Cambodia from infection of H5N1 (Wilson, 2023). Contact tracing identified a further infected individual though person-to-person transmission was not confirmed. Wild birds act as a reservoir for AIV, and their role in mammalian spillover is not fully understood. The high pinniped mortality was co-occurrent with large seabird mortality events (Stokholm *et al.*, 2023), with the leading hypothesis that infection was driven by sea lions consuming infected carrion. In human outbreak cases, most (including the Cambodia case (Wilson, 2023)) were due to proximity with infected poultry, often in less economically developed countries in Southeast Asia. Since 2021, six cases of H5N1 in people have been identified leading to two deaths. There is international concern for a repeat of outbreaks in the early 21st century when over 800 infections were detected with over 400 deaths. Mammalian spillover is considered the biggest step for significant human health concerns in zoonotic viruses.

In the UK, the statutory response to an AIV outbreak at a poultry holding includes culling the entire exposed flock (all those potentially in contact with infected birds) and restriction zones for trade and movements of birds from infected premises until demonstrated to be AIV-free(Bisdounis, 2022). During significant national outbreaks, all free-ranging poultry flocks of any size are legally required to be housed, something that has occurred during the last 3 winters (from 2022). If housing lasts longer than 16 weeks, this action removes the free-range status of flocks (DEFRA, 2023). During 2022 and despite precautionary measures, the UK recorded its highest number of AIV outbreaks on poultry farms.

The poultry industry in the UK provides one of the biggest sources of protein for human consumption, greater than that provided by beef, pork, fish, and other meat produce, with an annual revenue of £2.9 billion (Shabandeh, 2022). In September 2022, 94.2 million domesticated birds were slaughtered for use in the international food supply chain.

AIV spreads internationally with migrating waterfowl (Keawcharoen *et al.*, 2008) the majority of which do not typically coincide spatially with poultry holdings (Fox *et al.*, 2016). Consequently,

transmission of AIV from waterfowl to poultry is believed to be indirect and to involve one or more intermediate species, known as bridge species (Caron *et al.*, 2014). However, the routes of interspecific transmission remain unknown. It is considered that transmission between and from bridge species to poultry can be direct (i.e., via close contact) or indirect (i.e., via contaminated fomite), but the relative importance of each potential route has yet to be quantified.

Poultry farms themselves provide shelter and habitat in vast agricultural landscapes, with environmental factors such as hedgerows and trees providing wild bird attraction points close to poultry housing units. Food and water provisions (for both poultry and wild birds at feeding stations) within proximity to poultry holdings present potential resource provisions for wild birds bringing them into closer proximity with poultry holdings.

We sought to prioritise wild bird species by the potential AIV exposure risk that they pose to poultry by the frequency of their presence in three different zones of increasing risk of exposure. We posited that species in closest and most frequent contact with poultry likely posed the greatest exposure risks, and so merit further evaluation concerning their role in AIV epidemiology. A blended fieldwork and modelled approach were conducted to count and estimate the abundance of wild bird species around poultry farms across Yorkshire to inform exposure risk models, including models factoring in AIV prevalence estimates from Chapter 2. This research aims to identify the species most prevalent at our sampled poultry farms and compare these findings to the literature to identify differences and commonalities.

4.3 Materials and Methods

Between December 2019 and March 2020 (and during a pilot in February 2019), point counts were conducted at nine housed poultry units across Yorkshire, UK.

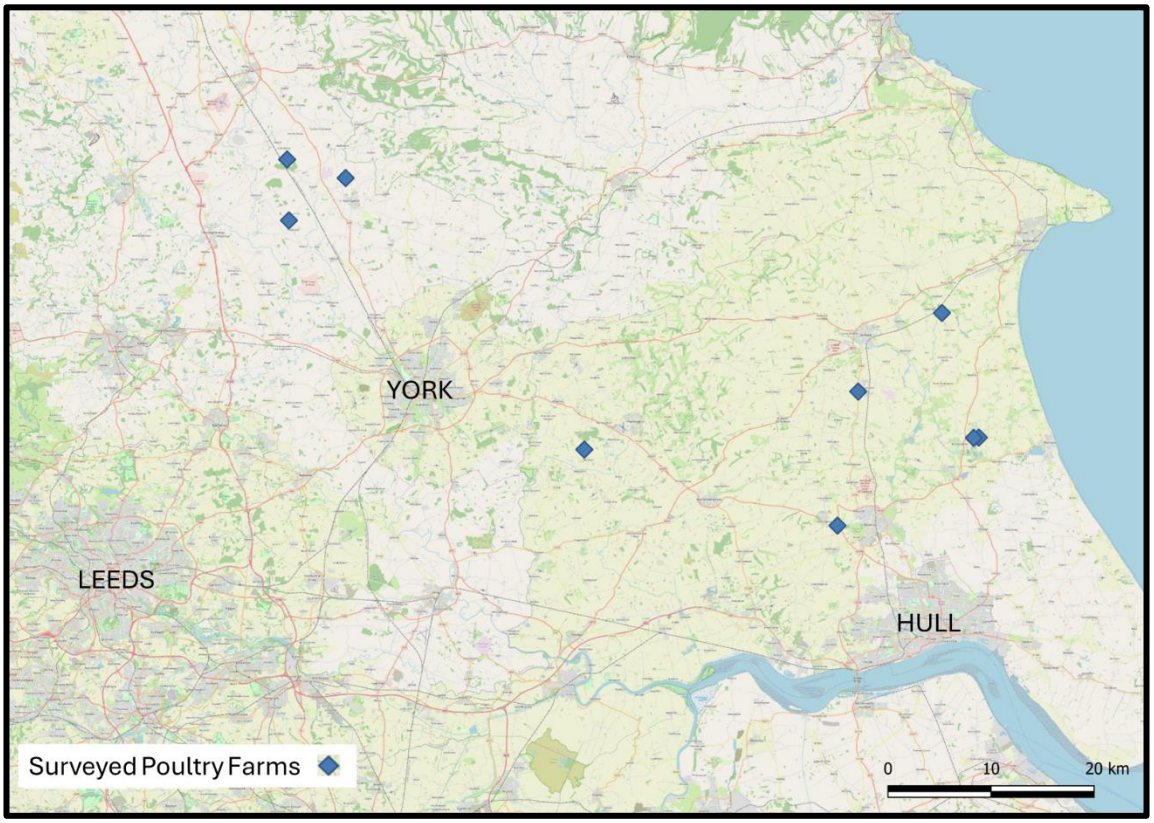

Figure 14: Mapped distribution of point count sampled poultry farms in Yorkshire, UK.

Counts were conducted of wild and free-roaming birds within 50 metres of a poultry holding with birds being recorded as flyover, present within 50m of the poultry shed, and present within the area inside of biosecurity fencing surrounding a poultry unit.

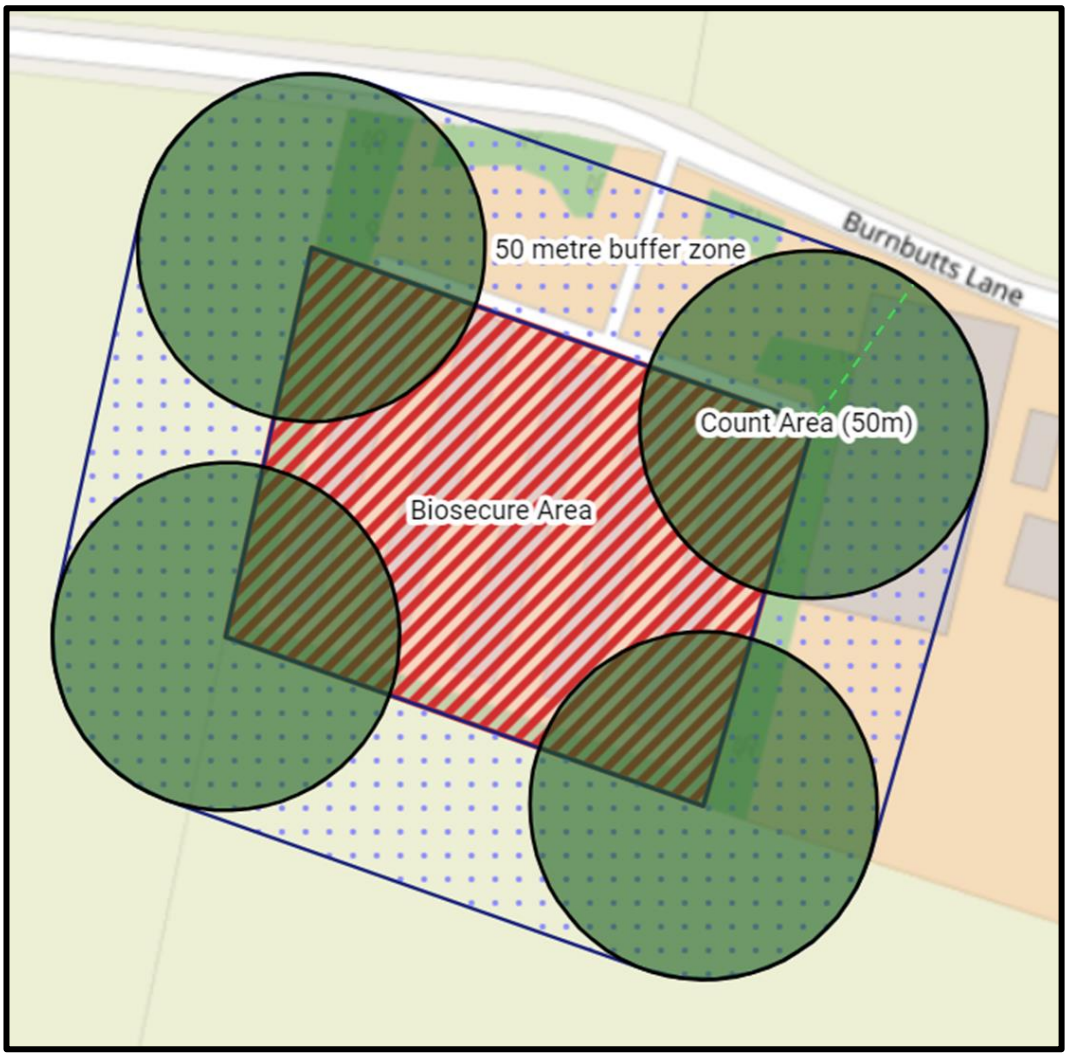

Figure 15: A representative example how non-overlapping point count samples taken from poultry farm sites.

Four counts by a single observer took place on each site on a survey morning, each lasting 10 minutes without spatial or temporal overlap. The location of each count was roughly associated with each corner of a quadrilateral poultry holding where possible, or as close as possible as to avoid count area overlap.

A zoonotic exposure model (ZEM) was constructed to the following formula:

Exposure Score (E)= Abundance of a species on point counts when present (a) multiplied by (total occasions a species is present across surveys (p) divided by total number of surveys (t))

$$E = a\,\frac{p}{t}$$

Error was calculated via Monte Carlo simulations (n=1000) through triangular distribution (mode, max and min for abundance and Jeffries lower and upper credible interval and proportion of time present for presence).

When considering avian influenza-specific exposure scores (AISES), the following formula was constructed:

Avian Influenza Specific Exposure score (E) = Abundance of a species on point counts when present (a) multiplied by (total occasions a species is present across surveys (p) divided by total number of surveys (t)) multiplied by mean body mass (Dunning, 2007) (m) multiplied by AIV prevalence rate (as calculated in chapter 2 of this thesis) (I).

$$E = a(\frac{p}{t})mI$$

Monte Carlo simulations (n=1000) were again used to inform error, with AIV prevalence taken from a triangular distribution based on AIV prevalence estimates for each species with Jeffreys Intervals used as credible intervals. Mean mass, taken from Dunning Jr, (2007)did not have data for

error in all cases and was not factored into the final models. Where mean mass was split by sex, a mean average was taken from both sexes presuming equal proportions within a given population.

Body mass (Dunning Jr, 2007) was used as a proxy metric for faecal size and lung capacity, as routes of viremia. Excretion per unit weight was assumed to be equal across all species in the absence of quantifiable data.

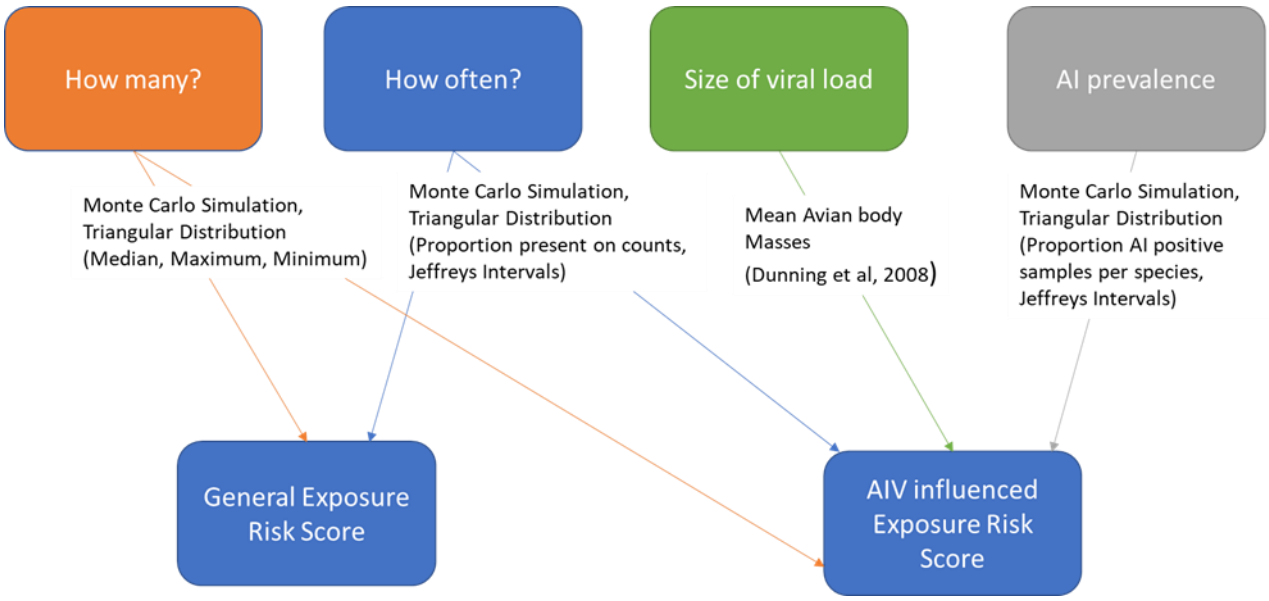

Figure 16: Model design for quantification of exposure risk within the 3 calculated risk areas at poultry holdings.

Each species-specific general zoonotic and avian influenza-specific exposure model was expressed with a mean and lower and upper quartile range. A model was constructed for each count zone (birds within the biosecurity fencing, birds utilising the area within 50m of the point count location and flyover birds). All model construction was done using R Studio (Team R, 2008, Carnell, 2022 ,Wickham, 2016 ,Venables and Ripley, 2002).

4.4 Results

A total of 8175 individuals of 73 wild and free-ranging species of bird were recorded across 148-point counts at 9 different poultry holding sites. Full species lists and totals and exposure scores are in the supplementary materials.

The mean Exposure Score for the ZEM (see Figure 17) for species recorded within the bio-secure fencing at the surveyed poultry sites was highest for Pied Wagtail (*Motacilla alba yarelli*, 0.76), Yellowhammer (*Emberiza citrinella*, 0.24), Eurasian Tree Sparrow (*Passer montanus*, 0.14), Dunnock (*Prunella modularis*, 0.08) and Eurasian Blue Tit (*Cyanistes caeruleus*, 0.06).

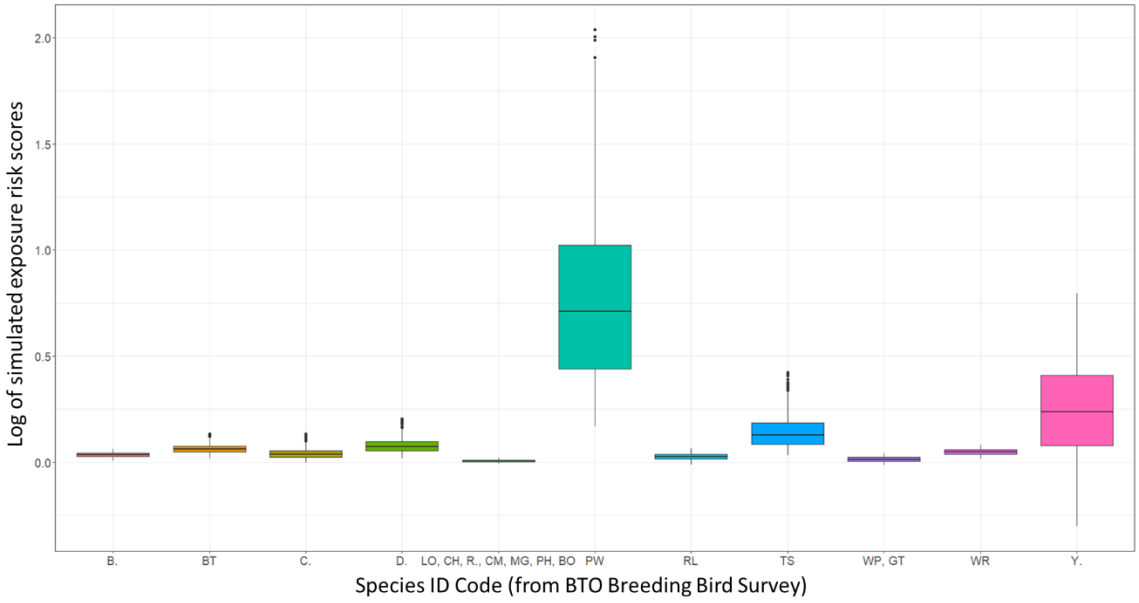

Figure 17: Boxplots of general exposure scores for species recorded within biosecurity fencing at poultry farms. BTO codes as follows B. Blackbird, BT Blue Tit, C. Carrion Crow, D. Dunnock, LO Little Owl, CH Chaffinch, R. Robin, CM Common Gull, MG Magpie, PH Pheasant, BO Barn Owl, PW Pied Wagtail, RL Red-legged Partridge, TS Tree Sparrow, WP Woodpigeon, GT Great Tit, WR Wren, Y. Yellowhammer.

Species' exposure scores to poultry farms from the ZEM for species detected within 50m of the bio-secure fencing (see Figure 18) varied from scores from species detected within the bio-secure fencing. The top-ranking species were Common Woodpigeon (*Columba palumbus*, 8.46), Eurasian

Starling (*Sturnus vulgaris*, 3.23), Common Blackbird (*Turdus merula*, 2.79), European Blue Tit (2.13) and Eurasian Tree Sparrow (1.99).

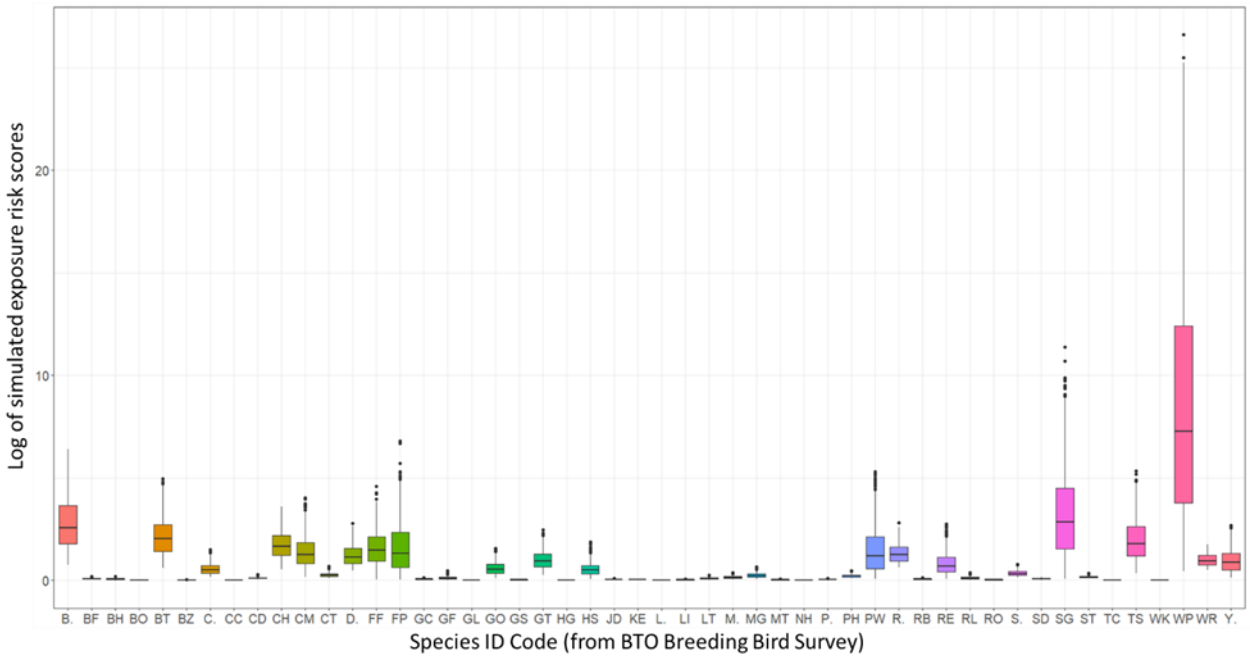

Figure 18: Boxplots of general exposure scores for species recorded outside of biosecurity fencing at poultry farms. BTO codes as follows. B. Blackbird, BF Bullfinch, BH Black-headed Gull, BO Barn Owl, BT Blue Tit, BZ Buzzard, C. Carrion Crow, CC Chiffchaff, CD Collared Dove, CH Chaffinch, CM Common Gull, CT Coal Tit, D. Dunnock, FF Fieldfare, FP Feral Pigeon, GC Goldcrest, GF Greenfinch, GL Grey Wagtail, GO Goldfinch, GS Great Spotted Woodpecker, GT Great Tit, HG Herring Gull, HS House Sparrow, JD Jackdaw, KE Kestrel, L. Lapwing, LI Linnet, LT Long-tailed Tit, M. Mistle Thrush, MG Magpie, MT Marsh Tit, NH Nuthatch, P. Grey Partridge, PH Pheasant, PW Pied Wagtail, R. Robin, RB Reed Bunting, RE Redwing, RL Red-legged Partridge, RO Rook, S. Skylark, SD Stock Dove, SG Starling, ST Song Thrush, TC Treecreeper, TS Tree Sparrow, WK Woodcock, WP Woodpigeon, WR Wren, Y. Yellowhammer.

Species' scores for the ZEM for species flying over poultry holding sites (Figure 19) varied from the 50m and inside fence models with highest scores for Common Woodpigeon (40.80), Common

Starling (28.23), European Herring Gull (*Larus argentatus*, 20.02), Western Jackdaw (*Corvus monedula*, 5.17) and Common Gull (*Larus canus*, 2.55).

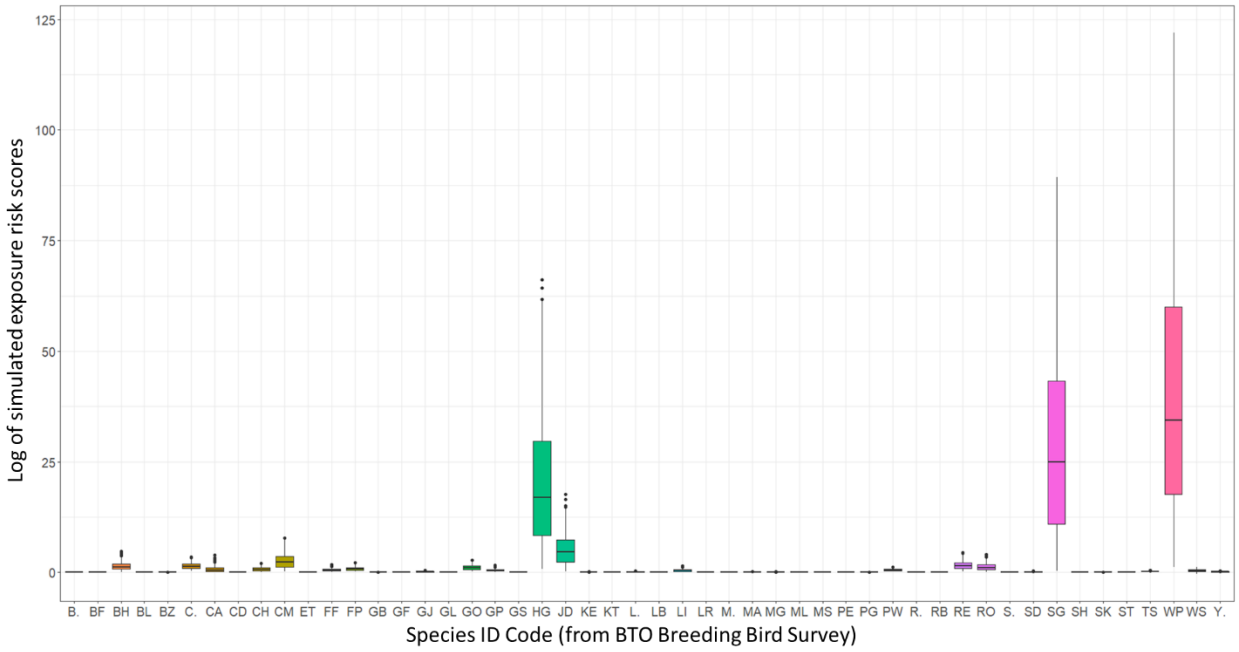

Figure 19: Boxplots of general exposure scores for species recorded as flyover at poultry farms. BTO codes as follows. B. Blackbird, BF Bullfinch, BH Black-headed Gull, BL Brambling, BZ Buzzard, C. Carrion Crow, CA Cormorant, CD Collared Dove, CH Chaffinch, CM Common Gull, ET Little Egret, FF Fieldfare, FP Feral Pigeon, GB Great Black-backed Gull, GF Greenfinch, GJ Greylag Goose, GL Grey Wagtail, GO Goldfinch, GP Golden Plover, GS Great Spotted Woodpecker, HG Herring Gull, JD Jackdaw, KE Kestrel, KT Red Kite, L. Lapwing, LB Lesser Black-backed Gull, LI Linnet, LR Lesser Redpoll, M. Mistle Thrush, MA Mallard, MG Magpie, ML Merlin, MS Mute Swan, PE Peregrine, PG Pink-footed Goose, PW Pied Wagtail, R. Robin, RB Reed Bunting, RE Redwing, RO Rook, S. Skylark, SD Stock Dove, SG Starling, SH Sparrowhawk, SK Siskin, ST Song Thrush, TS Tree Sparrow, WP Woodpigeon, WS Whooper Swan, Y. Yellowhammer.

When factoring in mass and average AIV prevalence data, scores were created for the AISES models

For AISES of species recorded within the bio-secure fencing at the surveyed poultry sites (Figure 20), the highest scoring species were Red-legged Partridge (*Alectoris rufa*, 2.71), Pied Wagtail (0.26), Eurasian Tree Sparrow (0.34), Carrion Crow (*Corvus corone corone*, 0.16) and Eurasian Magpie (*Pica pica*, 0.15)

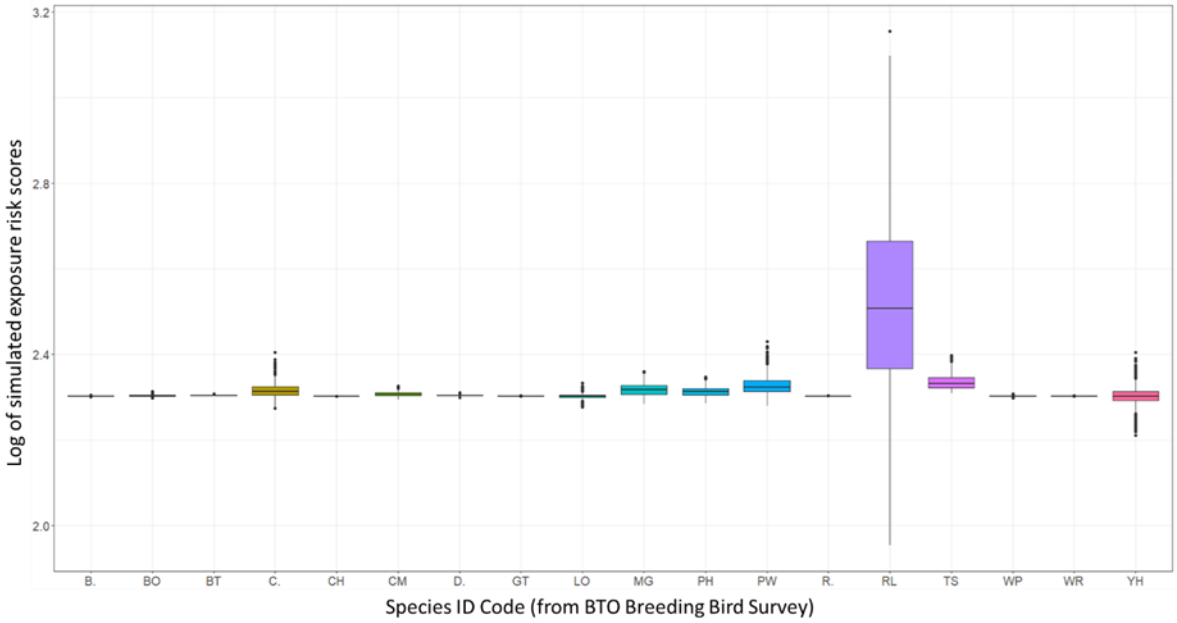

Figure 20: Boxplots of AIV influenced exposure scores for species recorded within biosecurity fencing at poultry farms. BTO codes as follows B. Blackbird, BT Blue Tit, C. Carrion Crow, D. Dunnock, LO Little Owl, CH Chaffinch, R. Robin, CM Common Gull, MG Magpie, PH Pheasant, BO Barn Owl, PW Pied Wagtail, RL Red-legged Partridge, TS Tree Sparrow, WP Woodpigeon, GT Great Tit, WR Wren, Y. Yellowhammer.

The AIVERS model scores for species utilising habitat within 50m of the bio-secure fencing (Figure 21) at surveyed poultry holding sites showed high scorers of Red-legged Partridge (12.26), Feral Pigeon (*Columba livia domestica*, 11.88), Common Gull (9.98), Eurasian Magpie (5.84) and Eurasian Tree Sparrow (4.67).

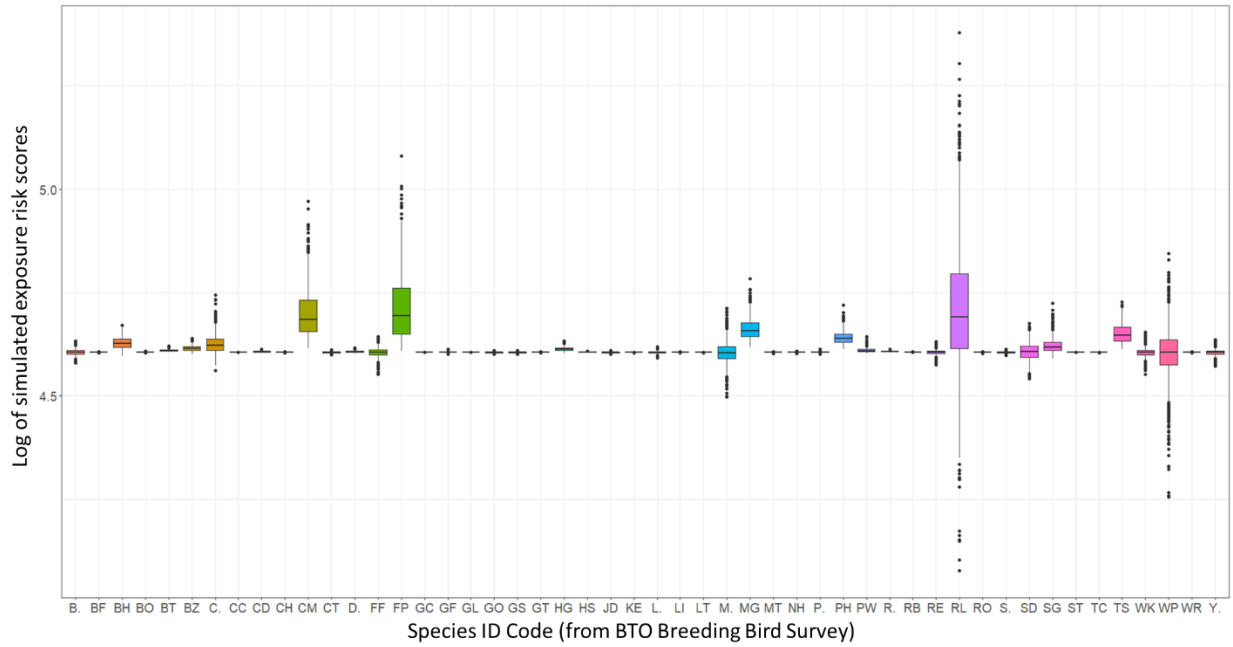

Figure 21: Boxplots of AIV influenced exposure scores for species recorded outside of biosecurity fencing at poultry farms. BTO codes as follows. B. Blackbird, BF Bullfinch, BH Black-headed Gull, BO Barn Owl, BT Blue Tit, BZ Buzzard, C. Carrion Crow, CC Chiffchaff, CD Collared Dove, CH Chaffinch, CM Common Gull, CT Coal Tit, D. Dunnock, FF Fieldfare, FP Feral Pigeon, GC Goldcrest, GF Greenfinch, GL Grey Wagtail, GO Goldfinch, GS Great Spotted Woodpecker, GT Great Tit, HG Herring Gull, HS House Sparrow, JD Jackdaw, KE Kestrel, L. Lapwing, LI Linnet, LT Long-tailed Tit, M. Mistle Thrush, MG Magpie, MT Marsh Tit, NH Nuthatch, P. Grey Partridge, PH Pheasant, PW Pied Wagtail, R. Robin, RB Reed Bunting, RE Redwing, RL Red-legged Partridge, RO Rook, S. Skylark, SD Stock Dove, SG Starling, ST Song Thrush, TC Treecreeper, TS Tree Sparrow, WK Woodcock, WP Woodpigeon, WR Wren, Y. Yellowhammer.

The final AISES model (Figure 22) for species recorded flying over poultry holding sites again demonstrated variance to its ZEM partner model, with Whooper Swan (*Cygnus cygnus*, 1435.79),

European Herring Gull (1183.26), Black-headed Gull (*Chroicocephalus ridibundus*, 47.85), Common Gull (18.50) and Great Cormorant (*Phalacrocorax carbo*, 16.9) scoring highest.

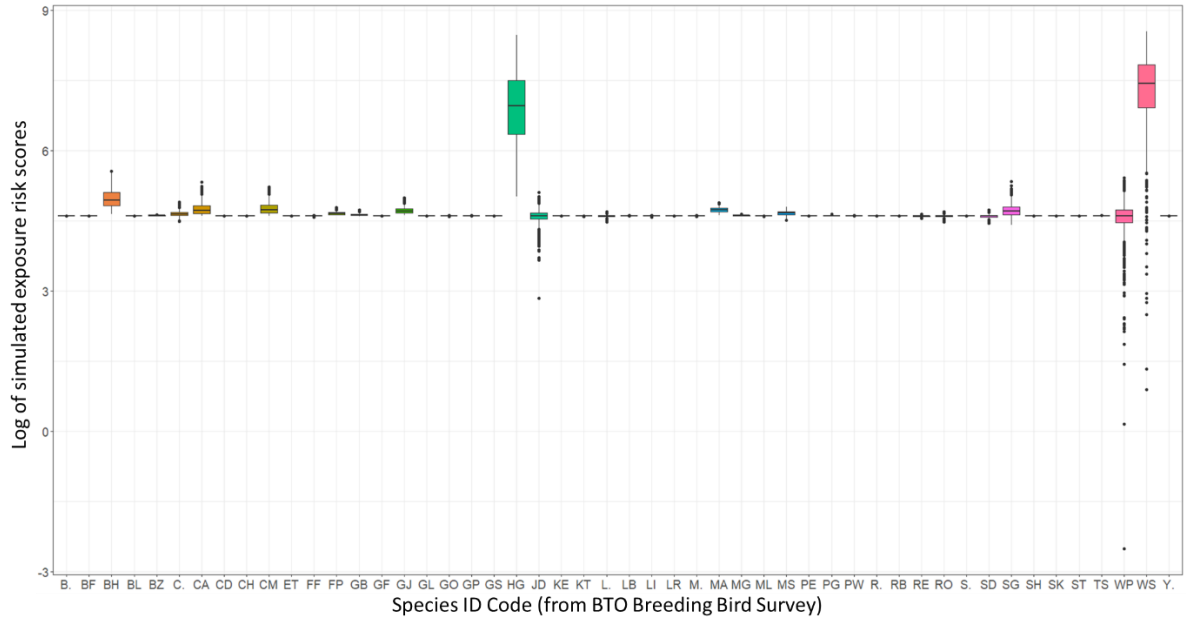

Figure 22: Boxplots of AIV influenced exposure scores for species recorded as flyover at poultry farms. BTO codes as follows. B. Blackbird, BF Bullfinch, BH Black-headed Gull, BL Brambling, BZ Buzzard, C. Carrion Crow, CA Cormorant, CD Collared Dove, CH Chaffinch, CM Common Gull, ET Little Egret, FF Fieldfare, FP Feral Pigeon, GB Great Black-backed Gull, GF Greenfinch, GJ Greylag Goose, GL Grey Wagtail, GO Goldfinch, GP Golden Plover, GS Great Spotted Woodpecker, HG Herring Gull, JD Jackdaw, KE Kestrel, KT Red Kite, L. Lapwing, LB Lesser Black-backed Gull, LI Linnet, LR Lesser Redpoll, M. Mistle Thrush, MA Mallard, MG Magpie, ML Merlin, MS Mute Swan, PE Peregrine, PG Pink-footed Goose, PW Pied Wagtail, R. Robin, RB Reed Bunting, RE Redwing, RO Rook, S. Skylark, SD Stock Dove, SG Starling, SH Sparrowhawk, SK Siskin, ST Song Thrush, TS Tree Sparrow, WP Woodpigeon, WS Whooper Swan, Y. Yellowhammer.

The AISES models utilised AIV prevalence data from Chapter 2 (see Appendices). Sample sizes and AIV detection rates varied between species, resulting in high variation in between-species prevalence estimates.

4.5 Discussion

Despite higher focus from researchers on AIV dynamics, Anatidae has little exposure to housed poultry sites. However, annual outbreaks within housed poultry sheds occur in the UK. This leads to speculation on how AIV is getting into these sites. When considering wild birds as a potential vector for AIV spread, generalist passerines had the largest exposure risk to captive poultry sheds with Pied Wagtail and Tree Sparrow scoring comparatively higher than other species detected within the biosecurity fencing count zone.

Each modelled count zone represents a different exposure landscape. The area within biosecurity fencing represents the landscape closest to poultry but falls shy of direct bird-to-bird exposure. Wild birds counted in this landscape present risk by deposition of fomite. Fomite refers to faecal matter, exhaled air, and saliva (Capua and Alexander, 2007). The source of fomite may be from the individual bird itself or transfer into the area on feathers or claws from its original deposition site and species. Wild birds within biosecurity fencing are the group most likely to find access to poultry-holding units through holes in walls. The risk is perceived to be reduced across the other two risk areas, though the understanding of how much remains uncalculated. Whilst outside the biosecurity fencing, risk comes from deposited fomite being brought into the bio-secure area by vectors moving into this area. Flyover risk further reduces the chance of fomite deposition whilst over poultry farm airspace (or close to it), which would then need to be transferred into poultry holdings through other means.

With the greatest perceived risk being posed by species present within biosecurity-fenced areas, it is important to consider the characteristics of all species registered within this area. Pied Wagtail stands out within this area as the species with the greatest general exposure risk, followed by Yellowhammer and Tree Sparrow. Uncertainty is higher for Yellowhammer; abundance was high in a few instances but rare within the count area otherwise. From counter observations, Pied Wagtails were most often seen on the roofs of poultry holding sheds. Other species were more regularly seen using floor space around the sites rather than the sheds themselves, bar both Great and Blue Tit and Tree Sparrow. Tree Sparrows and Blue Tits were both observed using holes in the side of poultry holding sheds likely once used to feed pipes into sheds and not covered over once the pipes

had been removed. From a wild bird perspective, poultry sheds provide provision of food, water and shelter for those species that can get inside them. In terms of infection risk, cavity-nesting species of small body mass are most likely to be able to access poultry sheds where direct exposure is possible. Birds using roofing such as Pied Wagtail will be depositing fomite which might contaminate the drainage system and find its way through leaks in structures. It is important to maintain human biosecurity before entering sheds as any materials picked up by foot on the outside of sheds could contain contaminated materials.

Species present in the landscape outside the biosecurity fencing are more numerous than those present within it. Woodpigeon stands out strongly amongst risk scores, followed by Starling. Below this, species are more closely matched. The epizootiological roles of these species refer to the deposition and movement of fomite into this count area. Species here don't present a risk of direct contact with poultry, but species present within the biosecurity fencing and outside it in the proximity landscape could move fomite from species only present outside the fencing into poultry sheds.

The final count area is species seen flying over the poultry holding sheds. These birds represent the smallest risk to poultry as their chances of deposition of fomite are, whilst difficult to quantify, undoubtedly small. Standout species with the highest risk scores from flyover counts were Woodpigeon, Starling, and Herring Gull. Whilst it is a sizeable piece of research to undertake, understanding the deposition rates of AIV-infected individual birds and the variances between them (and variance between species) would help to refine the models.

When factoring in AISES, the trend visible within each of the AISES scores is for larger species to be represented with higher risk scores due to the increased size of individually sourced fomite. Factoring in AIV prevalence data increases variance in modelled AISES often due to small sample sizes in AIV prevalence data for many of the species present on the counts. For example, Whooper Swan scores highest for flyover AISES scores, but in real terms, this is a single incidence of 57 birds flying over one site. Scores suggest this instance demonstrates higher risk than the total flyover abundance of over 400 Woodpigeons. Whooper Swan has an AIV prevalence estimate of 41.38%

with a Jeffreys interval of 17.21, whereas Wood-pigeon have an AIV prevalence estimate of 0% with a Jeffreys interval of 0.38.

Future refinement to the ZEM models would look to include a measure for the duration for individuals of a species within a count area. Currently, each count has the same duration (10 minutes) which acts as a proxy for to exact duration of wild bird presence at a poultry farm by calculating the proportion of times a species is listed as present. The implications of this are less nuance to the models, but the trends demonstrated should be characteristically similar.

The AISES models contain further assumptions, the first in the use of a proxy for excreted viral load, and the second being an unequal understanding of variation in AIV prevalence amongst species. The body mass proxy for excreted viral load follows the assumption that the viral load excreted is directly proportional to body mass. AIV prevalence rates were used from Chapter 2, which demonstrates considerable sampling bias between different families and species of wild birds. Jeffreys intervals were constructed to inform variation in prevalence due to sample size. However, each AIV prevalence estimate is for avian influenza in general, not strain-specific. Variation is to be expected between different strains and their subsequent transmissibility within and between different species. To improve margins of uncertainty within AISES, further research is needed into AIV prevalence rates (Caron, Cappelle and Gaidet, 2017). AIV sampling bias in wild birds is biased towards members of Anatidae, and as such the base knowledge on AIV prevalence is greater, with evidence for long-distance transmission, Anatidae species acting as natural AIV reservoirs, and asymptomatic infection (Hénaux *et al.*, 2012). Evidence of this kind of dynamics in other families is lacking, but without more sampling, we cannot be certain about further species' roles in the epidemiological landscape (Caron, Cappelle and Gaidet, 2017).

Whilst margin of error on mass was not used in the models, size regularity assumption states minimal variance in body size around the mean and as such this is not considered a major detraction from the model outputs.

Existing European risk scores for species from Veen et al, (2007) did not show a significant correspondence with the ZEM and AISES models. Whilst our AISES models need more data to

reduce uncertainty derived from AIV prevalence estimates, the project highlights that assumptions don't match across to studies attempting to quantify risk on a European basis and that a more spatially refined metric should be looked at. For example, Tree Sparrow is much more common within Yorkshire than in most areas of the UK (Woodward *et al.*, 2018). Variation amongst scores would be expected between different areas on a national and international basis.

The models take a working back approach to disease transmission, looking at which species is present at interest sites, rather than which species is infected; assuming that all birds could get infected rather than focussing on the species known as carriers. Very little is known about short-range transmission of AIV, and how long the virus lasts in the highest-risk-scoring candidate species (Yasué *et al.*, 2006). At this stage it is hard to decipher if a single large abundance event like a flyover of gulls commuting to a roost site presents a larger risk than a sedentary bird that is frequently active within the closest areas to poultry housing, or if birds consistently within biosecurity fencing are exposed consistently to species with known higher AIV prevalence in other areas of their ranges (i.e., Anatidae). Much of the research and metrics of risk at poultry farms are calculated from landscape factors, namely waterbody presence within the landscape due to the association of known high AIV prevalent species to these habitats. Risks identified with these metrics such as those published in Hill et al (Hill *et al.*, 2019) show high levels of overlap with outbreak locations (APHA, 2023a) but the exposure scores within this article suggest low levels of exposure from waterbody-centric species. The mismatch identified further highlights that the short-distance movement of AIV in the landscape needs further research to identify how AIV is getting into the UK poultry system. The increasing regularity of outbreaks within housed poultry suggests it is more likely that common exposure species are playing a greater role than occasional exposure from species identified by Hill et al (2019).

To reduce transmission risk between wild birds and poultry within poultry holding sheds, the simplest suggestion would be to ensure the covering of holes and other entry routes into the holdings. Any provision of grain within the sheds will attract granivorous birds if they can access the resource. Whilst free-ranging poultry sites have not been assessed in these models, the decision was made to look closely at housed poultry units as these represent all commercial sites during a

housing order where AIV is most regularly being detected in the wild bird population. Whilst the comparison with Veen et al. (2007) showed it was difficult to compare across the whole of Europe, Le Gall-Ladeveze et al. (2022) demonstrated similar species or groups of species (White Wagtail, a different subspecies to the UK's Pied Wagtail, and Sparrows (plural)) with high levels of interactions with domestic ducks at a free-range poultry farm in southwest France. A further recommendation would be to attract wild birds away from poultry holdings, potentially with habitat or food provisions away from poultry holding sites. Any discouragement of birds away from poultry holding sites should address the conservation status and implications for the affected species (Stanbury *et al.*, 2021). A further observation made during point counts was that many poultry holdings have mossy roofs and backed-up roof drainage. Both provide resources of utility to wild bird species, and both could be resolved to discourage site usage with routine clearing. Observations were made of bird feeders and boxes near poultry sheds, this would also likely attract a higher rate of exposure to the site from wild birds.

Further work should look beyond wild birds to other avenues of fomite transportation close to poultry farms. Rodents are abundant in the agricultural landscape and may act as a final vector through direct or indirect transmission into poultry holding sheds and have been proven to shed AIV in laboratory studies (Velkers *et al.*, 2017).

To conclude, the understanding of wild bird zoonotic risk at poultry farms is at this time reliant on the knowledge of who is present and for how long, until such a time as sampling effort on the present species can add a reliable variable for AIV or other zoonotic prevalence and regularity of viral excretion. Until this is further understood, generic zoonotic exposure models provide the best alternative to help quantify the risk posed by wild birds to housed poultry.

Whilst more traditionally associated AIV spreading species are present in low numbers in the landscape as flyovers, with Gulls also present in the landscape outside the biosecurity fencing, only a single incidence of one Common Gull represents a waterbody centric species within biosecurity fencing. All species within biosecurity fencing should be considered for future research, with a specific focus on Pied Wagtail.

Chapter 5 : Assessing the utility of citizen science in modelling avian influenza transmission networks at wetland sites

5.1 Abstract

There is a poor understanding of the risks and pathways from which wild birds can transmit AIV to poultry farms. This study constructs exposure risk models using Monte Carlo simulations from citizen science datasets of bird occurrences at waterbodies in East Yorkshire in an attempt to connect two key risk habitats. Three models were constructed looking at the frequency of occurrence of high poultry exposure risk species at waterbodies, one for their co-occurrence with Anatidae (a high AIV risk family) species at waterbodies, and an additional co-occurrence model including estimates for AIV prevalence and shedding rates in Anatidae. Risk scores for each poultry exposure risk species were largely uniform for each of the three models, with three distinct categories emerging, those with the highest comparative exposure risk, those with moderate comparative exposure risk and those with lower comparative exposure risk. Both Common Gull (*Larus canus*) and Wood-pigeon (*Columba palumbus*) scored in the higher category for each model as the most prevalent high poultry exposure risk species at waterbody sites, with Yellowhammer (*Emberiza citrinella*), Red-legged Partridge (*Alectoris rufa*), Little Owl (*Athene noctua*) and Barn Owl (*Tyto alba*) scoring lowest at the other end of the prevalence scale. Most high poultry exposure risk species represent species underprioritized for sampling for avian influenza prevalence and are common generalist species within the wider landscape. Whilst the mechanisms behind the spread between waterbodies and poultry sites are poorly understood, the models demonstrate insight into how poultry risk species occur within two high-interest landscapes for zoonotic exposure.

5.2 Introduction

The UK is currently undergoing the worst outbreak of AIV in its recorded history. In 2022, significant summer persistence of AIV was detected for the first time (HPAI H5N1) having substantial negative financial and biodiversity conservation consequences from both financial and conservation perspectives (Pearce-Higgins *et al.*, 2022). Wild birds provide a reservoir for AIV, with research suggesting a primary reservoir in waterfowl for H5N1, with spillover into other families

with research demonstrating bias towards the Anatidae family (Caron, Cappelle and Gaidet, 2017). Our understanding of the dynamics in AIV movements through species of other families is less formed, but evidence indicates lower AIV prevalence in most other families (see Chapter 2). The lack of knowledge of AIV prevalence outside Anatidae restricts our understanding of how AIV might move through the landscape and impact birds not associated with waterbodies or poultry farms.

AIV has had a global conservation impact on multiple bird species; within the UK the biggest concern is the internationally important seabird populations that breed in coastal areas and conservation organisations and the UK government have created an action plan to help seabird colonies (Pearce-Higgins *et al.*, 2022). Conservation measures that reduce virus transmission might include the removal of dead birds at colonies and roost sites and in extreme instances, dispersal of colonies where outbreaks occur, though both have undetermined results and effects on the birds in question (Lupiani and Reddy, 2009). Monitoring in the UK has found significant avian influenza-induced die-offs in seabird colonies, with a potential reduction of 60-70% in breeding territories for Great Skua (*Stercorarius skua*) since the last full census in 2015 in Foula, Scotland, with 1400 corpses located between May and August 2022 (Lupiani and Reddy, 2009) as one example. North Sea strandings of seabirds increased considerably, with 221 Northern Gannet (*Morus bassanus*) strandings in the Netherlands, an increase on the expected average of 22 over previous years (Camphuysen, Gear and Furness, 2022). It is hard to quantify how many corpses make landfall and how many remain lost to the sea, Paradell et al (2023) estimated a local population die-off of between 2993 and 3260 individual Gannets from colonies in Southern Ireland using aerial image analysis. On land, 2023 events have differed from those of 2022, with evidence of die-offs moving from seabirds in general to members of the Laridae family with sustained die-offs in Tern colonies around the North Sea and a substantial increase in die-off events at Black-headed Gulls (*Chroicocephalus ridibundus*) at inland wetland sites across Northern Europe (Paradell *et al.*, 2023). When AIV begins to be detected more regularly inland, the distance between known virus sites and poultry holding sites decreases and theoretically this increases the risk of spread of impacts into the UK's poultry economy. Outbreaks at poultry holding sites continue to increase in the winter months, as they have historically, with wild virus detections largely still coming from Anatidae,

waterbirds and birds of prey most frequently through the current detection systems. 188 UK poultry farm outbreaks have occurred between October 2022 and July 2023 (BTO, 2023).

Rates of mortality and other symptoms of infection seem to vary between bird families (APHA, 2023c). Often species detected by the UK's corpse detection monitoring systems include larger species such as swans and geese, with proportionately fewer detections in ducks relative to population sizes. Evidence suggests that sublethal infections are common in dabbling duck species (such as Mallard, *Anas platyrhynchos*, Wigeon, *Mareca penelope*, and Teal, *Anas crecca* (He *et al.*, 2023)) and asymptomatic infection plays a key role in cross-border movement of AIV. Anatidae demonstrated the highest prevalence of avian influenza from a large sample size (>226000 samples, 11.8% positive, Chapter 2) and are the most 'known' quantity when it comes to wild reservoirs for avian influenza, but are not alone in high testing prevalence rates, with Alcidae (auks), Scolopacidae (sandpipers), Pycnonotidae (bulbuls) and Acrocephalidae (reed warblers) all scoring particularly highly with much smaller sample sizes. Widely speaking sampling of a wide diversity of families and species has shown that most families have positive samples for avian influenza once you sample to a sufficient threshold. In the UK, a Eurasian Reed Warbler (Acrocephalus scirpaceus) was recently confirmed positive with HPAI H5N1 confirming the presence of the currently circulating strain in UK passerines (APHA, 2023c).

The current understanding of AIV in waterfowl suggests the importance of waterbodies as a core habitat which influences Anatidae distribution (and hence AI distribution) in the landscape. Whether used for roosting, feeding, or breeding, waterbodies are a key habitat for all waterfowl species found within the UK (Wade *et al.*, 2022). In wetland landscapes, the depth of waterbody, the area of waterbody and marginal vegetation may all be factors which define what species are present at a site (Svensson, Mullarney and Zetterstrom, 2023). It is important to acknowledge that whilst some bird species are centric to waterbodies, others may utilise them as a resource, if only briefly for hydration or feeding. Evidence of environmental persistence of AIV in waterbody samples shows that the length of persistence of the virus is linked to temperature (Hénaux *et al.*, 2012). Routes of transmission can be split by environmental exposure (to substrates and water infected by fomite) or direct exposure (bird-to-bird contact (Si, et al 2013)).

It is currently difficult to establish movements of birds between waterbodies and poultry sites, with only a newly developing understanding of which species occupy both spaces (explored in chapter 4 and within this following chapter) and through difficulties in suitable tracking devices for smaller bird species, which tend to dominate communities at poultry holding sites. It is reasonable to assume that there is an element of direct movement of birds between waterbodies and poultry holding sites, especially where those locations are nearby. Si et al (2013) report that 'poultry outbreaks increased with an increasing human population density combined with proximity to lakes or wetlands, increased temperatures and reduced precipitation during the cold season'.

To assess the exposure risk of poultry priority species at waterbodies, citizen science data was trained into models using eBird complete lists (lists where all birds seen over the count period are recorded). Citizen science, whilst caveated with assumptions, allows for a much larger sample size than would be attainable from a one-man fieldwork season, theoretically increasing the confidence in the dataset attained for modelling (Young *et al.*, 2019). The decision to use eBird over another citizen science repository for ornithological data, Birdtrack (Boersch-Supan and Robinson, 2021) was made on local knowledge of the use of both apps at migration hotspots, such as at the Spurn Peninsula, where eBird is the preference. Whilst unquantified, the level of observer competency at sites such as Spurn is perceived to be higher as birdwatchers seek the challenge of finding rarities at sites renowned as hotspots for lost migrants(Johnston *et al.*, 2020).

To quantify the risk of exposure between high exposure risk scoring species from poultry farms and Anatidae, risk models quantified via a Monte Carlo simulation approach (with a triangular distribution for quantifying uncertainty) were calculated for waterbodies in East Yorkshire. Three models were constructed, the first quantifying the frequency of exposure of species to waterbodies, the second investigating the frequency of co-occurrence between high poultry exposure risk species and Anatidae and a further model including variables for average avian influenza prevalence and a proxy for excretion size to the second model. This study aims to investigate the risk of avian influenza exposure to high poultry exposure risk species that occur in waterbodies, a habitat with regular detection of avian influenza viruses.

5.3 Material and Methods

To investigate species presence at waterbody sites, models were produced using lists from eBird (Cornell Lab of Ornithology, 2023) to build co-occurrence tables of species A being present at the same time as species B. eBird is an internationally used data recording service for bird-watchers to submit their sightings as lists containing presence and abundance data. Each observer can define if their submitted list is complete (contains all the birds recorded on their given route). The study period was defined as between September and March from 2018 to 2020 in line with a concurrent study looking at wild bird abundance at poultry farms (Chapter 4).

eBird data was downloaded via a data request to the Cornell Lab of Ornithology and filtered so that only complete lists at wetland sites within East Yorkshire were used in models. Lists were screened to look for duplicate data (some observers submitted matching lists during the survey window, essentially two bird-watchers recording alongside one another, duplicating the dataset). Finally, the species diversity for each count was calculated and counts outside the 95% quartile range ($n>61.5$ and $n<9$) were excluded from the analysis to factor out incorrect lists (i.e., where observers have recorded reasonably too few or too many species at a site, one observer recorded over 100 species on a count, which is against the trend and almost certainly inaccurate). Data was collected for presence and abundance for each species.

High poultry exposure risk species were defined as the species found within the biosecurity fencing of poultry holding sheds in Yorkshire as observed during field studies in Chapter 4. The mean mass for each bird species was taken (Dunning Jr, 2007)with prevalence estimates of avian influenza infection per species taken from the results of Chapter 2's systematic literature review.

A heatmap matrix was produced to demonstrate the proportion of eBird complete lists in the dataset where co-occurrence between two species of interest occurred.

Three models were constructed, the simplest (Model 1, Figure 23) looking at the presence and abundance of poultry farm priority species at waterbodies, hence calculating their modelled indirect exposure to waterbody sites.

Figure 23: Structure of model 1; each variable was constructed with a Monte-Carlo Simulation (n=1000) with a triangular distribution.

A further model (Model 2, Figure 24) looked at the level of overlap between poultry farm priority species and Anatidae species at waterbodies, factoring in the proportion of times both species are present on the same count and the mean abundance of both species when co-occurrence is recorded. This represents the modelled indirect exposure of poultry farm priority species to Anatidae at waterbodies.

A final model (Model 3, Figure 24) factors in the mean mass of Anatidae species recorded at waterbodies (as a proxy for the size of fomite) and their recorded avian influenza prevalence. This represents the modelled indirect exposure of poultry farm species to avian influenza sourced from Anatidae at waterbodies.

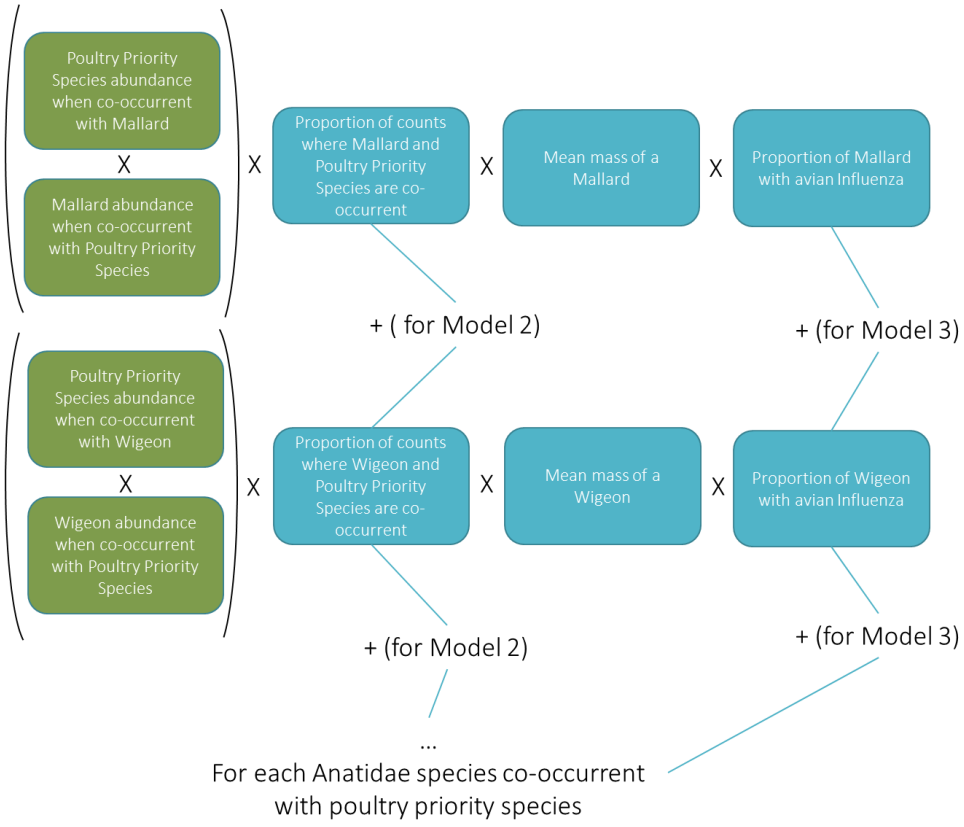

Figure 24: Construction of models 2 and 3; each variable represents a Monte Carlo simulation (n=1000) with a triangular distribution.

Models were created by multiplying variable datasets created using Monte Carlo simulations (n=1000) with triangular distributions, with abundance data variables constructed using mode, minimum and maximum values and frequency estimates using proportion data with lower and upper Jeffreys Intervals. Each species has a relative score, able to define their exposure risk amongst other selected species within two groups, Anatidae and high poultry exposure risk species.

5.4 Results

A total of 240 complete lists were extracted from eBird's data repository (Cornell Lab of Ornithology, 2023) for 27 terrestrial water bodies in the county of East Yorkshire, UK. All counts were between August 2018 and the end of 2020. A summary of the number of counts per site is included in the appendix.

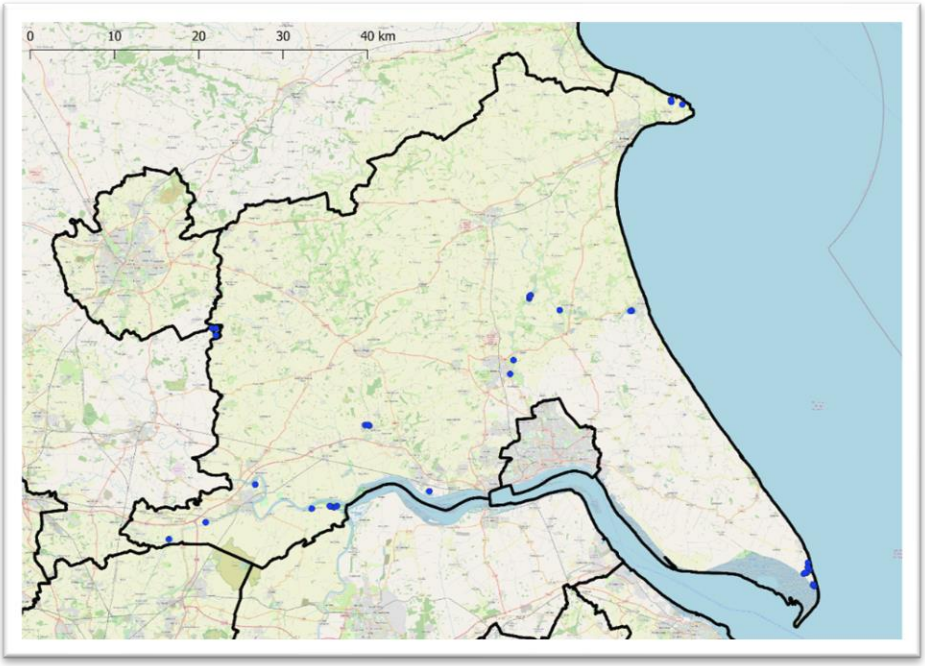

Figure 25: Distribution of waterbodies in East Yorkshire where eBird counts were collected and used in this study.

From the eBird list, data was extracted for poultry farm priority species (as defined in Chapter 4) and for species in the Anatidae family.

Table 18: List of species recorded from eBird complete checklist and modelled in this study

Poultry priority species present in eBird dataset	**Anatidae species present in eBird dataset**
Pied Wagtail (*Motacilla alba yarellii*)	Brent Goose (*Branta bernicla*)
Yellowhammer (*Emberiza citrinella*)	Canada Goose (*Branta canadensis*)
Tree Sparrow (*Passer montanus*)	Barnacle Goose (*Branta leucopsis*)
Blue Tit (*Cyanistes caeruleus*)	Greylag Goose (*Anser anser*)
Dunnock (*Prunella modularis*)	Pink-footed Goose (*Anser brachyrhynchus*)
Eurasian Wren (*Troglodytes troglodytes*)	White-fronted Goose (*Anser albifrons*)
Carrion Crow (*Corvus corone*)	Mute Swan (*Cygnus olor*)
Blackbird (*Turdus merula*)	Whooper Swan (*Cygnus cygnus*)
Red-legged Partridge (*Alectoris rufa*)	Shelduck (*Tadorna tadorna*)
Common Wood-pigeon (*Columba palumbus*)	Mandarin (*Aix galericulata*)
Great Tit (*Parus major*)	Garganey (*Spatula querquedula*)
Little Owl (*Athene noctua*)	Northern Shoveler (*Spatula clypeata*)
Chaffinch (*Fringilla coelebs*)	Gadwall (*Mareca strepera*)
Robin (*Erithacus rubecula*)	Eurasian Wigeon (*Mareca penelope*)
Common Gull (*Larus canus*)	Mallard (*Anas platyrhynchos*)
Eurasian Magpie (*Pica pica*)	Northern Pintail (*Anas acuta*)
Ring-necked Pheasant (*Phasianus colchiccus*)	Eurasian Teal (*Anas crecca crecca*)
Barn Owl (*Tyto alba*)	Common Pochard (*Aythya ferina*)
	Tufted Duck (*Aythya fuligula*)
	Greater Scaup (*Aythya marila*)
	Goldeneye (*Bucephala clangula*)
	Smew (*Mergellus albellus*)
	Goosander (*Mergus merganser*)

Co-occurrence with poultry priority species on waterbody counts was highest in Mallard (491.3), Greylag Goose (425.7), Teal (415.5), Wigeon (343.7) and Mute Swan (315.5) (calculated by adding together the co-occurrence probability of each species pairing, see Table 19). The five highest co-occurrence probabilities are for Woodpigeon with Mallard (57.28), Teal (49.03) and Greylag Goose (46.60), and for Carrion Crow (46.12) and Magpie (42.72) with Mallard.

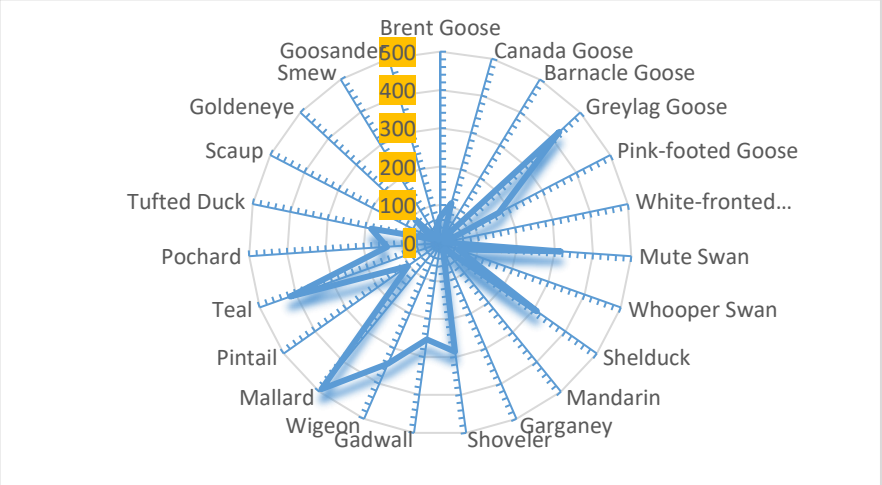

Figure 26: Cumulative co-occurrence scores of Anatidae species recorded on eBird counts with poultry priority species.

Table 19: Heatmap of species co-occurrence scores between poultry farm priority species for Yorkshire and members of the Anatidae family recorded on eBird complete lists at East Yorkshire waterbodies.

	Brent Goose	Canada Goose	Barnacle Goose	Greylag Goose	Pink-footed Goose	White-fronted Goose	Mute Swan	Whooper Swan	Shelduck	Mandarin	Garganey	Shoveler
Pied Wagtail	3.88	6.80	0.49	26.21	7.77	0.49	18.45	1.46	18.45	1.94	2.91	17.48
Eurasian Tree Sparrow	3.88	6.80	0.97	22.82	10.19	0.97	14.56	3.40	18.93	0.00	0.97	16.02
Eurasian Blue Tit	2.43	5.83	0.97	28.16	10.68	0.49	20.39	3.40	18.45	0.49	0.97	18.45
Dunnock	3.40	4.37	0.49	22.33	9.71	0.97	16.50	2.43	15.53	0.97	0.97	13.59
Eurasian Wren	4.37	3.88	0.97	26.70	12.14	0.97	21.36	2.43	18.45	1.94	0.97	17.96
Yellowhammer	1.46	0.00	0.00	1.94	2.43	0.00	1.94	0.49	2.91	0.00	0.00	1.46
Common Blackbird	2.43	4.85	0.97	26.21	9.71	0.49	18.45	3.40	17.96	1.46	0.97	15.53
Carrion Crow	6.31	11.17	0.97	38.35	16.99	0.49	30.58	4.37	27.18	1.46	1.94	28.64
Red-legged Partridge	0.97	0.00	0.49	0.97	0.97	0.00	0.97	0.49	0.49	0.00	0.00	0.00
Common Woodpigeon	6.80	13.59	1.46	46.60	16.50	0.49	33.98	4.85	35.44	1.46	2.91	32.04
Great Tit	3.88	6.31	0.49	27.67	10.68	0.97	22.82	3.40	18.45	1.46	0.97	20.39
Little Owl	0.00	0.49	0.00	0.49	0.00	0.00	0.49	0.00	0.49	0.00	0.00	0.00
Common Chaffinch	4.85	5.83	0.49	25.73	11.65	0.97	18.45	2.91	18.45	0.97	0.49	17.48
European Robin	3.40	5.83	0.97	26.70	10.68	0.97	18.93	2.91	18.93	1.94	0.97	16.50
Common Gull	6.80	11.65	1.46	35.44	12.62	0.97	25.73	3.88	28.64	1.46	2.43	23.30
Eurasian Magpie	5.34	11.17	1.46	34.47	16.99	0.97	25.24	4.37	27.18	1.46	0.97	23.79
Ring-necked Pheasant	5.83	8.74	0.97	28.64	10.68	0.49	20.39	3.40	17.96	1.46	1.46	19.90
Western Barn Owl	2.43	1.46	0.49	6.31	2.43	0.00	6.31	2.43	4.85	0.49	0.00	2.91

	Gadwall	Wigeon	Mallard	Pintail	Teal	Pochard	Tufted Duck	Scaup	Goldeneye	Smew	Goosander
Pied Wagtail	14.08	20.39	28.16	6.31	27.18	6.31	9.22	0.49	5.34	0.49	1.94
Eurasian Tree Sparrow	12.62	17.96	26.21	4.85	23.79	7.77	8.74	1.46	4.37	0.49	1.94
Eurasian Blue Tit	17.96	20.87	30.58	5.34	24.76	9.22	13.59	1.46	5.83	0.97	1.94
Dunnock	13.11	16.99	24.76	4.37	19.42	8.74	10.19	1.46	5.83	0.49	2.43
Eurasian Wren	17.48	20.87	30.58	5.34	26.21	8.74	11.17	0.97	5.83	0.49	3.40
Yellowhammer	1.94	2.43	2.43	0.97	2.43	0.49	1.46	0.97	0.97	0.49	0.49
Common Blackbird	14.56	19.90	28.16	4.85	24.27	9.22	12.14	1.46	6.31	0.49	3.40
Carrion Crow	25.73	31.07	46.12	10.19	40.78	11.17	17.48	1.94	6.31	1.46	2.91
Red-legged Partridge	0.00	0.49	0.97	0.00	0.49	0.49	0.00	0.00	0.49	0.00	0.00
Common Woodpigeon	27.67	40.78	57.28	15.53	49.03	12.14	16.50	2.91	7.28	0.97	3.40
Great Tit	20.87	22.33	31.07	4.85	26.21	10.19	15.53	0.97	6.31	0.97	2.91
Little Owl	0.00	0.49	0.49	0.49	0.49	0.00	0.00	0.00	0.00	0.00	0.00
Common Chaffinch	14.56	20.39	29.13	5.83	25.24	9.71	12.14	0.97	5.83	0.49	2.43
European Robin	15.53	19.42	31.07	2.91	23.79	12.14	13.11	1.94	5.34	0.97	3.40
Common Gull	17.48	32.52	41.75	13.59	34.95	9.22	13.59	2.43	8.25	0.49	2.91
Eurasian Magpie	21.84	30.58	42.72	9.22	34.47	11.17	15.05	2.43	6.80	0.49	3.40
Ring-necked Pheasant	15.05	21.36	33.50	7.77	25.73	9.22	11.65	1.46	5.34	0.49	2.43
Western Barn Owl	3.40	4.85	6.31	0.97	6.31	2.91	2.91	0.49	2.91	0.00	0.49

5.4.1 Model 1: Occurrence of poultry farm priority species at waterbodies.

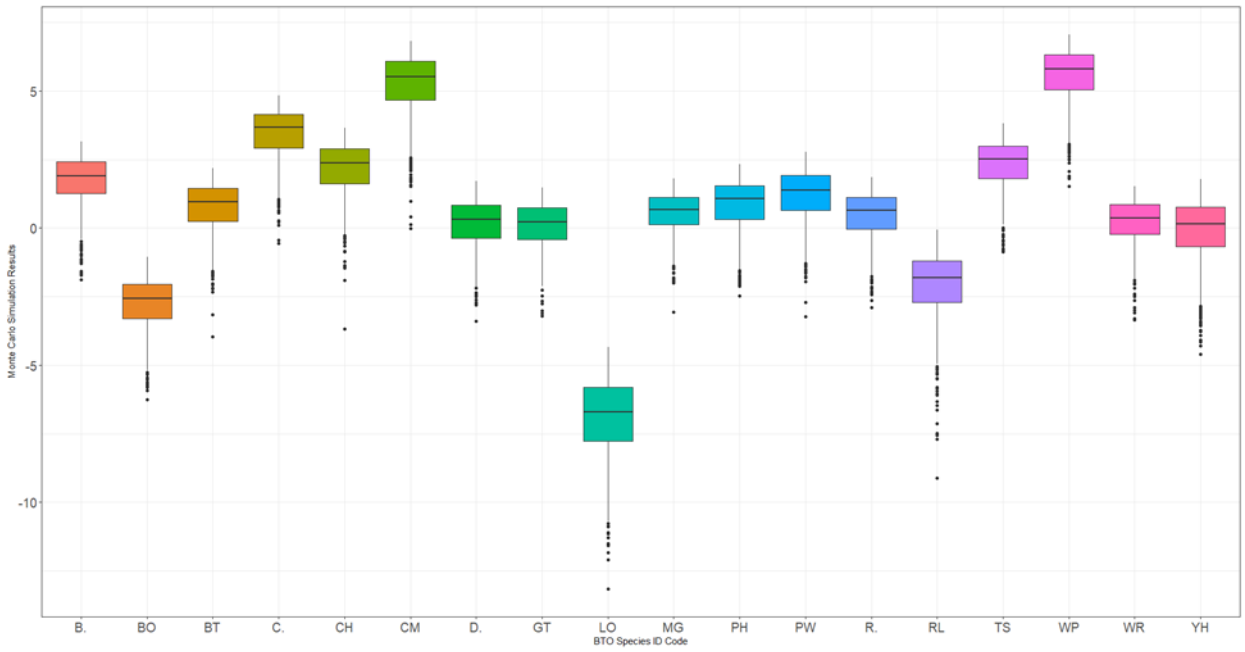

Figure 27: Boxplots of exposure risk of poultry priority species at waterbodies, boxplots represent interquartile range, with whiskers representing 95% data range. BTO codes as follows. B. Blackbird, BO Barn Owl, BT Blue Tit, C. Carrion Crow, CH Chaffinch, CM Common Gull, C. Dunnock, GT Great Tit, MG Magpie, PH Pheasant, PW Pied Wagtail, R. Robin, RL Red-legged Partridge, TS Tree Sparrow, WP Woodpigeon, WR Wren, YH Yellowhammer.

For poultry priority species, Wood-pigeon (3.62) and Common Gull (3.35) demonstrated higher exposure scores to waterbody sites. Blackbird (1.95), Carrion Crow (1.54), Tree Sparrow (0.52) and Chaffinch (0.39) all scored higher than the other priority species.

5.4.2 Model 2: Co-occurrence of poultry farm priority species and Anatidae species at waterbodies

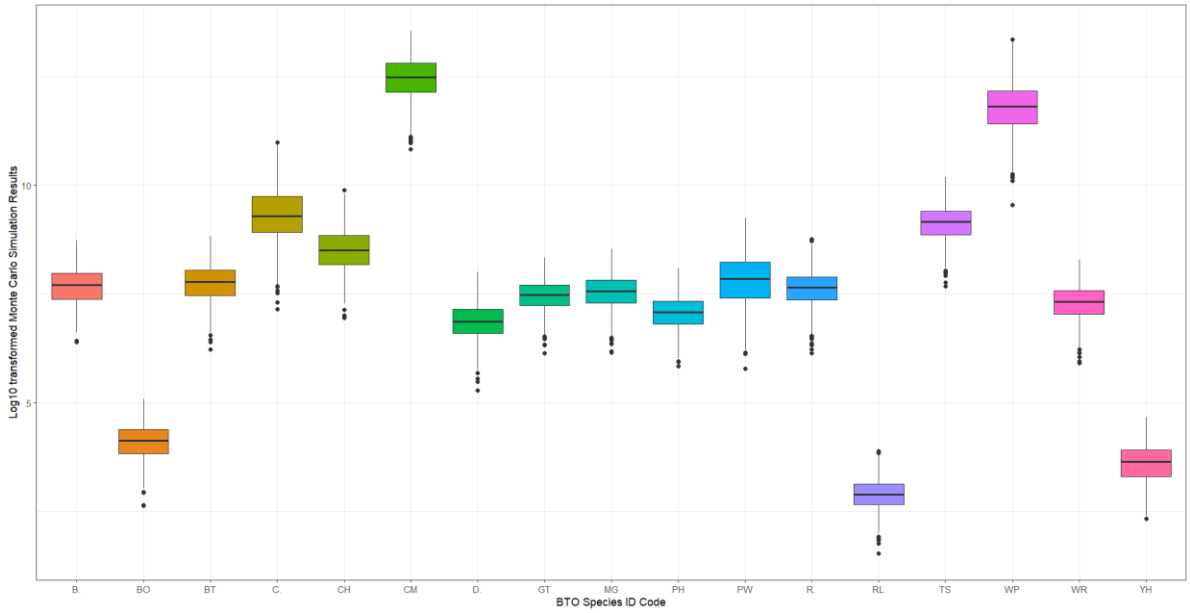

Figure 28: Boxplots of exposure risk of poultry priority species to Anatidae species at waterbodies, boxplots represent interquartile range, with whiskers representing 95% data range. BTO codes as follows. B. Blackbird, BO Barn Owl, BT Blue Tit, C. Carrion Crow, CH Chaffinch, CM Common Gull, C. Dunnock, GT Great Tit, MG Magpie, PH Pheasant, PW Pied Wagtail, R. Robin, RL Red-legged Partridge, TS Tree Sparrow, WP Woodpigeon, WR Wren, YH Yellowhammer.

Modelled exposure risk for poultry farm priority species from Anatidae at waterbodies was highest in Common Gull (285596) and Woodpigeon (154416), followed by Carrion Crow (13366), Tree Sparrow (9792) and Chaffinch (5642). The risk was comparatively low for Red-legged Partridge, Yellowhammer and Barn Owl.

5.4.3 Model 3: Risk of exposure to Anatidae-sourced avian influenza at waterbodies for poultry farm priority species.

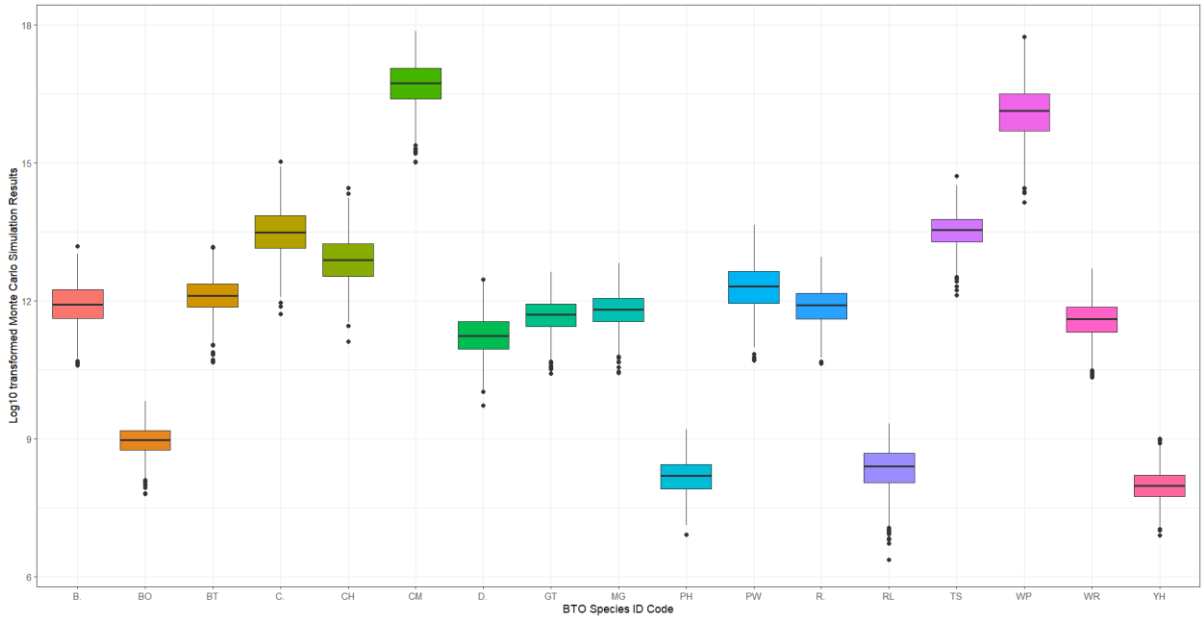

Figure 29: Boxplots of exposure risk of poultry priority species to avian influenza sourced from Anatidae species at waterbodies, boxplots represent interquartile range, with whiskers representing 95% data range. BTO codes as follows. B. Blackbird, BO Barn Owl, BT Blue Tit, C. Carrion Crow, CH Chaffinch, CM Common Gull, C. Dunnock, GT Great Tit, MG Magpie, PH Pheasant, PW Pied Wagtail, R. Robin, RL Red-legged Partridge, TS Tree Sparrow, WP Woodpigeon, WR Wren, YH Yellowhammer.

Modelled exposure risk for poultry farm priority species from estimated Anatidae sourced avian influenza at waterbodies followed a similar trend to the results of model 2 (direct exposure of poultry farm priority species to Anatidae), with Common Gull (20136339), Woodpigeon (11783672), Carrion Crow (842371), Tree Sparrow (796794) and Chaffinch (457293) scoring the highest risk. As well as Red-legged Partridge, Yellowhammer and Barn Owl, Pheasant also scored a low comparative risk.

5.5 Discussion

Anatidae co-occurrence with poultry farm exposure risk species demonstrated similar results across the three defined exposure models, with Common Gull and Woodpigeon demonstrating the highest co-occurrence on eBird complete lists. Conversely, Barn Owl, Pheasant, Red-legged

Partridge and Yellowhammer all consistently scored with the lowest co-occurrence with Anatidae at waterbodies.

The selection criteria for investigation as a poultry priority species was that a species had to have been recorded as interacting on the grounds of the area closest to poultry holding sheds, which in practice was within the biosecurity boundaries of each count site (see Chapter 4). This does exclude several species with other quantified risk scores at poultry holding sites which were encountered in the locality or as flyovers. Whilst both other categories pose a risk through local environmental contamination, it is presumed that species that occurred closest to poultry holding sheds produce the greatest risk of transmission to poultry, through direct transmission for birds that find their way into sheds (both Tree Sparrow and Blue Tit were observed to have done this during counts in chapter 4) and through close deposition of fomite which could be brought into the sheds through other means such as via rodents (Velkers *et al.*, 2017) or humans, though strict biosecurity measures were in place for workers at all study sites which reduces the risk of spread of infected fomite by humans.

Risk scores calculated at poultry holding sheds and waterbodies demonstrated that risk varied between these two key areas. For example, Pied Wagtail demonstrated the highest risk at poultry holding sites but was only the 6th highest risk of exposure to avian influenza at waterbodies in model 3. Yellowhammers were found to be the second highest exposure risk to poultry holding sheds but were largely absent at waterbodies. The link between these two key areas is largely underrepresented in the literature, and it is unquantified how often individuals of the poultry priority species move between both count sites. There are certainly more complex steps between waterbodies and poultry holding sheds and the direct link between the two remains unquantified in this study; future tracking studies may shed light on this, though few tracking devices are small enough for several of the smaller passerines in this category at present, though with technology getting smaller, and technologies such as the MOTUS network developing strongly, opportunities may arise in time (Mitchell et al., 2024).

However, the models do attempt to quantify the risk of exposure of potentially key wild bird species in the transmission chain of wild-sourced avian influenza in the UK poultry system in a way

previously untested. The three models showed large levels of overlap in exposure ratings for most of the poultry priority species, with three distinct groups emerging in the model outputs, species representing the lowest risk and highest risk standing out. Both Wood-pigeon and Common Gull demonstrated significantly higher risk scores than the other species in all three models. Carrion Crow and Tree Sparrow scored marginally higher than the middle group, but there was a noticeable degree of overlap with other species. Wood pigeons whilst quantified similarly to Common Gulls at waterbodies, would sensibly be considered a lower risk, as they do not interact directly with the waterbodies at the same frequency as Common Gulls, who roost on the open water and can swim. Wood-pigeon interaction with potentially infected waterbodies would largely be marginal, through washing and drinking from shallow edges. Common Gull represents the only species of bird that was found in the closest count areas at poultry farms that can readily swim and roost on open water.

Whilst both Common Gull and Wood-pigeons score highest exposure to waterbodies, at poultry holding sheds, both were only present on a couple of occasions. Neither are considered risks of entry to poultry holding sheds, as they are too large for any of the holes observed on poultry sheds during Chapter 4's point counts, but for free-ranging poultry, the degree of overlap could be significantly greater. During chapter 3, 50 shot Wood-pigeon were opportunistically sampled for avian influenza, though none returned a positive sample.

At the other end of the spectrum, Red-legged Partridge, Little Owl, Barn Owl and Yellowhammer scored particularly low for their exposure risk from waterbodies. All these species are mostly sedentary, with movements rarely seen (Svensson, Mullarney and Zetterstrom, 2023), reducing the risk of direct movement between poultry farms and waterbodies for all but the closest proximity sites. Barn and Little Owl will both predate on wild birds, with sick birds being particularly easy targets, potentially meaning the models under-represent these species' risks. Both also are hole-nesting and roosting species, often utilising barns, which aren't too dissimilar to poultry holding sheds. Little Owls were observed inside a poultry shed by workers on one of the count sites surveys in chapter 4 (pers comms, Avara Foods Ltd, 2019). Both owl species are nocturnal or crepuscular

unless food availability is low, this leads to lower detectability which increases the risk of under-detection on eBird complete lists.

The behaviour of Pied Wagtails at waterbodies, and their high prevalence at poultry farms (both in this thesis' study and in (Le Gall-Ladeveze *et al.*, 2022) leads to further inspection of risk, despite comparatively modest exposure scores at waterbodies. Pied Wagtails in the winter are observed to feed communally at waterbodies, with some territorial behaviour. Whilst roost counts are not considered in the models, Pied Wagtails readily roost over water in reedbeds, sometimes in flocks into the hundreds (Davies, 1976). Tree Sparrow show comparatively lower behavioural risks as their habitat preferences are more associated with scrub habitats than wetland edges, though as with Woodpigeon, they will use the shallow edges to wash and drink(Svensson, Mullarney and Zetterstrom, 2023).

The three model types demonstrate different quantifications of exposure risk. The first model only covers the exposure of poultry farm priority species to waterbodies, this is not nuanced by the species communities at a given site, but as such has a lower margin of error as few variables are considered. The model shows that species present at poultry farms are common at waterbodies, even though only Common Gull can be considered well adapted to a wetland habitat. These common generalist species are largely underrepresented in the literature when considering avian influenza transmission (see Chapter 2). When considering the co-occurrence of high poultry exposure risk species with Anatidae species (a better-known risk) at waterbodies (model 2), results only varied minimally with the same pattern of 3 groups emerging. Margins of error increase by adding more variables, but Wood-pigeon and Common Gull both still demonstrated higher risk scores. Adding avian influenza prevalence estimates and a proxy for the size of shed fomite, again demonstrated a similar pattern. It is considered that this is the case because the prevalence of common Anatidae and common high poultry exposure risk species was fairly level between counts (see appendix 5).

Limitations exist within the model structures used. Model 3 takes presence and abundance data for both poultry farm priority species and Anatidae from eBird counts. It was noted that in the complete lists, many of the figures were presumably rounded to the nearest hundred when

abundance was high at a site. Whilst these presumed estimates are useful, they do add a level of error that is not possible to quantify in the model. By using a 95% confidence level on species abundance on a count, the models do not account for the highest and lowest counts in the dataset. This was done to avoid incomplete or incorrect counts. Many of the complete lists had 'present' listed instead of a count for a species which limited the number of co-occurrent counts available to produce the data distributions for the species abundance Monte Carlo simulations. Overall, the increased sample size of using citizen science datasets over the period increased the available data for the models in a way that was not possible over the same period with a single fieldworker, which increased the accuracy of the distributions constructed for the Monte Carlo simulations.

Whilst the models account for species' frequency of occurrence at waterbodies, it doesn't specify how bird behaviour can increase exposure risk both directly with other birds and indirectly through exposure to environmental contamination of water and substrates. Fomite from an infected bird takes the form of breath, faecal matter or viscera from corpses. Infection through exhaled breath and associated infected water droplets affects mostly the closest proximity to its source bird; birds that flock together (including social roosting, breeding and feeding behaviours) likely increase the likelihood of direct transmission between individuals. Direct transmission through infected faecal matter occurs when individuals feed or probe on or around any infected faecal deposits; again, likely to be riskier when there is a higher density of birds in a given area. Exposure through viscera is more likely for avian consumers including predators and scavengers than for species reliant on other dietary requirements, but corpses present in high-density bird areas increase the risk of transmission through direct contact. The deposition of fomite into the environment and how long the risk of transmission from this source is feasible depends on the type of substrate or water (saline/freshwater) and the temperature of the water body (Hood *et al.*, 2020).

The eBird counts do not specify where on a waterbody each individual was located during the count period, and for how long they were present, the count data acts as a proxy for this without factoring this into exposure. Behavioural observations at waterbodies alongside counts would add this information for future models. The spread of locations of eBird complete lists was not uniform across included count locations. This will lead to variation in species communities found at

waterbodies and future studies could look to factor in variables for waterbody size, number of waterbodies on a site and waterbody depth.

The current models have not quantified the size of a water body and its physical attributes (such as depth, surface area, base profile, and distance from estuaries and poultry farms) which might affect its ability to attract birds (Si, de Boer and Gong, 2013), by quantifying this risk and comparing between waterbodies, it may be possible to quantify avian influenza risk through species communities at different sites.

Models 2 and 3 factors in Anatidae frequency of co-occurrence at waterbodies and all proxy metrics for their frequency of fomite shedding. Anatidae were selected as they are a better-understood family of birds when it comes to avian influenza prevalence, with international sample sizes being much higher than other families (see Chapter 2). Anatidae tend to have higher prevalence rates than other families, though it is yet to be conclusive if this is down to being tested more than other families. Once other families have increased tested sample sizes, they could be included in the models to investigate how this varies risk score outputs for poultry priority species. Mean mass is used as a proxy for the size of fomite shedding, but accuracy would increase by factoring in the rate of excretion for infected individuals, though to do this for every species is a very difficult metric to correctly calculate, especially in a wild study.

5.6 Conclusions

To conclude, at waterbodies, common generalist species classified as high poultry exposure risk demonstrate similar frequencies of encounter at both poultry holding sites and waterbodies, both important habitats in the transmission of avian influenza into the captive poultry sector. The only species adapted to waterbody habitats (Common Gull) was classified as the highest exposure risk to avian influenza at waterbodies in 2 of 3 constructed models (second in model 1) but only posed a risk of environmental deposition close to poultry holding sheds during chapter 4's point counts. Not all species that were common at poultry holding sheds were found regularly at waterbodies, reducing the perceived exposure risk to poultry for Yellowhammer, Red-legged Partridge, Little Owl and Barn Owl when not considering their feeding behaviours. Future research should work to

include how these species interact with waterbody environments such as how often are they present at the water's edge and interacting with semi and permanently 'wet' habitats as well as in areas where other species are in high abundance. Tracking studies focusing on high poultry exposure risk species and their movements between and around waterbodies and poultry farms would again allow a more targeted approach to future AIV sampling and expose the frequency of occurrence in and between these key avian influenza risk habitats.

Mist netting for waders with Humber Wader Ringing Group, Welwick Saltmarsh, East Yorkshire. 22/02/2019.

Chapter 6 General Discussion

6.1 Expanding the thesis conceptual model

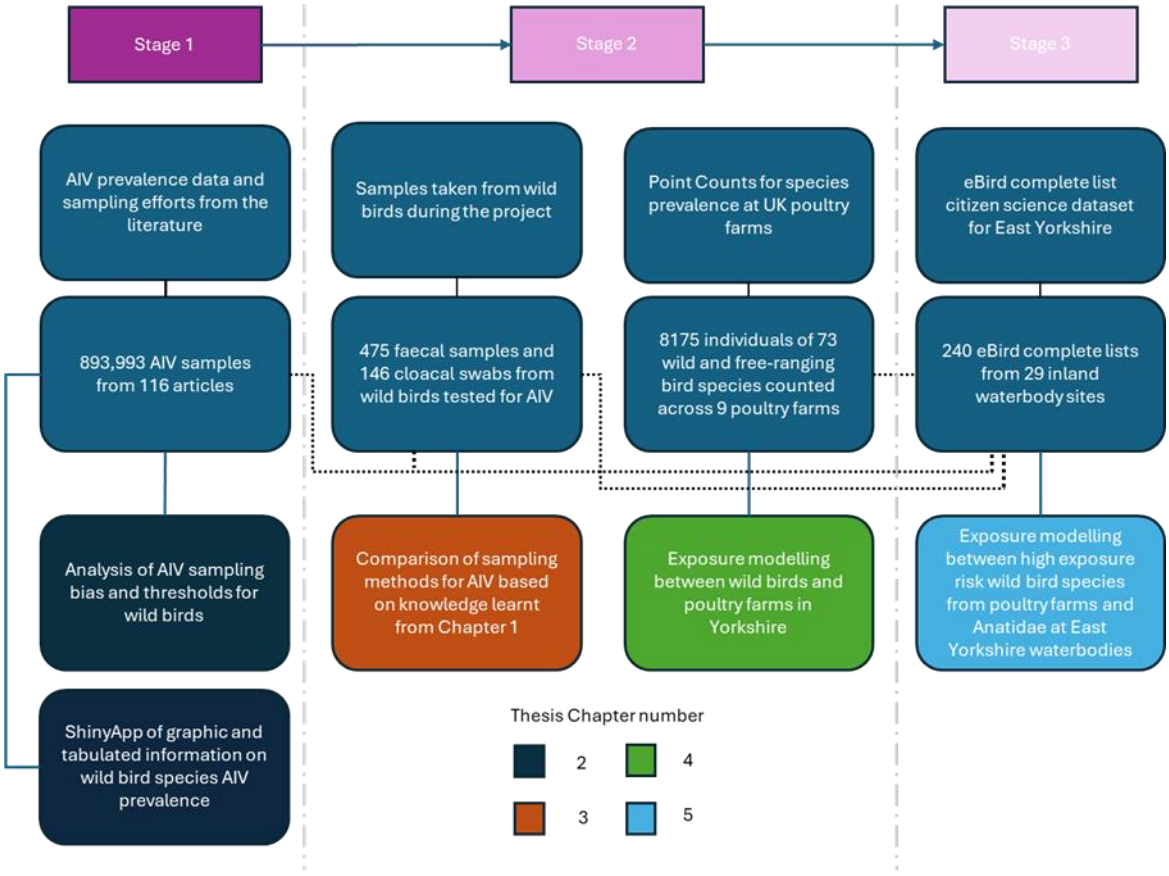

Figure 30 Expanded Conceptual model (Figure 2) demonstrating how the collected data contributed to the thesis research chapters and research outputs.

The expanded conceptual model (Figure 30) for this thesis introduced in Figure 2 of the literature review, represents the vision behind how data could be collected and used to inform the four research chapters of the proposed thesis. Data collected in the first stage of the thesis; a systematic literature review (Chapter 2) provided a comprehensive baseline to build the target species for AIV sampling (Chapter 3). Chapters 3 and 4's data collection ran concurrently during stage 2 of the thesis, adding newly collected fieldwork data to the existing databank created in Chapter 2. All data collected in stages 1 and 2 of the thesis informed the final exposure modelling in the final research chapter (stage 3).

6.1.1 Listed summary of key findings

The following key findings have been made following the completion of this thesis. These are expanded below and discussed in more detail in 6.2 below.

1. Anatidae AIV Prevalence may bias AIV research in wild birds
2. If you sample enough in a species or family, you are likely to find avian influenza
3. Importance of prompt AIV sampling and monitoring methods to mitigate risk
4. Lack of Anatidae presence at housed poultry farms
5. Comparison of Exposure Scores of wild birds at poultry farms
6. Generalist Species Presence at waterbodies and poultry farms
7. Suggestions of UK species that need further research interest for AIV spread

6.2 Key Findings

6.2.1 Results from Systematic Literature Review

Through a systematic review of the literature (Chapter 2), it is apparent that sampling bias for AIV samples within wild birds exists towards the Anatidae family. When included in models, Anatidae affected whether behavioural and environmental factors significantly predicted sample size and proportion prevalence of AIV, with species associated with wetland habitats demonstrating greater sample size and prevalence of AIV. In Anatidae-excluded models, this relationship was not statistically significant. The difference between the Anatidae excluded and inclusive models suggests that wetland-centric species do not demonstrate higher AIV prevalence than those centralized to non-wetland habitats. Anatidae seems to have the highest (or at the least one of the highest) family AIV prevalence rates within birds, which would lead to the logical assumption that species using the same habitats as waterfowl should on average experience higher exposure to AIV through both direct interaction with Anatidae, and through interaction with environments contaminated with AIV excreted from Anatidae hosts. The lack of disparity between wetland and non-wetland-centric species, and the differences between both these groups' exposure to Anatidae leads to questions about other more intricate behaviours, and the role of varying immunological responses.

Although still focused on the wetland-centric family Laridae, Arnal et al., (2015) highlights the similarities and differences between Laridae and Anatidae families. It is suggested that Laridae could be a key route for intercontinental spread as seabirds and differences in viral shedding are also key in understanding each family's role in AIV spread. The level of inspection demonstrated here must be sought to be conducted for high-risk AIV-spreading species to help piece together how AIV spreads in both a national and international context.

Sample sizes were frequently too small at a species level to infer strong confidence in population-level AIV prevalence estimates, with most species showing sizeable Jeffreys Intervals. The impact of this is high levels of uncertainty in most species' roles within AIV spread, both locally and long-distance. The findings of Chapter 2 have two main comparisons in the literature, one an article by Caron et al (2017) and the other by Alexander (2000). Both these articles used similar systematic review methods to investigate trends in sampling for AIV at an international scale. The sample size obtained in Chapter 2 of this thesis is greater than its two predecessor reviews and can take a more specific approach to analysis, refining analysis to species and family level as opposed to at order, which is the approach of the two aforementioned articles. Whilst having a larger sample size allows for analysis at a species level, the sensitivity analysis on sample size demonstrates variance on how we consider the roles of different species and families. Whilst a stricter threshold of >783 increases accuracy, it does lead to less certainty due to increased error margins. Stricter thresholds do not always have to be the answer, with different trains of thought on the appropriate cut-offs for inclusion (Bacchetti, 2010,(Rothman, Greenland and Lash, 2008)

Much of the established literature surrounding avian influenza takes a strong focus on the role of Anatidae within the transmission of AIV around a landscape, attributing it across different scales. Significantly fewer go further into looking at the role of other species in AIV movement dynamics, with proof widely available for the presence of AIV in other families. Since the start of this thesis in 2018, AIV has taken on a new threatening dynamic for wild birds worldwide, with widely evidenced infection and mortality in multiple species across multiple families, with seabirds being impacted across the globe. From a scientific perspective, seabirds congregate in high densities in multi-species and family colonies to breed (Falchieri *et al.*, 2022). The range of species showing mortality

of infection is wide-ranging and has importantly dispelled the myth that only waterfowl have a role to play in AIV epidemiology, something in agreeance with the findings of Chapter 2 (Haydon *et al.*, 2002). This research chapter, backed with the evidence of the last few years, demonstrates that sampling bias towards Anatidae has taken the focus away from studies into other species, which are now starting to see further research in response to the latest HPAI H5N1 outbreak trends.

Links between waterbody habitats and avian influenza have long been suggested by the literature, stemming from waterbodies being the primary habitat for Anatidae (Ahrens et al., 2022). After analysing results minus Anatidae for waterbody-centric species and prevalence, the results returned as non-statistically significant, suggesting there may be a more complex relationship at play when it comes to other families of birds at waterbodies. This may be due to several factors, firstly a lack of sampling in other families has not uncovered the true trends in most species of wild bird, or perhaps that models including Anatidae in analysis mask this trend. In either instance, there is a better understood and established relationship between Anatidae, waterbodies and avian influenza than there is for other families (Kjaer *et al.*, 2021). As a habitat, waterbodies are proven to host environmental AIV outside of their hosts (Zhang *et al.*, 2014), for what can be several weeks if the conditions are correct. The environmental persistence, if in high enough densities presents a considerable risk of exposure to wild birds interacting with these sites, including those drinking and washing at waterbodies. In scenarios with water scarcity that inevitably cluster wild birds together, the risk of proximity and exposure to AIV-infected environments likely increases (Ferenzci *et al.*, 2016). Waterbodies also act as a key stopover location for many different migratory bird species, and as such represent key areas for potential overlap of different AIV strains. This could allow for recombination and mutation of AIVs through co-infection within a host or environment. This represents a risk of a more lethal strain evolving, with more severe impacts on the conservation of wild birds and the biosecurity of captive ones (Richard *et al.*, 2017).

The systematic literature review was limited in several ways. Whilst a broad search term was used ('avian influenza'), the second stage of the review process was elimination through title relevance, looking specifically for titles referring to sampling in wild birds. This and the third stage, an abstract review, eliminated a large quantity of selected articles. Inevitably, some species samples from the

literature would have been missed from this technique, but the time requirement to fully review the number of papers the selection process returned was not possible within a PhD chapter. Due to the sample size produced by the chapter, whilst strongly caveated, it was deemed to be sufficient to address the aims of the chapter.

Despite the vast number of samples entered into the dataset, it became apparent that for most species and families, the sample sizes produced would not be able to investigate AIV prevalence estimates with strong confidence. An investigative approach was thus made to try and suggest what would be the target sample size for a sampling project going forward. An existing sample size calculator developed by Canon and Row (1982), estimated a sample size of >782 was necessary to discover a 2% prevalence rate in the population, which was greater than the 300-sample size threshold used by both Alexander (2000) and Caron et al (2017). Comparing these results demonstrated that minimal but important differences occurred in confidence levels and coefficients. Whilst fairly predictable, the results do show that sample sizes are important factors to consider and should be discussed thoroughly in the context of the results produced. An important tool for the future of research would be the ability to host a dataset that authors can openly upload their results into, to help extend our understanding of avian influenza prevalence estimates in less studied species.

Another important context to consider in the results is that the sample sizes in this study represent an entire species throughout its spatial range over an extended period of publishing time as a population. It is not captured but fully expected that temporal variation occurs in avian influenza prevalence across each species, with this also varying by strain (Berry *et al.*, 2022). Whilst some effort has been made at a coarse scale to understand prevalence estimates on a continental and national scale, birds do not follow international designations, and it is implicit that prevalence estimates vary across small spatial scales. A significant challenge when modelling risk from avian influenza is how to accurately reflect these parameters in epidemiological models and how to understand risk based on vastly complex conditions.

Another product of this chapter is a ShinyApp, allowing the user to search AIV prevalence estimates (and their Jeffreys Intervals) by family. The app presents the user with both table and

graphed representation of an extensive dataset, including all the AIV (not including serological sampling) samples collated in the systematic literature review. It is hoped this tool will be useful for researchers and decision-makers to be able to easily access an extensive dataset in a clearly presented format.

The findings of this research chapter have brought forward multiple speculations surrounding the role of other bird families in the spread of avian influenza. This systematic review is the first time the analysis of relationships has been scaled down to an interspecific analysis rather than at an inter-order level on such a scale. Several families scored highly for AIV prevalence including some associated with non-waterbody habitats. It is hard to pick a specific target family for further study from this chapter alone, as so many families do not have representative sample sizes that would allow for an accurate AIV prevalence estimate.

6.2.2 Sampling wild birds for AIV

The wild bird sampling element of this thesis (Chapter 3) explored different methods of active sampling for avian influenza in wild birds, comparing these against passive methods used by the APHA during 2019/2020 (APHA, 2023d). Results suggested that passerines migrating through Spurn had undetectable levels of, or no, avian influenza, but that sample size was not large enough to be conclusive. This was similarly represented when sampling common generalist species near to an outbreak site in Suffolk during early 2020. In contrast, waterfowl samples taken from hunter-harvested birds proved a successful method of sampling for avian influenza cost-effectively, with seven ducks of three species (Teal, Wigeon and Mallard, 4.7% of 146 samples) returning positive samples for avian influenza, some of which were HPAI H5 strain. Furthermore, active sampling from hunter-harvested waterfowl proved that it was possible to detect avian influenza during the autumn migration window sooner than through the currently implemented passive sampling method.

The decision to look at active sampling was a priority for the project since its inception, with initial plans to blood sample and cloacal swab to look at active infection and immunological history for sampled species, with Spurn Point acting as a sentinel site for avian influenza during east coast autumn bird migration. Location was defined by known ornithological knowledge; when birds

immigrate onto UK shores, they are best detected by bird observatories, which are set up in strategic locations around the country for the study of bird migration. Bird ringing and surveys have been an ongoing part of bird observatory studies (including Spurn) for much of the last century. Spurn Bird Observatory helped with the project from its inception and continues to support the study of avian influenza in the UK's wild bird populations. Unfortunately, logistical constraints meant it was not feasible to sample invasively (meaning through blood and internal swabs), so plans were adapted to collect faecal samples for virological testing. A method was formulated to sample from wild birds caught during standardized mist netting, with the decision to sample passerines coming from the literature and subsequently based on the results of Stage 1 of this thesis. Low levels of avian influenza are generally detected in most (if not all) families if a large enough sample size is obtained (as suggested in Chapter 2), and attempts were made to try and increase the literature's sample size when it comes to the species commonly migrating through Spurn during the autumn migration window. Whilst the risk of returning no positive samples was high and realised, not finding avian influenza in a sampled population still adds data to our ongoing understanding of how avian influenza crosses international borders. Sampling occurred over two autumn migrations in 2019 and 2020 at Spurn and extended to cover Filey Bird Observatory in October 2020, incorporating their annual 'Migweek' celebration ringing activities. Sampling of other passerines around a poultry outbreak in Suffolk was limited by how close established ringing sites were to the outbreak site, the closest that the study was able to ring at was 7.5km from the outbreak site. Future studies should look at obtaining permission to sample wild birds caught within the 5km exclusion zone put up around an outbreak site, specifically focusing on the wild birds present at poultry holding sites as established in Chapter 4. Chapter 2's systematic literature review revealed a predictable bias towards the Anatidae family, as was strongly suggested during the prior literature review process. Whilst the avian influenza prevalence estimates on the sampled population of wild birds demonstrated that other families can and do test with similar prevalence estimates to Anatidae, it did also highlight that Anatidae do have an avian influenza prevalence estimate of around 11.8%, meaning that they are a suitable target family for disease monitoring projects. With this in mind, and through a review of other international methods of monitoring (such as that in the United States, see Bevin et al, 2016), when the opportunity arose to sample hunter-harvested

waterfowl from a hobbyist hunter, cloacal swabs were obtained (this is not regulated in corpses as it is in live birds in the UK), and subsequently proved to be a suitable and cost-effective method of actively sampling for avian influenza. Whilst the hunter-harvesting approach was shown to be most effective, using bird ringing techniques and mobilization of these specialist practitioners, could allow for a more targeted approach to sampling live waterfowl, rather than reliance on hunting and corpse collection, which may change in legality with future conservation implications for a number of the legal quarry species (Stroud, Pain and Green, 2021).

The concept of using hunter-harvested waterfowl to monitor for avian influenza is not a new one. The US Geological Society of America requests a sample of hunter-harvested waterfowl to be tested for zoonotics such as AIV with Bevin et al., 2016, using similar methods to effectively study AIV in migratory Anatidae in North America. Anatidae as a family have a long association with avian influenza and their increased research focus has meant that more is known about their AIV prevalence estimates. At a rate around 11.8%, theoretically, a smaller sample size is required to detect geographical spikes in wild AIV prevalence estimates compared to species in other families, though this may be because of smaller sample sizes producing lower confidence in AIV prevalence estimations in other families as highlighted in Chapter 2.

Currently, and at the time of the data collection period (autumn 2019 and 2020) of chapter 3, the APHA use a DEFRA hotline (APHA and DEFRA, 2023a) for members of the public, alongside land managers to report dead birds to collection teams. These birds are then couriered to testing laboratories to confirm if AIV is the cause of death, using a post-mortem examination supplemented with PCR testing of corpse samples. The approach allows the potential for a large sample size to be obtained over an outbreak but does elicit several biases. Firstly, larger and brighter species will stand out more than smaller and duller individuals (Johnston *et al.*, 2014). An example from a different field in Schwartz et al (2018) demonstrated that of roadkill places on roads at 9am, 62% of the starling sized carcasses had been removed within 2 hours. Smaller carcasses are easier to scavenge reducing detectability. Secondly, open habitats where corpses would be more obvious will likely offer larger sample sizes than more enclosed habitats, which might not be truly reflective of the actual distribution of AIV corpses in the landscape (APHA, 2023b). Within a densely populated

country such as the UK, the chances of detection of an AIV corpse are likely higher than in many other less densely populated countries, but bias may exist here as population spread is not uniform in humans or Anatidae in the UK. The whole strategy is determined by the public and land managers across the country being aware of the APHA's strategy, which is not guaranteed. Furthermore, during extensive outbreaks, the APHA must have enough personnel to collect, send and test corpses for AIV. At current the APHA has varying collection thresholds, whereby varying numbers of corpses for different species or families must be found together to prompt collection (APHA and DEFRA, 2023b). Whilst targeted approaches for species known to have a high prevalence of AIV in the UK are likely to yield the highest returns from a monitoring perspective, it is also important to reflect that this stratagem is unlikely to uncover unknown avenues of investigation, though this is not the primary objective of the APHA's passive monitoring protocol. A Eurasian Reed Warbler tested positive for H5N1 HPAI in Yorkshire in July 2023 (APHA, 2023b), though the exact reason behind the sampling is unknown to the author at current, this is an afro-palearctic migratory passerine, breeding in the UK in reedbeds and riparian habitats and possibly represents overspill from Anatidae reservoirs.

In comparison to the passive approach, the hunter-harvested active sampling method allows for the sampling of birds that have not been found dead but are killed for sport and sampled. By following an active sampling protocol, a study can sample from both the symptomatic and asymptomatic AIV-infected populations and get a more reflective prevalence from the species that can legally be sampled. Legal quarry in the UK is mostly limited to most (not all) species within the Anatidae family (The British Association for Shooting and Conservation, 2022), with the legal hunting window running between 1st August and the end of February. It is important to consider two scenarios within the critical analysis of these methods; the first being the scenario that AIV is present year-round in the UK, the second being that AIV influxes occur during the autumn migration window. In scenario one, a hunter-harvested monitoring approach is limited temporally, with increases within the sampled population on detectable over the legal quarry open season. In scenario 2, which covers autumn migration of wintering Anatidae into the UK, this monitoring scheme is more efficient at detecting a seasonal increase at its earliest stage, allowing for mitigation responses to be made by the poultry industry. During the sampling period for Chapter 3, the

second scenario was true, with the first scenario, less likely to be co-occurring, but untested. As of August 2023, there is a good chance that both scenarios occur at once, and Anatidae as a family with known high prevalence, can act as strong sentinels for increasing AIV prevalence in wild populations.

As a method of AIV monitoring, sampling predominantly for migrating passerines during autumnal migration proved to not produce positive samples. As a method of monitoring, quite simply, Anatidae demonstrate a higher AIV prevalence rate and hence provide a more understood and reliable method of monitoring AIV trends, whereas non-Anatidae require increased sample sizes to be more certain of AIV prevalence estimates, something Chapter 3 contributes to. Though our study did not find them, explaining the reasons behind positive samples, such as the Reed Warbler mentioned here, (APHA, 2023b) (overspill from reservoir species, strain-specific reservoirs, short-term infections, asymptomatic infections, etc.) should be a key objective of future studies. For the same reasons as the scenarios described in the previous paragraph, there were temporal limits to the study, but in this instance, the study could be repeated at a different time of year akin to the outbreak response method. Some of the reasons no positive samples were found in migratory passerines include that AIV prevalence during the sampling period was non-existent, or extremely low leading to no detections being made. It could also be that infected individuals were unable to successfully migrate (Hoye, Fouchier and Klaassen, 2012) or perished from infection.

The outbreak response AIV sampling method was limited by legal requirements prohibiting bird ringing surrounding poultry holding sites (BTO, 2023), and the lack of established ringing sites that exist within a reasonable radius beyond this.

Discussing bird-ringing methods as a method of AIV surveillance in general, there is importance in noting that if the bird ringer is licenced appropriately, the scope of what can be sampled is much heightened compared to a hunter-harvested method. Sampling in this study focused more on less studied species, on the chance that our understanding of these species was missing a key element of the AIV transmission puzzle, but the sample size was not large enough to be definitive. It is important to note, that chapter 3 discovered AIV in cloacal samples from waterfowl and was absent from faecal samples from waders and passerines. A sensitivity analysis on the detectability of AIV

from faecal samples under different conditions and storage times is an important step in fully understanding these results, with the suspicion being that faecal samples underestimate viremia by an unknown proportion.

An issue in sampling for AIV in the UK is the ability to take invasive samples for live birds is regulated by the Animals Scientific Procedures Act (1986). This is an important law requiring strict training and licencing to be able to take invasive samples (which includes cloacal and oropharyngeal swabs, blood samples and plucked feathers). We need to sample large numbers of birds due to low levels of natural prevalence in AIVs if we are going to find out the true AIV prevalence estimates in UK species. At current, research efforts lack resources (trained people, equipment, sample testing, sample transit). The low availability of these resources restricts scientific research in this area, but it must be noted that most of the current structures are built around regulatory surveillance rather than furthering understanding. As our understanding of AIV changes, it will be important to assess the needs of a science-led structure, and what potential insights might to do make surveillance easier. It is no easy task for the APHA to process the number of samples they will be receiving from both the wild birds reported nationally and captive birds from infected poultry sites in a way which could act as an early warning system. If a research-led approach is chosen, it will be important for future consideration of which sampling methods for monitoring are used and look carefully at the most efficient and effective ways of gathering reliable data.

Whilst this chapter focused on sampling for AIV via wild bird vectors, there are other methods of monitoring the landscape. Firstly, and as previously mentioned, small mammals such as mice and rats are likely to have the ability to enter poultry housing sheds and as such could act as vectors for viral transmission to poultry. Rodents have in the past tested positive for AIV (Houston *et al.*, 2017) so should not be overlooked when discussing the research focus.

A further method involves the collection and sampling of environmental DNA (or eDNA) to investigate virus presence in the landscape. At present, most research focuses on the detection of AIV infection (or evidence of past infection through a seroprevalence approach) in bird hosts. What has not been fully investigated is how much, and how often AIV is present in the environment. Alfano et al., (2021) completed a study investigating the presence of mammalian virus

diversity at waterbodies using eDNA, and insect DNA (iDNA) from leeches. Whilst this study collected its samples from Malaysia and Tanzania and had issues with a lack of described baselines for many of the isolated virus lineages, the UK has access to a diverse sequenced array of current circulating viruses on a national and international level. The opportunity to enhance and evolve an eDNA approach can be complemented with sequencing of bird DNA assays to look at which species have shared a waterbody with environmental AIV and could be further complemented by counts and surveys before the eDNA collection period. A similar approach in the UK would allow for the detection of AIV in the landscape, and if proven to be an effective method, may present the easiest form of sample collection for spatial surveillance. Paired with quantifying wild bird abundance of proven infected sites, it will be possible to define better the wild bird species which should be the focus of future sampling.

6.2.3 AIV risk from wild birds at poultry farms

During Stage Two of the thesis, field data was also collected at poultry farms for wild bird abundance. Chapter 4 looked at the exposure risk these wild birds hold to poultry at poultry farms concerning their potential roles as spreaders of AIV. The sites surveyed for this element were all housed poultry units rather than free-range flocks. This selection was made during high avian influenza risk time periods for the UK, the legal order during these periods is to house poultry, and hence attempt to reduce the potential for contact between captive birds and potentially infected wild birds or other sources. Despite this, outbreaks frequently occur in housed poultry during these ordered confinements indicating routes of spread are not being eliminated by current measures (DEFRA and APHA, 2023). Surveys consisted of four-point counts covering as much of a poultry holding site as possible without overlapping. This provided strong estimates for the presence and abundance of wild bird species interacting with different spaces and habitats close to poultry holding sheds. Birds counted during the surveys were categorized as interacting with three different areas, first the airspace above a poultry holding site, answering what species fly over poultry sheds. Birds counted using the terrestrial habitats at each site were split into their presence inside biosecurity fencing and in the 50 metres outside of it. Biosecurity fencing is designed to reduce the spread of viruses and pathogens between sites by human vectors. Workers had to abide by strict biohazard protocols and procedures to mitigate risk. Species exposure risk scores were created for

the three count zones. Whilst unquantified between models, flyover risk was considered the lowest exposure risk of the three models, with each species only providing a risk of deposition of fomite into the poultry shed areas. Future nuance would involve recording defecation frequencies for flyover species, and timing how long each species spends in the airspace above the different grounded count zones. For those species external to the biosecurity fencing, the risk comes from the deposition of fomite into the landscape as with flyover birds but is considered a higher risk as birds were observed to spend longer times in this area, though this was unquantified. Birds inside biosecurity fencing represent the highest risk for they have the potential to deposit fomite at the closest proximity to poultry. Whilst this is unquantified within the sheds, observations were made of both Tree Sparrow and Blue Tit entering sheds through old pipe holes in walls, which represents, presumably, direct sharing of compact airspaces between wild birds and poultry, in even strict bio-secure areas. Wild birds are likely to utilize food and water resources should they be able to access them (especially during winter resource shortages), and as such, any holes in sheds increase the risk of avian influenza spread from small birds, which as defined in chapters 2 and 3, have uncertain levels of avian influenza prevalence amongst their populations. Whilst the qualitative observations of both Tree Sparrow and Blue Tit entering poultry holding sheds are unquantified and the following statement untested, both species represent hole-nesting birds, who readily access similarly sized holes to explore breeding locations, especially during spring and warmer weather in the late winter period (Svensson, Mullarney and Zetterstrom, 2023).

Pied Wagtail stood out as a particularly high exposure risk scoring species within the biosecurity fencing of studied poultry farms with the following observations being made during point counts. Pied Wagtails regularly feed on the roofs of poultry holding sheds, especially those with mosses and other short-sward plants present on them. It seems vegetated rooves offered a habitat with food and/or water sources for the birds to utilise, with Pied Wagtails adapted to the short vegetation and open habitat (Svensson, Mullarney and Zetterstrom, 2023).

On a couple of occasions, a temporary pool of water formed in an unsown, bare field outside one of the poultry holding sites. This attracted several Wagtails not seen on other counts (50+ on one occasion). These birds were seen to fly to and from the pool from the poultry holding roofs

increasing their interaction across short spatial scales. Common Gulls were also observed on this waterbody, a species with high exposure risk at waterbodies in East Yorkshire (see Chapter 5).

Yellowhammers were represented with the second highest exposure risk scores, though their occurrence within the poultry holding fences was occasional, there was an incidence of 37 individuals feeding upon spilt grain in a single flock. Tree Sparrows were recorded entering poultry holding sheds but were widely common in small flocks at poultry farms in East Yorkshire. It is important to note that Tree Sparrows are not an abundant species in most other areas of the UK, so the risk they represent, whilst regionally significant, highlights the need for replication in other areas of the country (BTO, 2023a).

One of the key findings within Chapter 4 demonstrated that the birds present at the surveyed poultry farms were different from those studied the most for avian influenza in an international context (see Chapter 2). Anatidae were largely absent, with the only exceptions being flyover occurrences, and Larids were present in a larger quantity but only a single occurrence was made of an individual within poultry farm security fencing. The vast majority of species present within biosecurity fencing at poultry holding sites represented generalist passerines, generally or locally common within the landscape utilizing a habitat and opportunity that many other species are not adapted for.

This obvious disparity between how science is studying avian influenza in wild birds, and the difference in bird species present around poultry farms demonstrates that a virology-led approach within the UK could be missing a key element of the transmission chain from wild birds to captive food chains

Caron et al., (2010), looked at a model-based approach in Zimbabwe to highlight the risk between wild bird communities, Anatidae and poultry holding units for viruses (namely AIV). The study looked to quantify how risk scores changed over time at poultry sites, but also looked at which species represented the largest risk between domestic poultry industries and waterbody sites. The families identified as greatest risk here included Ploceidae (Weavers), Hirundinidae (Swallows and Martins) and Estrildidae (Estrildid finches). None of these families represents species traditionally

associated with waterbody-centric habitat requirements, though Hirundinids do utilise them for feeding and roosting (and have tested positive for AIV in Africa at reedbed roost sites previously (Caron *et al.*, 2017). The key species detailed in the study are all common generalists, and whilst the climate and habitats discussed are vastly different, the results in this key area match.

A western Palearctic relevant example (Le Gall-Ladeveze *et al.*, 2022) collected data over a similar period to Chapter 4 located at free-ranging duck farms in Southern France. The key identified species of interest fully matched the Chapter 4 study with White Wagtail (Pied Wagtail being a subspecies of White) and Sparrows (both Tree and House) being the most frequently detected at poultry farms. Further network analysis also claims that the identified species have a role in linking the farmed ducks to other wild bird species. Both the White Wagtail and the sparrow species were also observed perching and interacting with feeders located in the farmed duck enclosures. Whilst this study represents free-range and not housed, it does confirm that across neighbouring countries, similar results were concluded for both studies down to a species-specific level.

Whilst both studies looked more in-depth and had a larger quantity of data to analyse, they highlight that looking at wild bird communities is a key piece of the AIV transmission chain and focus need to be highlighted for the species suggested by ecology-focused studies. Camera trap studies in the Netherlands were conducted by Elbers and Gonzales (2019) found that direct transmission at free-range sites was absent, with all potential transmission coming from birds utilizing the same space at different times, highlighting the importance of fomite deposition in areas where captive poultry are present.

In the field, Chapter 4 took upon a point count approach, with 4 non-overlapping point counts occurring in a morning at a count site. Future methods might consider a perimeter transect approach, but both would produce widely interchangeable results. The key issue would be double counting of birds at a site, as they move between count zones which were unable to be counted concurrently during this study. Before each count a settle period of 1-minute before a count allows birds to familiarize themselves with counter presence and reduce the frequency of disturbance and hence dispersal around a site. This approach was selected over a transect count for this merit, but there are arguments that both cases would be the best approach. La Gall-Deleveze et al. (2022),

used an elevated vantage point survey at a single site in their study, whilst other studies used camera traps (Scott et al., 2018] and Burns et al., 2012)) used a point count study not dissimilar from that used in Chapter 4.

Several challenges were met throughout the modelling phase of chapter 4, namely on how to accurately quantify several key variables. The first challenging variable to consider was the time element of species presence at a site; how long was an individual bird present within a count window. The logistics behind this study means that due to the abundance of birds to count during each 10-minute window, it was challenging to record the duration of presence during a count. A future refinement to all the produced models would be to include data for the target species highlighted in Chapter 4, specifically focusing on how long individuals spend within the count zone and integrating this data into the metric alongside species abundance and frequency of occurrence during the study. In future, a count method that focuses on individuals and how long they spend at a poultry site would add data, and robustness to our exposure risk models.

For the models attempting to model AIV into their risk scores, challenges multiplied, mostly due to data absence on key elements of how diseases are spread. This led to models that focus on exposure to AIV rather than transmission. Not all of these elements have easy-to-acquire answers, but the following would need to be obtained before it will be possible to accurately model for AIV in the target species identified.

- At current, there is not enough data available to accurately model the volume of shed virus in wild bird excretions. The model takes a proxy approach of mass, assuming that excretion size will have links to the size of the bird excreting it.
- Frequency of excretion. At current, we do not know how often many of the birds highlighted in the study excrete, and when they do, what variables affect the frequency of excretion. This element is not factored into the model as there is no proxy available (and is thus assumed constant across species), but future laboratory-based studies may be the answer to investigate this through experimental infection.
- Unknown AIV prevalence within target species populations. Most of the species in the study have relatively small AIV-tested sample sizes, leading to insecurity in the values being

used. For this study, AIV prevalence estimates were taken from Chapter 2's systematic literature review, and a triangular distribution was constructed for the Monte-Carlo simulations using Jeffries Intervals, which were often broad in nature. Future testing of these target species will reduce the modelled margins of error and lead to more accuracy from the modelled risk scores.

The study created three modelled areas and communities, those detected as flyovers, those detected within 50 metres of the poultry sheds (outside the biosecurity fencing) and those counted within biosecurity fencing. Ranking risk between these count areas is tricky, and not strictly valid due to the differences in count area, and the nature of the birds being counted. Even if count areas were equal in size, the inaccuracies in AIV prevalence are key to being able to infer the relative risk between scores and so are dependent on further research. However, on a strictly qualitative basis, proximity to housed poultry would be the easiest metric to consider which modelled area represents the most severe risk (i.e., the counts within the biosecurity fencing).

One area that the study was unable to count was within poultry sheds, with only external observations of wild birds entering sheds being obtained for this important count area. To factor this in fully, either counts within the sheds or camera trap studies on key entry points and resources (such as food and water) should be undertaken to understand the frequency of these interactions, if they happen at all.

6.2.4 Anatidae and Generalist species at waterbodies

The final research chapter of this thesis acts as an accumulation of the knowledge and results gained through the previous three chapters. Risk modelling was once again used, focusing on the species with the highest perceived exposure risk of spreading avian influenza to captive birds at poultry farms, but this time using citizen science to enhance understanding of these species occurrences at a key habitat highlighted by the literature, inland water bodies. Three model constructions were built, two of which looked at the co-occurrence of high poultry exposure risk species with Anatidae, which were found to be carrying avian influenza in East Yorkshire (on the Humber Estuary) during active sampling studies in Chapter 2. Chapter 5 utilized avian influenza

prevalence estimates constructed through Chapter 2's systematic literature review, for species identified as demonstrating high exposure risks.

Many of the generalist target species identified in Chapter 4 were present at waterbodies during eBird citizen science counts, frequently at the same time as Anatidae of multiple different species. Woodpigeons and Common Gull were the species from the poultry farm target list that recorded the highest exposure scores at waterbodies, contrasting with the highest exposure scores to housed poultry (Pied Wagtail and Tree Sparrow), though Tree Sparrow did score relatively highly compared to the average. The high exposure scoring Anatidae at waterbodies were Mallard, Greylag Goose, Teal, Wigeon and Mute Swan, representing a mix of long-distance migrants, short-distance migrants and sedentary species.

Chapter 2's systematic literature review looked specifically at the significance of waterbody-centric species AIV prevalence estimates in comparison to species considered to be non-waterbody-centric in their habitat requirements. Waterbody-centric species were found to test significantly higher for AIV presence across three different count thresholds, but interestingly not so when Anatidae were removed from the model, indicating the bias this family has in our understanding of AIV in wild habitats. These results underpinned the decision to use Anatidae co-occurrence in Chapter 5's model, with the relatively small margins of error for AIV prevalence (due to high sample size) allowing for more accurate estimates for use in the final models. Additionally, the sampling that took place in Chapter 3, demonstrated that Anatidae in East Yorkshire during the counting window had detectable levels of AIV (4.8% (n=148)), lower than the Chapter 2 international average of 11.8% (n=226,192).

As highlighted in Chapter 4, Anatidae are widely absent at Yorkshire's housed poultry farms, so it is perceived that they represent a low risk of direct transmission to housed poultry, and also to deposition of infected fomite within proximity to the count sites. This leads to hypothesising that other wild bird species or other vectors are important in the final steps of the transmission chain between high Anatidae abundance habitats and poultry farms.

Except Little Owl, all species counted within the biosecurity fencing at poultry farms were also recorded at waterbodies on eBird complete lists, indicating a wide potential for movement of individuals between habitats. It is now important to reflect upon the complex array of options for transmission between these habitats. One of the key features would be the risk of direct movement of infected target species from waterbodies (hypothetically the site of infection from fomite deposited by infected Anatidae) and poultry farms. They would then either directly interact with poultry causing infection to spill into the captive population, or deposit fomite which is either transmitted through further vectors into contact with or picked up directly by the captive population. There is also a further possibility that other habitats play currently unknown roles in the transmission of AIV through the landscape, with this thesis hypothesizing the role of generalist species with the ability to utilize multiple niches driving the movement away from the key infection sites (waterbodies).

A similar study was conducted over a longer count window (July 2020 to June 2021) modelling an entire year of counts (Caron *et al.*, 2017) focussing on poultry farms. The approach used by this model was to first calculate the Shannon and Simpson diversity indices alongside the Pielou evenness index. This compares to our model approach in using a triangular distribution to model species frequency of occurrence. In this model species co-occurrences (wild birds and free-ranging ducks) were visualised using an undirected weighted network, with bird species as nodes and co-occurrence frequencies as edges. Epidemiological modelling, especially in wild animals outside of laboratory settings, is challenging. Two concepts need to be understood to estimate the rate of transmission of viruses like AIV, the probability of pathogen transmission in a contact event, and the rate at which contacts occur. It is widely accepted that calculating the rate of transmission is challenging to estimate, with numerous variables leading to increasing uncertainty, and as such most studies focus on the rate at which contacts occur (Craft, 2015). Whilst Chapter 5 does its best to include the rate of transmission, it is still beholden to this increasing uncertainty. A factor missing from the eBird dataset-derived models is that co-occurrence in a habitat does not quantify species interaction, with behaviour at key sites important in AIV transmission whether that be direct interaction with infected individuals or indirect interaction through infected fomite, substrates or water (Velde *et al.*, 2021).

The utility of eBird complete counts in models allowed for a much greater sample size than would have been obtained otherwise. However, it is important to note, that it is not possible to account for the reliability of each counter submitting complete lists. Whilst largely impossible to police, efforts were made to look through the species sampled and eliminate lists that made claims of locally very unlikely species to occur. Another method used to reduce the risk was a 95% confidence interval surrounding species diversity of a count. This was performed to eliminate outstanding lists with statistically outlying diversity. Whilst this may have eliminated several reliable lists, it was thought that this would not have a significant effect on the results of the simulations.

Similarly, to Chapter 4, Monte-Carlo simulations had limitations regarding the inability to count the duration of presence of key species at waterbodies alongside abundance due to the number of birds involved. Anatidae likely spend a significant degree of time on or adjacent to waterbodies, or moving between waterbody sites with feeding, roosting and social behaviours focused on this habitat. However, with the generalist target species identified at poultry farms, it is currently more difficult to estimate the duration of time each individual spends at waterbody sites. A further similarity to Chapter 4 is the inability to accurately measure AIV prevalence due to small sample sizes and lack of data to calculate shedding potential for generalist target species. Due to a larger sample size, AIV prevalence estimates for Anatidae had enough confidence to be reliably modelled. Again, mass was used as a proxy for the size of the shed viral load.

The simulations run during Chapter 5 focused upon two key risk groups, Anatidae and the species identified within biosecurity fencing at poultry farms. A more comprehensive model could be run by looking at all the species present on the eBird complete lists, looking at co-occurrence matrices between all species, not just those done during this study. Whilst this would be more comprehensive and might identify more important co-occurrences at waterbody sites, including species recorded on the ground and as flyovers during Chapter 4's poultry farm counts, simulations begin to become wildly complex the more species you add into the matrices. A future effort could be made to include this in the analysis, but it is likely to garner more impactful results if conducted once further sampling effort has been done to identify AIV prevalence among the whole species communities at waterbody sites.

6.3 Recommendations and suggestions for further research

It is considered that the research contained within this thesis presents several suggestions and recommendations for further research in both the zoological and epizootiological fields.

Firstly, due to the findings from both the systematic review and the exposure risk models it is recommended that increased sampling effort is achieved to aid our understanding of AIV in less studied species, with a focus in the UK on generalist passerines sharing space and habitats near both poultry and Anatidae (as an identified higher AIV prevalence rate family) at waterbodies. This could also be proposed on a wider international scale as supported by research by Caron, et al. (2017) and Alexander (2000). It is suggested that this research should include serological sampling to understand the infection history of sampled species as well as sampling for live viruses. The larger the sample sizes produced, the more utility the associated AIV prevalence scores will have in future exposure risk models and research.

Within the UK, sampling for live wild birds could utilize the Bird Observatories Council to collect samples, as hubs of ornithological research. Further sampling at wetland sites could be supported by the Wildfowl and Wetlands Trust, RSPB and other conservation organisations who have invested interest in wild bird conservation. Future sampling at waterbodies should look to try and sample Anatidae and other species which represent an overspill risk from Anatidae (as an AIV reservoir). This could also be supported by refined eDNA/eRNA sampling of the environment for viral presence to help better our understanding of viral transmission at waterbody sites, as the systematic review suggests that when Anatidae is excluded from models, there is no significant difference in AIV levels in wetland centric and non-wetland centric species.

In the future, it is felt that the ShinyApp produced for this thesis can act as an easily accessible tool for researchers to find out the AIV prevalence rates and sample sizes for species of interest. This could be built upon by creating functionality for researchers to submit samples and for the app to update regularly to reflect this additional data. It is also felt that the dataset could be strengthened through the inclusion of national sampling efforts not recorded in the literature, for example, those held by the World Organisation for Animal Health (WOAH, 2023).

Hunter-harvested waterfowl present an alternative sampling strategy to the corpse collection strategy currently used by the APHA in the UK. It is recommended that the APHA explore the feasibility of the use of hunter-harvested waterfowl as an early warning system to assist in informing risk to the UK poultry sector. This would involve testing upon reception of samples as close to possible from sample collection rather than retrospective testing the following summer.

Generalist passerines are the most abundant group found close to poultry farms. As well as a target for further sampling research, work should be encouraged to understand how birds move around the landscape. Tracking studies can use a multitude of options (including the MOTUS network (Reimann *et al.*, 2023) and PIT tagging (Green, Robinson and Baillie, 2019)) with a targeted list of species as per the list of birds recorded within biosecurity fencing at poultry farms. It will be important to establish which birds are commuting between waterbodies and poultry farms to understand the role of generalist passerines in an epizootiological context. It should also be recommended that efforts to sample as many live wild birds as possible be made around captive bird outbreak sites as soon as they are identified.

Our exposure risk models do not have the information of what is happening inside poultry sheds. It is recommended that to fill these gaps, camera trap studies are used to evaluate the occurrence of wild birds (Houston *et al.*, 2017) and other potential AIV vectors such as rodents (Velkers *et al.*, 2017) visiting resources such as food and water.

To improve exposure risk scores, a study focusing on the duration of time individuals of high-scoring exposure risk species spend at both waterbodies and poultry farms should be conducted. At present, it is assumed that the duration of occurrence is uniform between each individual when recorded on a point count, which we know to be incorrect. This metric would increase the accuracy of our exposure risk scores. In future, co-occurrence at waterbodies and their associated models could be run on more species than the high poultry exposure risk species and Anatidae families, as it is unknown if any other species and their interspecies interactions represent important steps in AIV transmission.

6.4 Concluding remarks

In March of 2023, the culmination of extensive research efforts resulted in the release of a UK-centred report by an autonomous panel of scientific experts. This report, which emanated from the Scientific Advisory Group specializing in highly pathogenic avian influenza (HPAIG) operating within Defra's Science Advisory Council's Exotic and Emerging Animal Diseases subgroup (SAC-ED), carries extensive recommendations for the future of research in safeguarding the UK poultry industry and comprehending the mitigation of HPAI's impact on wild bird populations.

The research encapsulated within this PhD thesis has worked to identify the pivotal intermediary species, a highlighted research focus request within the HPAIG report, assessing their prevalence at poultry farms and aquatic habitats. These recommendations notably align with the suggestion for an improved monitoring infrastructure as outlined in the report. Furthermore, Chapter 3 expanded the sampled population for AIV among lesser-known species, further concurring with recommendations. Chapter 5 introduces the utility of live eBird complete counts, which could provide a near-real-time approach to monitoring temporal fluctuations within important wild bird families, such as Anatidae.

Although this thesis predates the recommendations issued by DEFRA and the APHA, it intersects with several of the key tenets put forth in these directives, substantiating its relevance and foresight. This thesis's overarching objective was to challenge the prevailing research bias towards Anatidae, convincingly arguing that diverse wild bird species have the potential to serve as transmission vectors across the landscape. The findings from Chapter 2's systematic literature review underscore a resounding message: diligent scrutiny can unearth AIV presence within a broad spectrum of wild bird species, not confined solely to Anatidae. This underscores the need for comprehensive sampling across the entirety of the UK's avian landscape.

Another pivotal goal of this thesis was to assess the effectiveness of current passive monitoring techniques in detecting real-time AIV prevalence among wild bird populations within the UK. Chapter 3 introduces a viable alternative sampling method, harnessing the efforts of hobbyists and volunteers to proactively ascertain AIV presence before a nationally implemented passive

monitoring strategy. The success of this approach has led to a follow-up study examining the feasibility of real-time implementation, examining cost-effectiveness and efficiency. Chapter 4 adopts a novel reverse methodology, striving to pinpoint the presence of wild birds near poultry facilities. Through point counts and Monte-Carlo simulations, it illuminates a discrepancy between the focus of AIV sampling and the species encountered near Yorkshire's poultry farms. This chapter substantiates an uncharted role for generalist bird species in the landscape-scale movement of AIV, a role corroborated by findings in the existing literature. The risk assessments presented in Chapter 4 pave the way for the development of target species lists for poultry farms, forming the bedrock for subsequent Monte-Carlo simulations in Chapter 5. These simulations delve into the co-occurrence of target species with Anatidae, which, as previously established in Chapter 2 and confirmed through localized sampling in Chapter 3, exhibit high AIV prevalence. The simulation results, while indicating the presence of target species at waterbodies, reveal variable exposure risk scores between sites, rendering any definitive distinction elusive.

This thesis, thus, offers a multifaceted framework for directing future research endeavours concerning AIV in the UK. The primary focus remains on mitigating the risk of AIV spillover from wild birds into the UK's poultry sector. It efficaciously ascertains a roster of target species for future AIV sampling initiatives and tracking studies, which would further foster a deeper comprehension of AIV dynamics across the landscape. It proposes cost-effective adaptations to the UK's prevailing AIV monitoring techniques, challenging the preconceived bias towards Anatidae-centric research. This thesis, in essence, is hoped to serve as a catalyst to future focus on avian ecology in aiding our understanding of the epizootiology of AIV within the UK and beyond.

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Appendices

Chapter 2: Appendix 1: List of data extracted papers in systematic literature review:

Acc number	Number of Samples compiled to analysis	Authors	Year of Publication	Title of Publication	Journal of Publication
1	8	V. I. K. Shearn-Bochsler, S.//Ip, H.	2019	Lethal Infection of Wild Raptors with Highly Pathogenic Avian Influenza H5N8 and H5N2 Viruses in the USA, 2014-15	Journal of Wildlife Diseases
2	3392	O. A. Torrontegi, V.//Acevedo, P.//Gerrikagoitia, X.//Hofle, U.//Barral, M.	2019	Long-term avian influenza virus epidemiology in a small Spanish wetland ecosystem is driven by the breeding Anseriformes community	Veterinary Research
3	12	C. B. Adlhoch, A.//Kuiken, T.//Miteva, A.//Mulatti, P.//Smietanka, K.//Staubach, C.//Gogin, A.//Guajardo, I. M.//Baldinelli, F.//European Food Safety Authority//European Ctr Dis Prevention//European Union Reference Lab Avian	2018	Avian influenza overview August - November 2018	Efsa Journal

4	67	C. B. Adlhoch, A.//Kuiken, T.//Mulatti, P.//Smietanka, K.//Staubach, C.//Guajardo, I. M.//Verdonck, F.//Amato, L.//Baldinelli, F.//European Food Safety Authority//European Food Safety Authority//European Ctr Dis Prevention C	2018	Avian influenza overview February - May 2018	Efsa Journal
5	299	J. H. Amery-Gale, C. A.//Vaz, P. K.//Marenda, M. S.//Owens, J.//Eden, P. A.//Devlin, J. M.	2018	Avian viral surveillance in Victoria, Australia, and detection of two novel avian herpesviruses	Plos One
6	1213	J. P. Araujo, M. V.//Fabrizio, T.//Walker, D.//Ometto, T.//Thomazelli, L. M.//Scherer, A. L.//Serafini, P. P.//Neto, I. S.//Krauss, S.//Webster, R. G.//Webby, R. J.//Durigon, E. L.	2018	Migratory birds in southern Brazil are a source of multiple avian influenza virus subtypes	Influenza and Other Respiratory Viruses
7	868	C. S. P. Bahnson, R. L.//Krauss, S.//Webster, R. G.//Stallknechtl, D. E.	2018	Neutralizing Antibodies to Type a Influenza Viruses in Shorebirds at Delaware Bay, New Jersey, USA	Journal of Wildlife Diseases
9	15	B. P. Bozic, V.//Vucicevic, I.//Vidanovic, D.//Vaskovic, N.//Prodanov-Radulovic, J.//Aleksic-Kovacevic, S.	2018	Morphological Differences of Pancreatic Lesions in Mute Swans and Hens Naturally Infected with Highly Pathogenic Avian Influenza Virus H5n8	Acta Veterinaria-Beograd
10	6	B. M. B. Chandranaik, S. M.//Venkatesha, M. D.//Ramesha, K. R.//Nandini, P.//Reddy, P.//Bindu, K. V. T.//Shivashankar, B. P.//Shankar, B. P.//Rani, M. S.	2018	Virus isolation and molecular epidemiology of highly pathogenic avian influenza A(H5N8) from an outbreak in free-ranging wild birds, India 2016	European Journal of Wildlife Research

12	388	X. L. Q. Chen, Y. P.//Wang, H. H.//Wang, Y. T.//Wang, H. X.//Ni, H. B.	2018	Prevalence of Multiple Subtypes of Avian Influenza Virus Antibodies in Egg Yolks of Mallards (Anas platyrhynchos) and White-winged Terns (Chlidonias leucopterus) in the Northeastern Republic of China	Journal of Wildlife Diseases
13	125	N. G. Curland, F.//van Neer, A.//Ziegler, L.//Heffels-Redmann, U.//Lierz, M.//Baumgartner, W.//Wohlsein, P.//Volker, I.//Lapp, S.//Bello, A.//Pfankuche, V. M.//Braune, S.//Runge, M.//Moss, A.//Rautenschlein, S.//Jung, A.//Teske, L.//Strube, C.//Schulz, J.//Bodewes, R.//Osterhaus, A. D. M. E.//Siebert, U.	2018	Investigation into diseases in free-ranging ring-necked pheasants (Phasianus colchicus) in northwestern Germany during population decline with special reference to infectious pathogens	European Journal of Wildlife Research
14	320	N. L. Gaidet, I.//Batejat, C.//Grassin, Q.//Daufresne, T.//Manuguerra, J. C.	2018	Avian Influenza Virus Surveillance in High Arctic Breeding Geese, Greenland	Avian Diseases
15	52	A. S. Globig, C.//Sauter-Louis, C.//Dietze, K.//Homeier-Bachmann, T.//Probst, C.//Gethmann, J.//Depner, K. R.//Grund, C.//Harder, T. C.//Starick, E.//Pohlmann, A.//Hoper, D.//Beer, M.//Mettenleiter, T. C.//Conraths, F. J.	2018	Highly Pathogenic Avian Influenza H5N8 Clade 2.3.4.4b in Germany in 2016/2017	Frontiers in Veterinary Science
16	422	W. A. Grimaldi, D. G.//Massaro, M.	2018	Multi-year serological evaluation of three viral agents in the Adelie Penguin (Pygoscelis adeliae) on Ross Island, Antarctica	Polar Biology

18	300	S. M. G. Hird, H.//Eisen, J. A.//Boyce, W. M.	2018	The Cloacal Microbiome of Five Wild Duck Species Varies by Species and Influenza A Virus Infection Status	Msphere
20	237	H. K. K. Kim, H. J.//Noh, J. Y.//Phan, L. V.//Kim, J. H.//Song, D.//Na, W.//Kang, A.//Nguyen, T. L.//Shin, J. H.//Jeong, D. G.//Yoon, S. W.	2018	Serological evidence of H5-subtype influenza A virus infection in indigenous avian and mammalian species in Korea	Archives of Virology
21	17	O. G. Krone, A.//Ulrich, R.//Harder, T.//Schinkothe, J.//Herrmann, C.//Gerst, S.//Conraths, F. J.//Beer, M.	2018	White-Tailed Sea Eagle (Haliaeetus albicilla) Die-Off Due to Infection with Highly Pathogenic Avian Influenza Virus, Subtype H5N8, in Germany	Viruses-Basel
22	61	S. H. M. Li, W. Y.//Liu, D. P.//Yang, Q. Q.//Chen, L. X.//Dai, Q.//Ma, T.//Gao, R. Y.//Ru, W. D.//Li, Y. F.//Yu, P. B.//Lu, J.//Zhang, G. G.//Tian, H. Y.//Chai, H. L.//Li, Y. B.	2018	Migratory Whooper Swans Cygnus cygnus Transmit H5N1 Virus between China and Mongolia: Combination Evidence from Satellite Tracking and Phylogenetics Analysis	Scientific Reports
23	1647	H. Z. X. Liu, C. C.//Chen, J.//Chen, G.//Zhang, J.//Li, Y.//Xiong, Y. P.//Wang, R. K.//Cao, Y.//Chen, Q. J.//Liu, D.//Wang, H. Z.//Chen, J. J.	2018	Two genetically diverse H7N7 avian influenza viruses isolated from migratory birds in central China	Emerging Microbes & Infections
24	1	Y. P. L. Liu, D. H.//Chen, L. H.//Lin, Y. J.//Li, W. C.//Hu, S. C.//Chen, Y. P.//Swayne, D. E.//Lee, M. S.	2018	Detection of reassortant H5N6 Glade 2.3.4.4 highly pathogenic avian influenza virus in a black-faced spoonbill (Platalea minor) found dead, Taiwan, 2017	Infection Genetics and Evolution

29	8	H. O. Nakagawa, K.//Kawabata, T.//Matsuu, A.//Takase, K.//Kuwahara, M.//Toda, S.//Ozawa, M.	2018	Genetic characterization of low-pathogenic avian influenza viruses isolated on the Izumi plain in Japan: possible association of dynamic movements of wild birds with AIV evolution	Archives of Virology
31	11185	M. J. B. Poen, T. M.//Vuong, O.//Scheuer, R. D.//van der Jeugd, H. P.//Kleyheeg, E.//Eggink, D.//Lexmond, P.//van den Brand, J. M. A.//Begeman, L.//van der Vliet, S.//Muskens, G. J. D. M.//Majoor, F. A.//Koopmans, M. P. G.//Kuiken, T.//Fouchier, R. A. M.	2018	Local amplification of highly pathogenic avian influenza H5N8 viruses in wild birds in the Netherlands, 2016 to 2017	Eurosurveillance
32	1502	K. K. Poltep, N.//Paungpin, W.//Prompiram, P.//Sedwisai, P.//Chamsai, T.//Puthavathana, P.//Ratanakorn, P.	2018	A Long-Term Serosurvey of Avian Influenza H5 among Wild Birds in Nakhon Sawan Province, Thailand	Journal of Zoo and Wildlife Medicine
34	1097	P. S. Ratanakorn, S.//Wiriyarat, W.//Eiamampai, K.//Chaichoune, K.//Wiratsudakul, A.//Sariya, L.//Puthavathana, P.	2018	Satellite telemetry tracks flyways of Asian Openbill storks in relation to H5N1 avian influenza spread and ecological change	Bmc Veterinary Research
35	5048	A. B. H. Reeves, J. S.//Poulson, R. L.//Donnelly, T.//Stallknecht, D. E.//Ramey, A. M.	2018	Influenza A virus recovery, diversity, and intercontinental exchange: A multi-year assessment of wild bird sampling at Izembek National Wildlife Refuge, Alaska	Plos One

37	4342	D. P. Venkatesh, M. J.//Bestebroer, T. M.//Scheuer, R. D.//Vuong, O.//Chkhaidze, M.//Machablishvili, A.//Mamuchadze, J.//Ninua, L.//Fedorova, N. B.//Halpin, R. A.//Lin, X. D.//Ransier, A.//Stockwell, T. B.//Wentworth, D. E.//Kriti, D.//Dutta, J.//van Bakel, H.//Puranik, A.//Slomka, M. J.//Essen, S.//Brown, I. H.//Fouchier, R. A. M.//Lewis, N. S.	2018	Avian Influenza Viruses in Wild Birds: Virus Evolution in a Multihost Ecosystem	Journal of Virology
39	51	A. G.-R. Afanador-Villamizar, C.//Diaz, A.//Ruiz-Saenz, J.	2017	Avian influenza in Latin America: A systematic review of serological and molecular studies from 2000-2015	Plos One
40	1187	A. T. Barbara, O.//Camacho, M. C.//Barral, M.//Hernandez, J. M.//Hofle, U.	2017	Avian Influenza Virus Surveillance in South-Central Spain Using Fecal Samples of Aquatic Birds Foraging at Landfills	Frontiers in Veterinary Science
41	493764	I. M. Brown, P.//Smietanka, K.//Staubach, C.//Willeberg, P.//Adlhoch, C.//Candiani, D.//Fabris, C.//Zancanaro, G.//Morgado, J.//Verdonck, F.//European Food Safety Authority//European Ctr Disease Prevention Co//European Ctr Disease Prevention Co//European Union Reference Lab Avian	2017	Avian influenza overview October 2016-August 2017	Efsa Journal
43	86670	A. C. Caron, J.//Gaidet, N.	2017	Challenging the conceptual framework of maintenance hosts for influenza A viruses in wild birds	Journal of Applied Ecology

44	417	A. C. Caron, N.//Mundava, J.//Abolnik, C.//Dondona, A. C.//Scacchia, M.//Gaidet, N.	2017	Avian Viral Pathogens in Swallows, Zimbabwe Infectious Diseases in Hirundinidae: A Risk to Swallow?	Ecohealth
47	3518	M. M. H. Hassan, M. A.//Debnath, N. C.//Yamage, M.//Klaassen, M.	2017	Are Poultry or Wild Birds the Main Reservoirs for Avian Influenza in Bangladesh?	Ecohealth
49	484	N. J. H. Hill, I. T. M.//Davis, K. R.//Ma, E. J.//Spivey, T. J.//Ramey, A. M.//Puryear, W. B.//Das, S. R.//Halpin, R. A.//Lin, X. D.//Fedorova, N. B.//Suarez, D. L.//Boyce, W. M.//Runstadler, J. A.	2017	Reassortment of Influenza A Viruses in Wild Birds in Alaska before H5 Clade 2.3.4.4 Outbreaks	Emerging Infectious Diseases
51	813	D. D. A. Houston, S.//Lundy, C. W.//Sato, Y.//Guo, B. Q.//Blanchong, J. A.//Gauger, P. C.//Marks, D. R.//Yoon, K. J.//Adelman, J. S.	2017	Evaluating the role of wild songbirds or rodents in spreading avian influenza virus across an agricultural landscape	Peerj
52	107	C. S. C. Jennelle, M.//Hildebrand, E. C.//Wolf, P. C.//Grear, D. A.//Ip, H. S.//Cornicelli, L.	2017	Surveillance for Highly Pathogenic Avian Influenza in Wild Turkeys (Meleagris gallopavo) of Minnesota, USA during 2015 Outbreaks in Domestic Poultry	Journal of Wildlife Diseases
54	242	E. S. Kleyheeg, R.//Bodewes, R.//Rijks, J. M.//Spierenburg, M. A. H.//Beerens, N.//Kelder, L.//Poen, M. J.//Stegeman, J. A.//Fouchier, R. A. M.//Kuiken, T.//van der Jeugd, H. P.	2017	Deaths among Wild Birds during Highly Pathogenic Avian Influenza A(H5N8) Virus Outbreak, the Netherlands	Emerging Infectious Diseases

57	11	D. H. S. Lee, K.//Swayne, D. E.//Kurskaya, O.//Sobolev, I.//Kabilov, M.//Alekseev, A.//Irza, V.//Shestopalov, A.	2017	Novel Reassortant Clade 2.3.4.4 Avian Influenza A(H5N8) Virus in Wild Aquatic Birds, Russia, 2016	Emerging Infectious Diseases
58	188	D. H. T. Lee, M. K.//Killian, M. L.//DeLiberto, T. J.//Swayne, D. E.	2017	Reoccurrence of Avian Influenza A(H5N2) Virus Clade 2.3.4.4 in Wild Birds, Alaska, USA, 2016	Emerging Infectious Diseases
60	304	E. N. M. Liberda, R.//Charania, N. A.//Davey, R.//Tsuji, L. J. S.	2017	Avian influenza prevalence among hunter-harvested birds in a remote Canadian First Nation community	Rural and Remote Health
61	379	E. E.-K. Lindh, C.//Isomursu, M.//Alasaari, J.//Vaheri, A.//Vapalahti, O.//Huovilainen, A.	2017	Genetic Characterization of H13 and H16 Influenza a Viruses in Gulls (Larus Spp.) with Clinically Severe Disease and Concurrent Circovirus Infection	Journal of Wildlife Diseases
63	251	S. H. Meier, D.//Hofmann, M.//Renzullo, S.//Vogler, B. R.//Sigrist, B.//Hoop, R. K.//Albini, S.	2017	Outbreak of Highly Pathogenic Avian Influenza H5N8 in November 2016 in Wild Birds in Switzerland	Schweizer Archiv Fur Tierheilkunde
65	4268	Z. C. Papp, R. G.//Parmley, E. J.//Leighton, F. A.//Waldner, C.//Soos, C.	2017	The ecology of avian influenza viruses in wild dabbling ducks (Anas spp.) in Canada	Plos One
66	299	E. D. d. A. Petersen, J.//Kruger, L.//Seixas, M. M.//Ometto, T.//Thomazelli, L. M.//Walker, D.//Durigon, E. L.//Petry, M. V.	2017	First detection of avian influenza virus (H4N7) in Giant Petrel monitored by geolocators in the Antarctic region	Marine Biology
67	7759	L. A. L. Preskenis, B. S.//Gelb, J.	2017	Identification of Type A Influenza Viruses from Wild Birds on the Delmarva Peninsula, 2007-10	Avian Diseases

68	400	D. J. D. Prosser, C. L.//Hindman, L. J.//Iwanowicz, D. D.//Ottinger, C. A.//Iwanowicz, L. R.//Driscoll, C. P.//Nagel, J. L.	2017	Low Pathogenic Avian Influenza Viruses in Wild Migratory Waterfowl in a Region of High Poultry Production, Delmarva, Maryland	Avian Diseases
69	1408	A. M. H. Ramey, N. J.//Cline, T.//Plancarte, M.//De La Cruz, S.//Casazza, M. L.//Ackerman, J. T.//Fleskes, J. P.//Vickers, T. W.//Reeves, A. B.//Gulland, F.//Fontaine, C.//Prosser, D. J.//Runstadler, J. A.//Boyce, W. M.	2017	Surveillance for highly pathogenic influenza A viruses in California during 2014-2015 provides insights into viral evolutionary pathways and the spatiotemporal extent of viruses in the Pacific Americas Flyway	Emerging Microbes & Infections
72	31	N. L. C. Sinai, P. S.//Andrle, K. M.//Jefferis, C.//Senties-Cue, C. G.//Pitesky, M. E.	2017	A Serosurvey of Greater Sage-Grouse (Centrocercus urophasianus) in Nevada, USA	Journal of Wildlife Diseases
73	2046	T. J. L. Spivey, M. S.//Meixell, B. W.//Smith, K. R.//Puryear, W. B.//Davis, K. R.//Runstadler, J. A.//Stallknecht, D. E.//Ramey, A. M.	2017	Maintenance of influenza A viruses and antibody response in mallards (Anas platyrhynchos) sampled during the non-reeding season in Alaska	Plos One
74	21	G. M. G. Stoimenov, G. V.//Nikolov, B.//Petrova, R.//Teneva, A.//Dimitrova, I.	2017	Histopathological findings in Dalmatian pelicans (Pelecanus crispus) naturally infected with avian influenza subtype A H5N1 in Bulgaria	Journal of the Hellenic Veterinary Medical Society
77	50	F. F. S. Vaz, P. P.//Locatelli-Dittrich, R.//Meurer, R.//Durigon, E. L.//de Araujo, J.//Thomazelli, L. M.//Ometto, T.//Sipinski, E. A. B.//Sezerban, R. M.//Abbud, M. C.//Raso, T. F.	2017	Survey of pathogens in threatened wild red-tailed Amazon parrot (Amazona brasiliensis) nestlings in Rasa Island, Brazil	Brazilian Journal of Microbiology

79	977	M. L. Wille, K.//Muradrasoli, S.//Olsen, B.//Jarhult, J. D.	2017	Urbanization and the dynamics of RNA viruses in Mallards (Anas platyrhynchos)	Infection Genetics and Evolution
80	958	Y. F. S. Yao, Z. Y.//He, B.//Yang, W. H.//Chen, J. J.//Zhang, T.//Chen, X. B.//Chen, J.	2017	Characterization of a reassortant H11N9 subtype avian influenza virus isolated from bean goose along the East Asian-Australian flyway	Virus Genes
84	40	D. S. Bengtsson, K.//Avril, A.//Fiedler, W.//Wikelski, M.//Gunnarsson, G.//Elmberg, J.//Tolf, C.//Olsen, B.//Waldenstrom, J.	2016	Does influenza A virus infection affect movement behaviour during stopover in its wild reservoir host?	Royal Society Open Science
87	1240	Y. H. L. Bi, H. Z.//Xiong, C. C.//Liu, D.//Shi, W. F.//Li, M. X.//Liu, S. L.//Chen, J.//Chen, G.//Li, Y.//Yang, G. X.//Lei, Y. S.//Xiong, Y. P.//Lei, F. M.//Wang, H. Z.//Chen, Q. J.//Chen, J. J.//Gao, G. F.	2016	Novel avian influenza A (H5N6) viruses isolated in migratory waterfowl before the first human case reported in China, 2014	Scientific Reports
88	117	M. H. Camacho, J. M.//Lima-Barbero, J. F.//Hofle, U.	2016	Use of wildlife rehabilitation centres in pathogen surveillance: A case study in white storks (Ciconia ciconia)	Preventive Veterinary Medicine
93	1250	A. S. M. Gonzalez-Reiche, M. L.//Ortiz, L.//Cordon-Rosales, C.//Perez, D. R.	2016	Prevalence and Diversity of Low Pathogenicity Avian Influenza Viruses in Wild Birds in Guatemala, 2010-2013	Avian Diseases
94	164	M. H. Guimaraes, R.//Bello, C.//Vanstreels, R.//Ferreira, A.	2016	Surveillance for Newcastle Disease Virus, Avian Influenza Virus and Mycoplasma Gallisepticum in Wild Birds Near	Brazilian Journal of Poultry Science

				Commercial Poultry Farms Surrounded by Atlantic Rainforest Remnants, Southeastern Brazil	
95	41	C. M. K. Hartby, J. S.//Merkel, F.//Holm, E.//Larsen, L. E.//Hjulsager, C. K.	2016	First Characterization of Avian Influenza Viruses from Greenland 2014	Avian Diseases
96	162	S. C. M. Hill, R. J.//Schulenburg, B.//Shell, W.//Wikramaratna, P. S.//Perrins, C.//Sheldon, B. C.//Brown, I. H.//Pybus, O. G.	2016	Antibody responses to avian influenza viruses in wild birds broaden with age	Proceedings of the Royal Society B-Biological Sciences
97	214	B. J. M. Hoye, V. J.//Huig, N.//de Vries, P.//Oosterbeek, K.//Tijsen, W.//Klaassen, M.//Fouchier, R. A. M.//van Gils, J. A.	2016	Hampered Performance of Migratory Swans: Intra- and Inter-Seasonal Effects of Avian Influenza Virus	Integrative and Comparative Biology
98	905	R. D. A. Hurtado, S. M.//Vanstreels, R. E. T.//Fabrizio, T.//Walker, D.//Rodrigues, R. C.//Seixas, M. M. M.//de Araujo, J.//Thomazelli, L. M.//Ometto, T. L.//Webby, R. J.//Webster, R. G.//Jerez, J. A.//Durigon, E. L.	2016	Surveillance of Avian Influenza Virus in Aquatic Birds on the Brazilian Amazon Coast	Ecohealth
99	1761	H. S. D. Ip, R. J.//Bodenstein, B.//Torchetti, M. K.//DeBruyn, P.//Mansfield, K. G.//DeLiberto, T.//Sleeman, J. M.	2016	High Rates of Detection of Clade 2.3.4.4 Highly Pathogenic Avian Influenza H5 Viruses in Wild Birds in the Pacific Northwest During the Winter of 2014-15	Avian Diseases

104	2694	B. W. A. Meixell, T. W.//Lindberg, M. S.//Smith, M. M.//Runstadler, J. A.//Ramey, A. M.	2016	Detection, prevalence, and transmission of avian hematozoa in waterfowl at the Arctic/sub-Arctic interface: co-infections, viral interactions, and sources of variation	Parasites & Vectors
106	6802	D. P.-J. Muzyka, M.//Spackman, E.//Smith, D.//Rula, O.//Muzyka, N.//Stegniy, B.	2016	Isolation and Genetic Characterization of Avian Influenza Viruses Isolated from Wild Birds in the Azov-Black Sea Region of Ukraine (2001-2012)	Avian Diseases
107	3692	R. P. Nallar, Z.//Leighton, F. A.//Epp, T.//Pasick, J.//Berhane, Y.//Lindsay, R.//Soos, C.	2016	Ecological Determinants of Avian Influenza Virus, West Nile Virus, and Avian Paramyxovirus Infection and Antibody Status in Blue-Winged Teal (Anas Discors) in the Canadian Prairies	Journal of Wildlife Diseases
109	50	H. E. L. Pearson, S. J.//Hernandez-Jover, M.//Toribio, J. A. L. M. L.	2016	Pathogen Presence in European Starlings Inhabiting Commercial Piggeries in South Australia	Avian Diseases
110	71	L. B. O. Pinto, T.//Araujo, J.//Thomazelli, L. M.//Seixas, M. M.//Barbosa, C. M.//Ramos, D. G. S.//Melo, A. L. T.//Pinho, J. B.//Durigon, E. L.//Aguiar, D. M.	2016	Investigation of Influenza A, West Nile and Newcastle Disease Viruses in Birds from the Pantanal Wetlands of Mato Grosso, Brazil	Brazilian Journal of Poultry Science
111	8805	M. J. V. Poen, J. H.//Manvell, R. J.//Brown, I.//Bestebroer, T. M.//van der Vliet, S.//Vuong, O.//Scheuer, R. D.//van der Jeugd, H. P.//Nolet, B. A.//Kleyheeg, E.//Muskens, G. J. D. M.//Majoor, F. A.//Grund, C.//Fouchier, R. A. M.	2016	Lack of virological and serological evidence for continued circulation of highly pathogenic avian influenza H5N8 virus in wild birds in the Netherlands, 14 November 2014 to 31 January 2016	Eurosurveillance

113	15122	M. V. Steensels, D.//Linden, A.//Houdart, P.//van den Berg, T. P.//Lambrecht, B.	2016	One Decade of Active Avian Influenza Wild Bird Surveillance in Belgium Showed a Higher Viroprevalence in Hunter-Harvested Than in Live-Ringed Birds	Avian Diseases
116	298	J. K. W. Wong, B. R.//Fojtik, A.//Poulson, R. L.//Stallknecht, D. E.	2016	Antibodies to Influenza A Viruses in Wintering Snow Geese (Chen caerulescens) in Texas	Avian Diseases
117	27	L. C. L. Zhou, J.//Pei, E. L.//Xue, W. J.//Lyu, J. M.//Cai, Y. T.//Wu, D.//Wu, W.//Liu, Y. Y.//Jin, H. Y.//Gao, Y. W.//Wang, Z. H.//Wang, T. H.	2016	Novel Avian Influenza A(H5N8) Viruses in Migratory Birds, China, 2013-2014	Emerging Infectious Diseases
120	11	Y. H. Z. Bi, Z. J.//Liu, W. J.//Yin, Y. B.//Hong, J. M.//Li, X. D.//Wang, H. M.//Wong, G.//Chen, J. J.//Li, Y. F.//Ru, W. D.//Gao, R. Y.//Liu, D.//Liu, Y. X.//Zhou, B. P.//Gao, G. F.//Shi, W. F.//Lei, F. M.	2015	Highly Pathogenic Avian Influenza A(H5N1) Virus Struck Migratory Birds in China in 2015	Scientific Reports
121	1	M. H. A. Body, Abdulmajeed H.//Alhubsy, Saif S.//Saravanan, Nirmala//Rajmony, Sunil//Mansoor, Muhammad Khalid	2015	Characterization of Low Pathogenic Avian Influenza Virus Subtype H9N2 Isolated from Free-Living Mynah Birds (Acridotheres tristis) in the Sultanate of Oman	Avian Diseases
123	3984	A. S. N. Bowman, Jacqueline M.//Massengill, Rose//Baker, Joseph//Workman, Jeffrey D.//Slemons, Richard D.	2015	Influenza A Virus Surveillance in Waterfowl in Missouri, USA, 2005–2013	Avian Diseases

124	1	V. N. O. Bui, H.//Hussein, I. T. M.//Hill, N. J.//Trinh, D. Q.//AboElkhair, M.//Sultan, S.//Ma, E.//Saito, K.//Watanabe, Y.//Runstadler, J. A.//Imai, K.	2015	Genetic characterization of a rare H12N3 avian influenza virus isolated from a green-winged teal in Japan	Virus Genes
125	148	D. G. Cano-Terriza, R.//Lecollinet, S.//Cerda-Cuellar, M.//Cabezon, O.//Almeria, S.//Garcia-Bocanegra, I.	2015	Epidemiological survey of zoonotic pathogens in feral pigeons (Columba livia var. domestica) and sympatric zoo species in Southern Spain	Comparative Immunology Microbiology and Infectious Diseases
128	359	H. L. V. Ferreira, Didier//Van Borm, Steven//Poncin, Olivier//Dumont, Nathalie//Ozhelvaci, Orkun//Munir, Muhammad//van den Berg, Thierry//Lambrecht, Benédicte	2015	Differential Viral Fitness Between H1N1 and H3N8 Avian Influenza Viruses Isolated from Mallards (Anas platyrhynchos)	Avian Diseases
131	5581	J. S. R. Hall, R. E.//Franson, J. C.//Soos, C.//Dusek, R. J.//Allen, R. B.//Nashold, S. W.//TeSlaa, J. L.//Jonsson, J. E.//Ballard, J. R.//Harms, N. J.//Brown, J. D.	2015	Avian Influenza Ecology in North Atlantic Sea Ducks: Not All Ducks Are Created Equal	Plos One
133	5505	M. A. B. Hoque, G. W.//Cheam, A. L.//Skerratt, L. F.	2015	Epidemiology of avian influenza in wild aquatic birds in a biosecurity hotspot, North Queensland, Australia	Preventive Veterinary Medicine
134	1811	D. M. Karmacharya, S.//Sharma, A.//Bhatta, T.//Adhikari, P.//Sherchan, A. M.//Shrestha, B.//Bista, M.//Rajbhandari, R.//Oberoi, M.//Bisht, K.//Hero, J. M.//Dissanayake, R.//Dhakal, M.//Hughes, J.//Debnath, N.	2015	Surveillance of Influenza A Virus and Its Subtypes in Migratory Wild Birds of Nepal	Plos One

135	1451	W. M. G. Kistler, S. E. J.//Stallknecht, D. E.//Yabsley, M. J.	2015	Wood ducks (Aix sponsa) as potential reservoirs for avian influenza and avian paramyxoviruses	Avian Pathology
136	79	C. M. Mathieu, V.//Pedersen, J.//Jeria, J.//Agredo, M.//Gutierrez, C.//Garcia, A.//Vasquez, M.//Avalos, P.//Retamal, P.	2015	Avian Influenza in wild birds from Chile, 2007-2009	Virus Research
137	13574	R. P. Nallar, Z.//Epp, T.//Leighton, F. A.//Swafford, S. R.//DeLiberto, T. J.//Dusek, R. J.//Ip, H. S.//Hall, J.//Berhane, Y.//Gibbs, S. E. J.//Soos, C.	2015	Demographic and Spatiotemporal Patterns of Avian Influenza Infection at the Continental Scale, and in Relation to Annual Life Cycle of a Migratory Host	Plos One
139	7	M. M. Ozawa, A.//Tokorozaki, K.//Horie, M.//Masatani, T.//Nakagawa, H.//Okuya, K.//Kawabata, T.//Toda, S.	2015	Genetic diversity of highly pathogenic H5N8 avian influenza viruses at a single overwintering site of migratory birds in Japan, 2014/15	Eurosurveillance
140	185	A. M. R. Ramey, A. B.//Poulson, R. L.//Wasley, J.//Esler, D.//Stallknecht, D. E.	2015	Sampling of Sea Ducks for Influenza A Viruses in Alaska during Winter Provides Lack of Evidence for Epidemiologic Peak of Infection	Journal of Wildlife Diseases
141	2842	A. M. R. Ramey, A. B.//Sonsthagen, S. A.//TeSlaa, J. L.//Nashold, S.//Donnelly, T.//Casler, B.//Hall, J. S.	2015	Dispersal of H9N2 influenza A viruses between East Asia and North America by wild birds	Virology
142	7233	M. D. H. Samuel, Jeffrey S.//Brown, Justin D.//Goldberg, Diana R.//Ip, Hon//Baranyuk, Vasily V.	2015	The dynamics of avian influenza in Lesser Snow Geese: implications for annual and migratory infection patterns	Ecological Applications

145	148	J. G. B. K. van Dijk, E.//Soons, M. B.//Nolet, B. A.//Fouchier, R. A. M.//Klaassen, M.	2015	Weak negative associations between avian influenza virus infection and movement behaviour in a key host species, the mallard Anas platyrhynchos	Oikos
146	6756	J. H. v. d. J. Verhagen, H. P.//Nolet, B. A.//Slaterus, R.//Kharitonov, S. P.//de Vries, P. P.//Vuong, O.//Majoor, F.//Kuiken, T.//Fouchier, R. A.	2015	Wild bird surveillance around outbreaks of highly pathogenic avian influenza A(H5N8) virus in the Netherlands, 2014, within the context of global flyways	Eurosurveillance
147	1	J. X. Yuan, L. L.//Bao, L. L.//Yao, Y. F.//Deng, W.//Li, F. D.//Lv, Q.//Gu, S. Z.//Wei, Q.//Qin, C.	2015	Characterization of an H9N2 avian influenza virus from a Fringilla montifringilla brambling in northern China	Virology
149	2942	C. Abolnik	2014	A current review of avian influenza in pigeons and doves (Columbidae)	Veterinary Microbiology
151	108	S. K. Albini, L.//Sigrist, B.//Guttinger, R.//Keller, R.//Hoop, R. K.	2014	Shedding of zoonotic pathogens and analysis of stomach contents in great cormorants (Phalacrocorax carbo sinensis) from Switzerland between 2007 and 2012	Schweizer Archiv Fur Tierheilkunde
155	507	A. G. Caron, V.//Etter, E.//Gaidet, N.//de Garine-Wichatitsky, M.	2014	Bridge hosts for avian influenza viruses at the wildlife/domestic interface: An eco-epidemiological framework implemented in southern Africa	Preventive Veterinary Medicine

156	441	H. D. Chang, F. Y.//Liu, Z. L.//Yuan, F. Z.//Zhao, S. Y.//Xiang, X.//Zou, F. C.//Zeng, B. Q.//Fan, Y. T.//Duan, G.	2014	Seroprevalence Survey of Avian influenza A (H5) in wild migratory birds in Yunnan Province, Southwestern China	Virology Journal
157	8623	J. M. E. Curran, Trevor M.//Robertson, Ian D.	2014	Surveillance of Charadriiformes in Northern Australia Shows Species Variations in Exposure to Avian Influenza Virus and Suggests Negligible Virus Prevalence	Avian Diseases
158	239	V. L. G. D'Amico, P. M.//Baker, A. J.//Buehler, D. M.//Bertellotti, M.	2014	Multi-year surveillance of selected avian pathogens in the migrant shorebird Red Knot (Calidris canutus rufa) at its main stopover site in Patagonia, Argentina	Journal of Ornithology
159	330	J. d. A. de Araujo, S. M.//Gaidet, N.//Hurtado, R. F.//Walker, D.//Thomazelli, L. M.//Ometto, T.//Seixas, M. M. M.//Rodrigues, R.//Galindo, D. B.//da Silva, A. C. S.//Rodrigues, A. M. M.//Bomfim, L. L.//Mota, M. A.//Larrazabal, M. E.//Branco, J. O.//Serafini, P.//Neto, I. S.//Franks, J.//Webby, R. J.//Webster, R. G.//Durigon, E. L.	2014	Avian Influenza Virus (H11N9) in Migratory Shorebirds Wintering in the Amazon Region, Brazil	Plos One
160	167	M. A. D. De Marco, M.//Sivay, M.//Sharshov, K.//Yurlov, A.//Cotti, C.//Shestopalov, A.	2014	Virological Evaluation of Avian Influenza Virus Persistence in Natural and Anthropic Ecosystems of Western Siberia (Novosibirsk Region, Summer 2012)	Plos One

161	997	M. A. V. De Marco, A.//Foni, E.//Savarese, M. C.//Cotti, C.//Chiapponi, C.//Raffini, E.//Donatelli, I.//Delogu, M.	2014	Is there a relation between genetic or social groups of mallard ducks and the circulation of low pathogenic avian influenza viruses?	Veterinary Microbiology
162	1078	R. J. H. Dusek, G. T.//Ip, H. S.//Jonsson, J. E.//Sreevatsan, S.//Nashold, S. W.//TeSlaa, J. L.//Enomoto, S.//Halpin, R. A.//Lin, X. D.//Fedorova, N.//Stockwell, T. B.//Dugan, V. G.//Wentworth, D. E.//Hall, J. S.	2014	North Atlantic Migratory Bird Flyways Provide Routes for Intercontinental Movement of Avian Influenza Viruses	Plos One
163	14	S. T. Z. Fan, L. C.//Wu, D.//Gao, X. L.//Pei, E. L.//Wang, T. H.//Gao, Y. W.//Xia, X. Z.	2014	A novel highly pathogenic H5N8 avian influenza virus isolated from a wild duck in China	Influenza and Other Respiratory Viruses
166	118735	S. R. D. Groepper, Thomas J.//Vrtiska, Mark P.//Pedersen, Kerri//Swafford, Seth R.//Hygnstrom, Scott E.	2014	Avian Influenza Virus Prevalence in Migratory Waterfowl in the United States, 2007–2009	Avian Diseases
167	881	J. S. H. Hall, G. T.//Suwannanarn, K.//Sreevatsen, S.//Ip, H. S.//Magnusdottir, E.//TeSlaa, J. L.//Nashold, S. W.//Dusek, R. J.	2014	Avian influenza virus ecology in Iceland shorebirds: Intercontinental reassortment and movement	Infection Genetics and Evolution
168	452	Y. Y. R. Huang, G. J.//Ojkic, D.//Whitney, H.//Lang, A. S.	2014	Diverse inter-continental and host lineage reassortant avian influenza A viruses in pelagic seabirds	Infection Genetics and Evolution

169	1642	Y. Y. W. Huang, M.//Benkaroun, J.//Munro, H.//Bond, A. L.//Fifield, D. A.//Robertson, G. J.//Ojkic, D.//Whitney, H.//Lang, A. S.	2014	Perpetuation and reassortment of gull influenza A viruses in Atlantic North America	Virology
170	1288	J. K. Jeong, H. M.//Lee, E. K.//Song, B. M.//Kwon, Y. K.//Kim, H. R.//Choi, K. S.//Kim, J. Y.//Lee, H. J.//Moon, O. K.//Jeong, W.//Choi, J.//Baek, J. H.//Joo, Y. S.//Park, Y. H.//Lee, H. S.//Lee, Y. J.	2014	Highly pathogenic avian influenza virus (H5N8) in domestic poultry and its relationship with migratory birds in South Korea during 2014	Veterinary Microbiology
171	72	J. A. D. Johnson, L. H.//Ruthrauff, D. R.//Krauss, S.//Hall, J. S.	2014	Avian Influenza Virus Antibodies in Pacific Coast Red Knots (Calidris canutus roselaari)	Journal of Wildlife Diseases
172	284	L. S. Jurinovic, V.//Balenovic, M.//Lisicic, D.//Lucic, V.	2014	Virological and serological investigation of avian influenza in black-headed gulls captured on a rubbish dump in Zagreb, Croatia	Veterinarski Arhiv
173	66	S. U. B. Khan, L.//Haider, N.//Gerloff, N.//Rahman, M. Z.//Shu, B.//Rahman, M.//Dey, T. K.//Davis, T. C.//Das, B. C.//Balish, A.//Islam, A.//Teifke, J. P.//Zeidner, N.//Lindstrom, S.//Klimov, A.//Donis, R. O.//Luby, S. P.//Shivaprasad, H. L.//Mikolon, A. B.	2014	Investigating a crow die-off in January-February 2011 during the introduction of a new clade of highly pathogenic avian influenza virus H5N1 into Bangladesh	Archives of Virology
175	200	K. B. P. Ku, E. H.//Yum, J.//Kim, J. A.//Oh, S. K.//Seo, S. H.	2014	Highly Pathogenic Avian Influenza A(H5N8) Virus from Waterfowl, South Korea, 2014	Emerging Infectious Diseases

179	694	K. M. Pedersen, David R.//Arsnoe, Dustin M.//Afonso, Claudio L.//Bevins, Sarah N.//Miller, Patti J.//Randall, Adam R.//DeLiberto, Thomas J.	2014	Avian Paramyxovirus Serotype 1 (Newcastle Disease Virus), Avian Influenza Virus, and Salmonella spp. in Mute Swans (Cygnus olor) in the Great Lakes Region and Atlantic Coast of the United States	Avian Diseases
180	6782	A. M. P. Ramey, R. L.//Gonzalez-Reiche, A. S.//Wilcox, B. R.//Walther, P.//Link, P.//Carter, D. L.//Newsome, G. M.//Muller, M. L.//Berghaus, R. D.//Perez, D. R.//Hall, J. S.//Stallknecht, D. E.	2014	Evidence for Seasonal Patterns in the Relative Abundance of Avian Influenza Virus Subtypes in Blue-Winged Teal (Anas discors)	Journal of Wildlife Diseases
181	3896	P. H. Schwemmer, B.//Geiter, O.//Gunther, K.//Corman, V. M.//Garthe, S.	2014	Weather-related Winter Mortality of Eurasian Oystercatchers (Haematopus ostralegus) in the Northeastern Wadden Sea	Waterbirds
183	7790	M. J. W. Slusher, B. R.//Lutrell, M. P.//Poulson, R. L.//Brown, J. D.//Yabsley, M. J.//Stallknecht, D. E.	2014	Are Passerine Birds Reservoirs for Influenza a Viruses?	Journal of Wildlife Diseases
185	122	G. R. Z. Wang, T.//Li, X. W.//Jiang, Z. B.//Jiang, Q.//Chen, Q. J.//Tu, X. B.//Chen, Z.//Chang, J. Y.//Li, L. X.//Xu, B.	2014	Serological evidence of H7, H5 and H9 avian influenza virus co-infection among herons in a city park in Jiangxi, China	Scientific Reports
186	3276	M. H. Wille, Y. Y.//Robertson, G. J.//Ryan, P.//Wilhelm, S. I.//Fifield, D.//Bond, A. L.//Granter, A.//Munro, H.//Buxton, R.//Jones, I. L.//Fitzsimmons, M. G.//Burke, C.//Tranquilla, L. M.//Rector, M.//Takahashi,	2014	Evaluation of Seabirds in Newfoundland and Labrador, Canada, as Hosts of Influenza A Viruses	Journal of Wildlife Diseases

		L.//Kouwenberg, A. L.//Storey, A.//Walsh, C.//Hedd, A.//Montevecchi, W. A.//Runstadler, J. A.//Ojkic, D.//Whitney, H.//Lang, A. S.			

Chapter 2 Appendix 2: Number if samples collected for AIV and the prevalence of positives per bird family

Family	Total number of Samples	Total Number of Positives	Percentage of samples positive (%)	Jeffreys Interval (+-)
Anatidae	226192	26698	11.8	-0.27
Pycnonotidae	1004	99	9.86	-3.69
Acrocephalidae	1440	111	7.71	-2.76
Alcidae	3187	195	6.12	-1.66
Laridae	13776	648	4.7	-0.71
Estrildidae	871	39	4.48	-2.75
Scolopacidae	7660	261	3.41	-0.81
Passeridae	1506	42	2.79	-1.67
Muscicapidae	1382	38	2.75	-1.73
Ardeidae	8870	242	2.73	-0.68
Corvidae	2783	71	2.55	-1.17
Ploceidae	1630	41	2.52	-1.52
Columbidae	3809	64	1.68	-0.82
Tyrannidae	1028	16	1.56	-1.53
Sturnidae	1827	28	1.53	-1.13
Hirundinidae	1696	26	1.53	-1.18
Rallidae	14065	194	1.38	-0.39
Odontophoridae	1341	17	1.27	-1.21
Phylloscopidae	1372	15	1.09	-1.11
Sylviidae	1325	14	1.06	-1.11
Parulidae	3007	29	0.96	-0.70
Accipitridae	2967	28	0.94	-0.70
Turdidae	3150	29	0.92	-0.67
Falconidae	1147	10	0.87	-1.09
Emberizidae	4463	38	0.85	-0.54
Paridae	952	8	0.84	-1.18
Phalacrocoracidae	11161	87	0.78	-0.33
Motacillidae	975	7	0.72	-1.08
Pelecanidae	1197	7	0.58	-0.88
Phasianidae	2548	14	0.55	-0.58
Spheniscidae	2073	8	0.39	-0.54
Ciconiidae	2944	11	0.37	-0.45
Charadriidae	2100	7	0.33	-0.50
Procellariidae	2644	8	0.3	-0.43
Fringillidae	2447	7	0.29	-0.43
Icteridae	1055	2	0.19	-0.57
Gruidae	1415	1	0.07	-0.32
Hydrobatidae	1157	0	0	-0.22

Chapter 2 Appendix 3 : ShinyApp UI, server and data link.

```
library(shiny)

library(readxl)

library(dplyr)

library(ggplot2)

library(plotly)

library(scales)

library(flextable)

library(kableExtra)

# Define UI

ui <- fluidPage(

  tags$img(src = "https://findvectorlogo.com/wp-content/uploads/2019/04/university-of-hull-vector-logo.png", height = 100, width = 200),

  titlePanel("Avian Influenza prevalence rates and error in wild bird species"),

  h4("Developed by Daniel Wade, as part of his PhD thesis titled 'The Epizootiology of Avian Influenza in Wild Birds and Its Risk to the UK Poultry Sector' at the University of Hull. This app is a culmination of a systematic literature review."),

  fluidRow(

    column(

      width = 3,
```

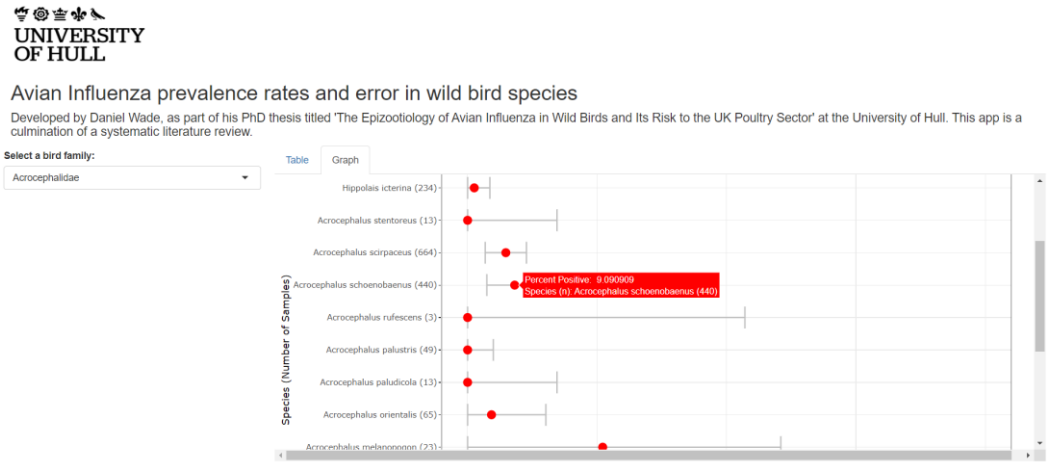
```
      style = "padding: 5px;",  # Reduce the padding around the sidebarPanel
      selectInput("Family", "Select a bird family:",
                  choices = unique(data$Family),
                  width = "100%"  # Adjust the width of the dropdown box
      )
    ),
    column(
      width = 9,
      tabsetPanel(
        tabPanel("Table", tableOutput("table")),
        tabPanel("Graph", div(
          style = "width: 100%; overflow-x: scroll;",
          plotlyOutput("graph")  # Use plotlyOutput to display the plot
        ))
      )
    )
  )
)

# Define server
server <- function(input, output) {
```

```
data <- reactive({
  Species_AI_Prevalence_and_Jeffries_Intervals
})

output$graph <- renderPlotly({
  filtered_data <- data() %>% filter(Family == input$Family)
  filtered_data$Species_n <- paste(filtered_data$Species, filtered_data$`Species (n)`)

  # Calculate the height of the graph based on the number of rows
  num_rows <- nrow(filtered_data)
  graph_height <- 400 + num_rows * 30

  p <- ggplot(filtered_data, aes(x = `Percent Positive`, y = `Species (n)`)) +
    geom_errorbar(aes(xmin = `Lower CI`, xmax = `Upper CI`), colour = 'grey') +
    geom_point(aes(x = `Percent Positive`, y = `Species (n)`), color = "red", size = 3) +
    xlim(0, 100) +
    theme_bw() +
    theme(legend.position = 'none') +
    xlab('Proportion positive and Jeffreys Interval') +
    ylab('Species (Number of Samples)') +
```

```
    theme(plot.margin = margin(1, 1, 1, 1, "cm"))


  # Convert ggplot to plotly

  p <- ggplotly(p, height = graph_height)


  # Customize plotly layout

  p <- p %>% layout(margin = list(l = 50, r = 50, b = 50, t = 50))


  p

 })


 output$table <- renderTable({

  filtered_data <- data() %>% filter(Family == input$Family)

  filtered_data$Species <- cell_spec(filtered_data$Species, "html", italic = TRUE)

  filtered_data$`Total Number of Samples` <- format(filtered_data$`Total Number of Samples`,
big.mark = ",")

  filtered_data$`Total Number of Positive Samples` <- format(filtered_data$`Total Number of
Positive Samples`, big.mark = ",")

  dplyr::select(filtered_data, Species, `Total Number of Samples`, `Total Number of Positive
Samples`, `Percent Positive`, `Jeffreys Interval`)

 }, sanitize.text.function = function(x) x)
```

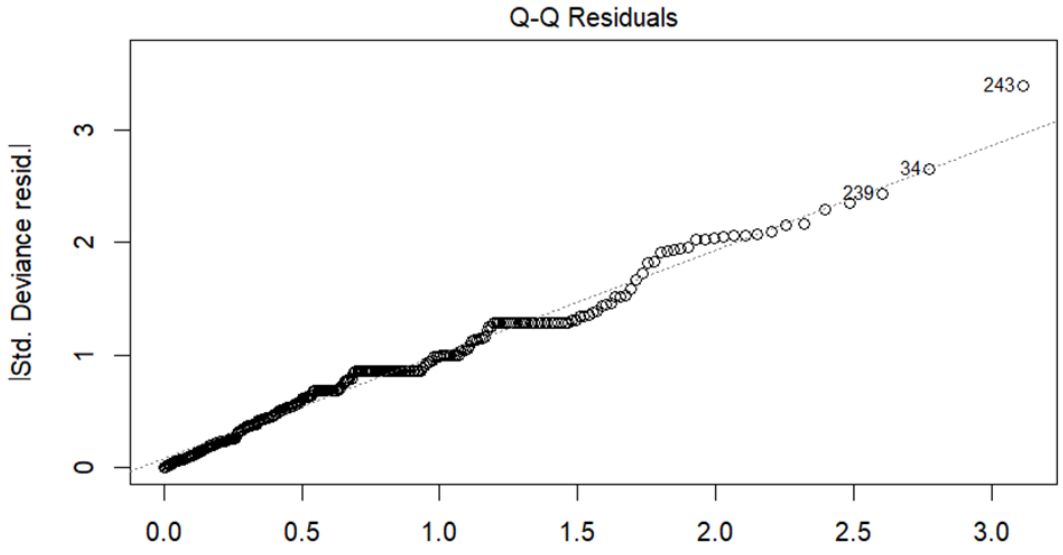
```
}

# Run the app

shinyApp(ui, server)
```

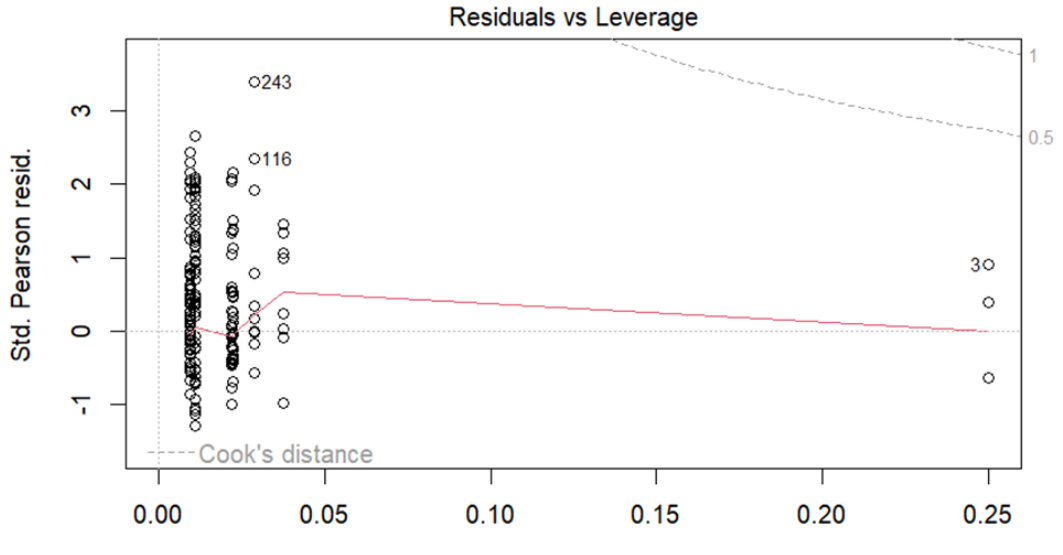
The screenshot below demonstrates the output of this code, an interactive graph and table demonstrating the sample size and AIV prevalence estimates classified by species.

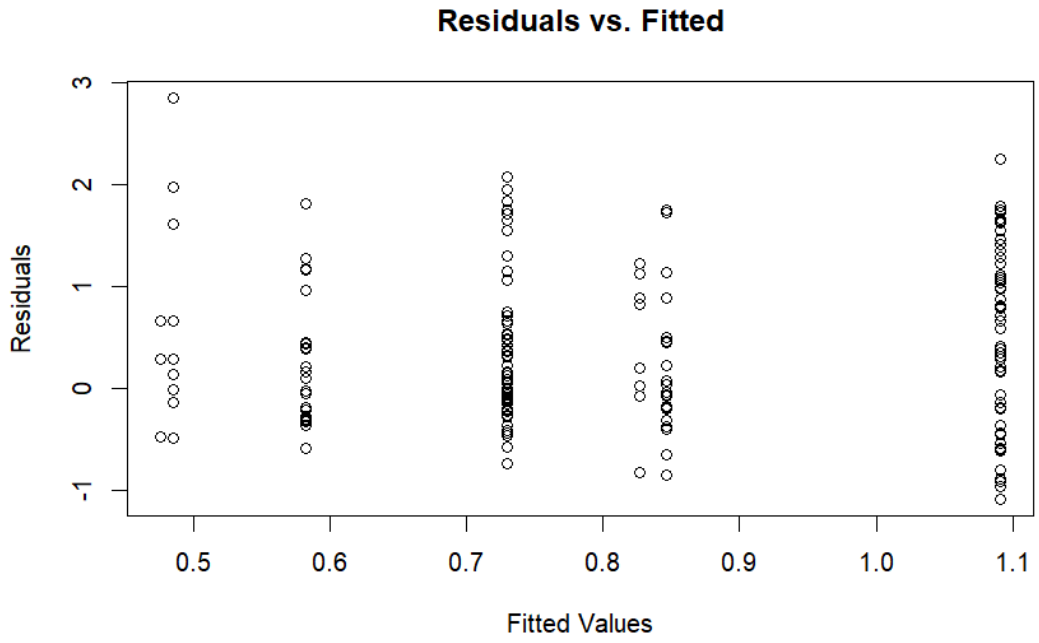


Chapter 2 Appendix 4: GLM assumptions data

>100 samples species AIV prevalence GLM

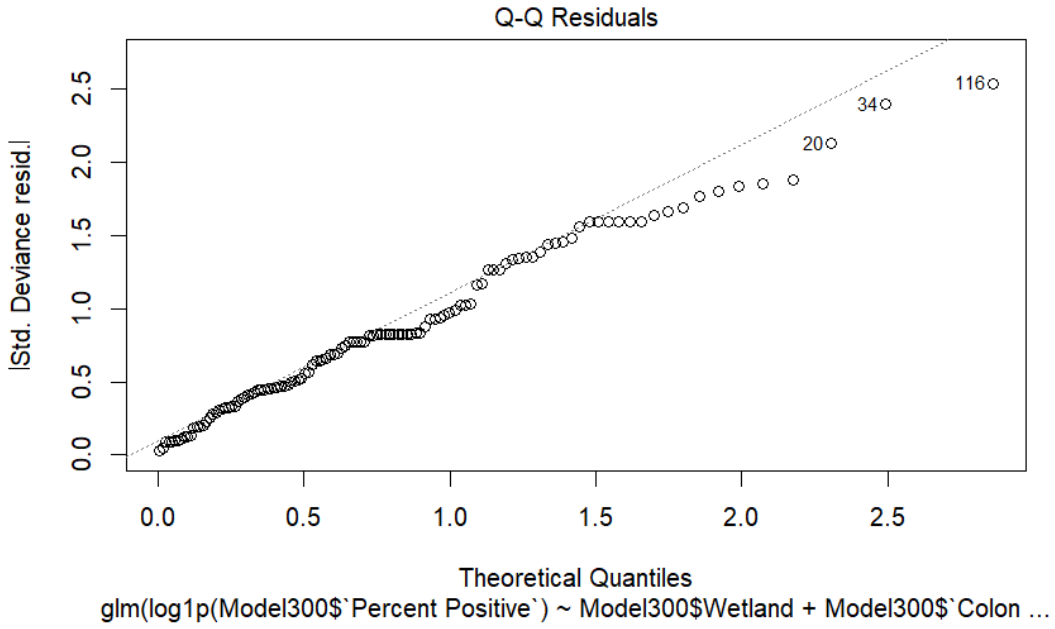


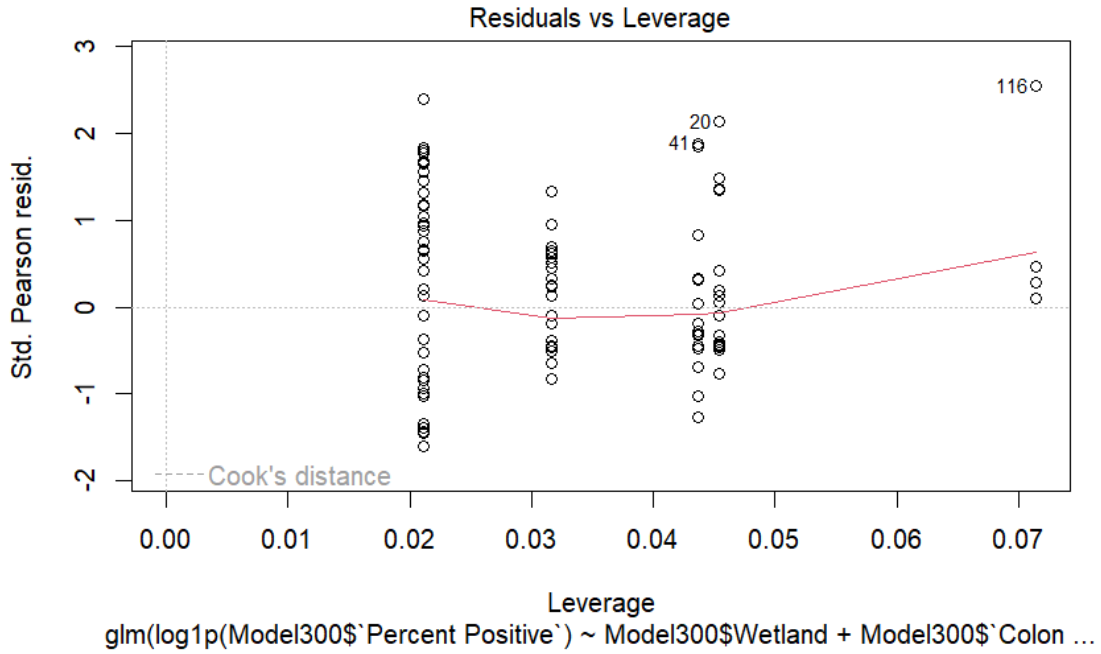


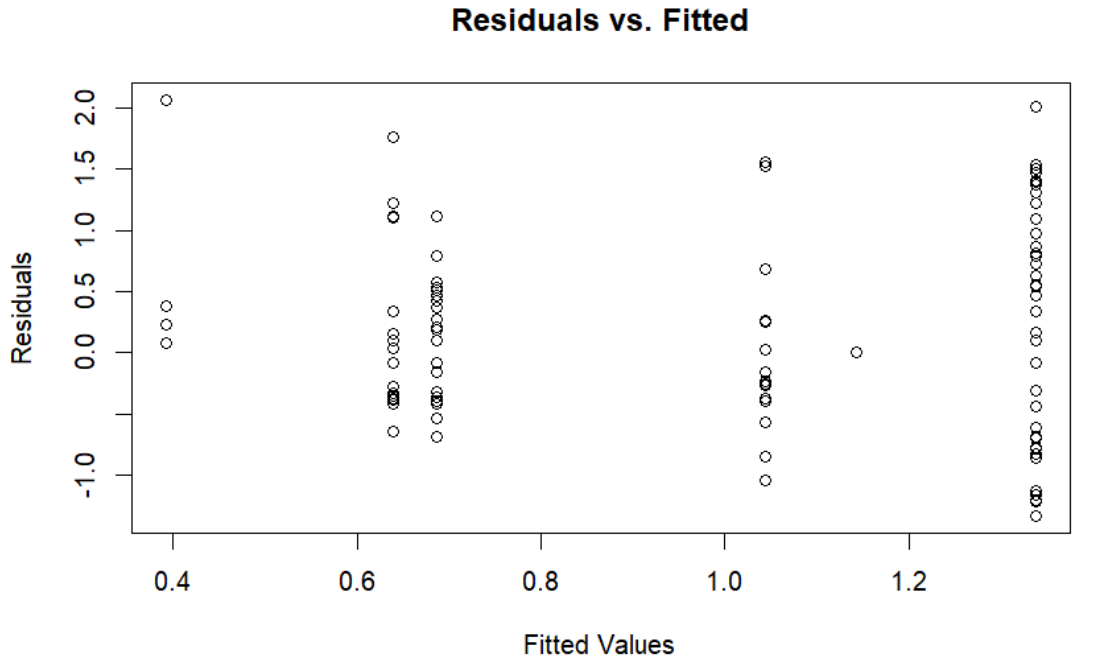


The ratio of Deviance to Degrees of Freedom= 0.73 (no overdispersion)

>300 samples species AIV prevalence GLM

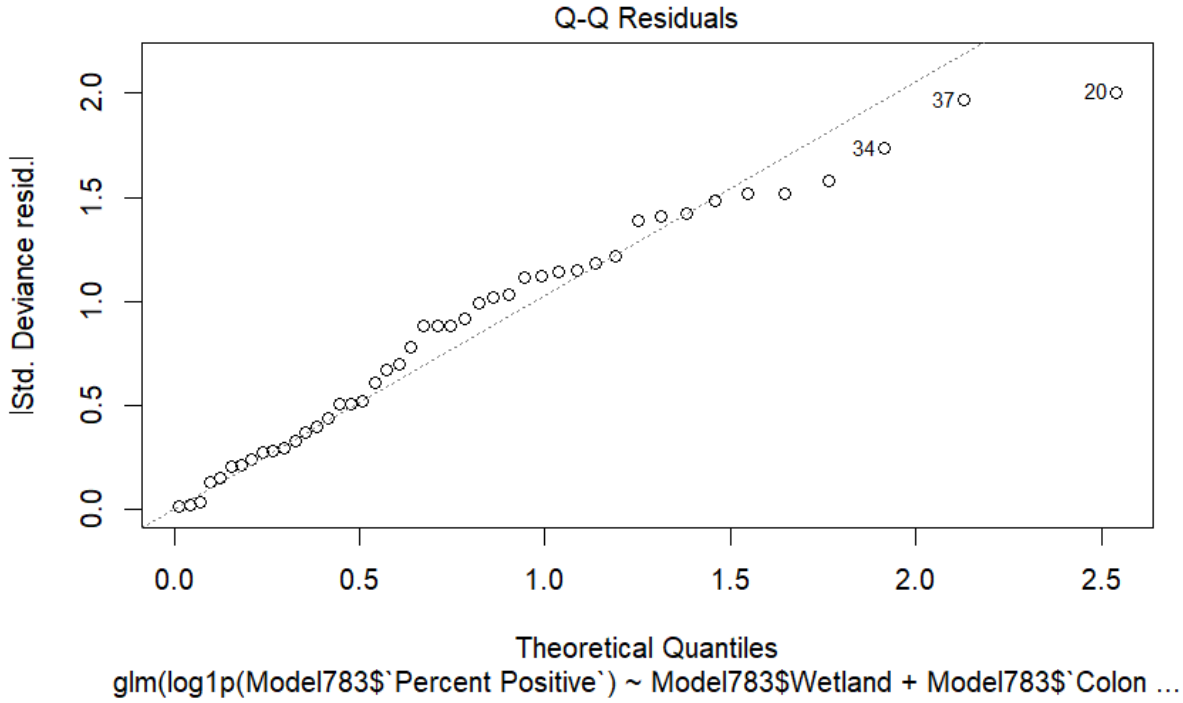


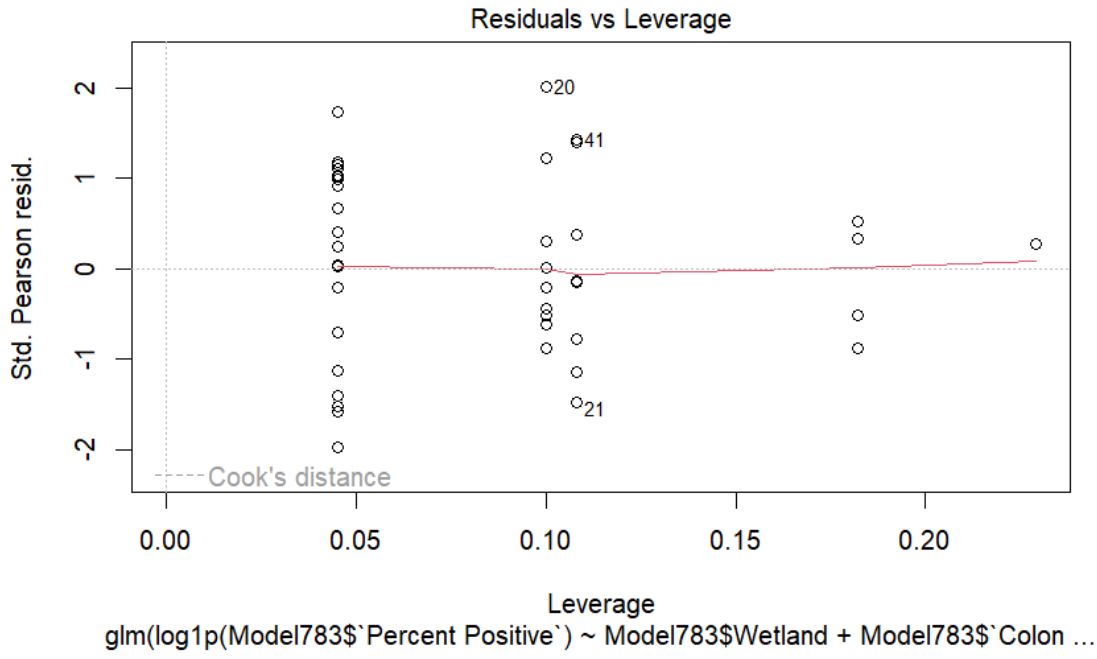


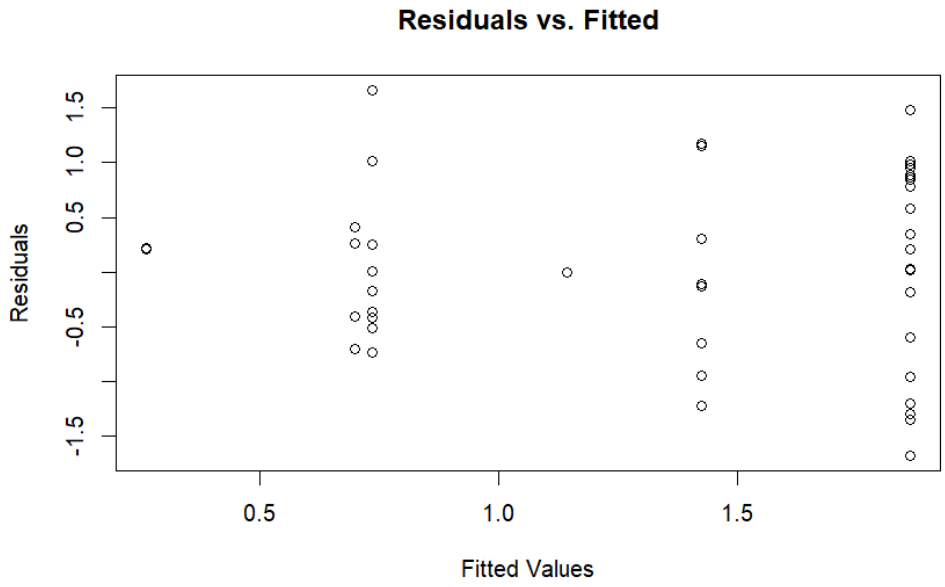


The ratio of Deviance to Degrees of Freedom= 0.71 (no overdispersion)

>783 samples species AIV prevalence GLM

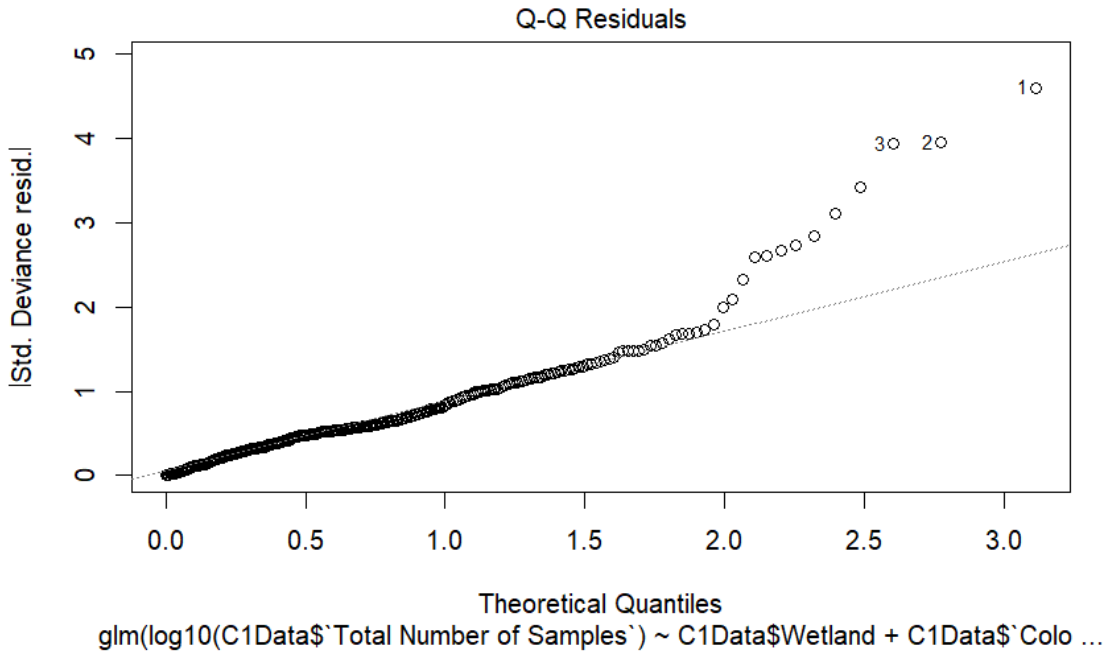


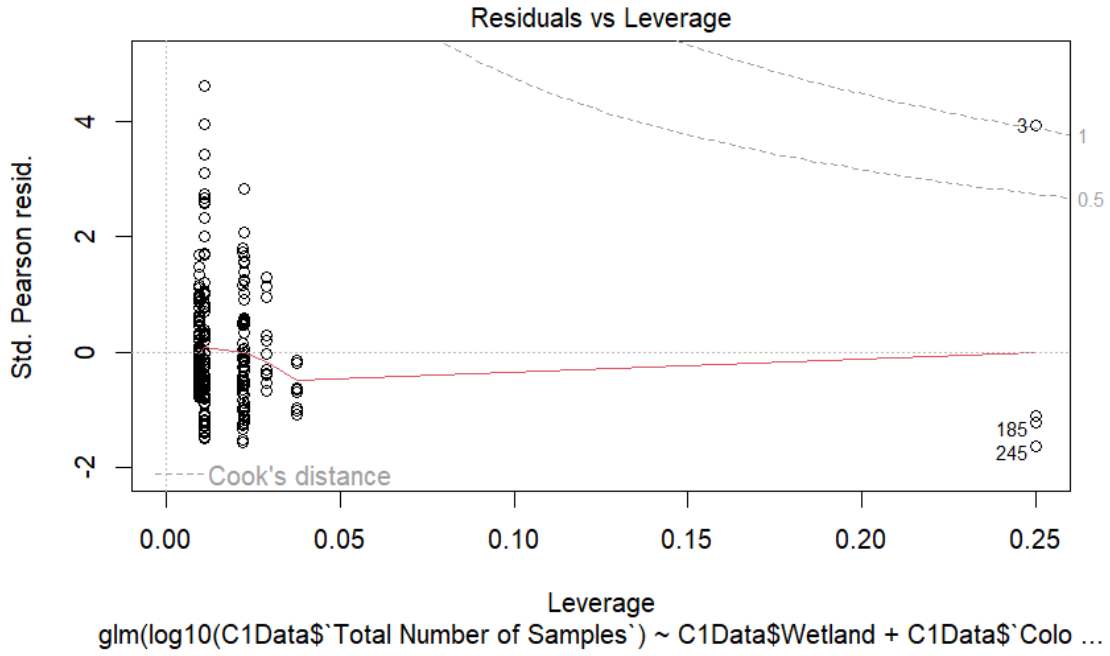


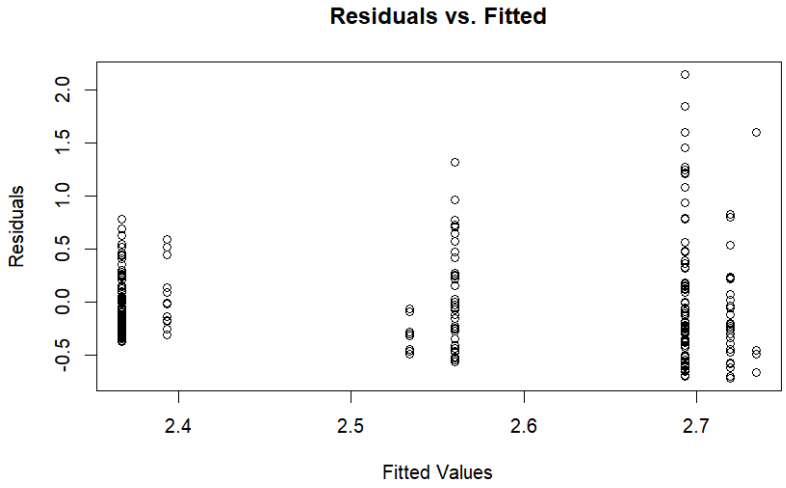


The ratio of Deviance to Degrees of Freedom= 0.77 (no overdispersion).

>100 samples species GLM

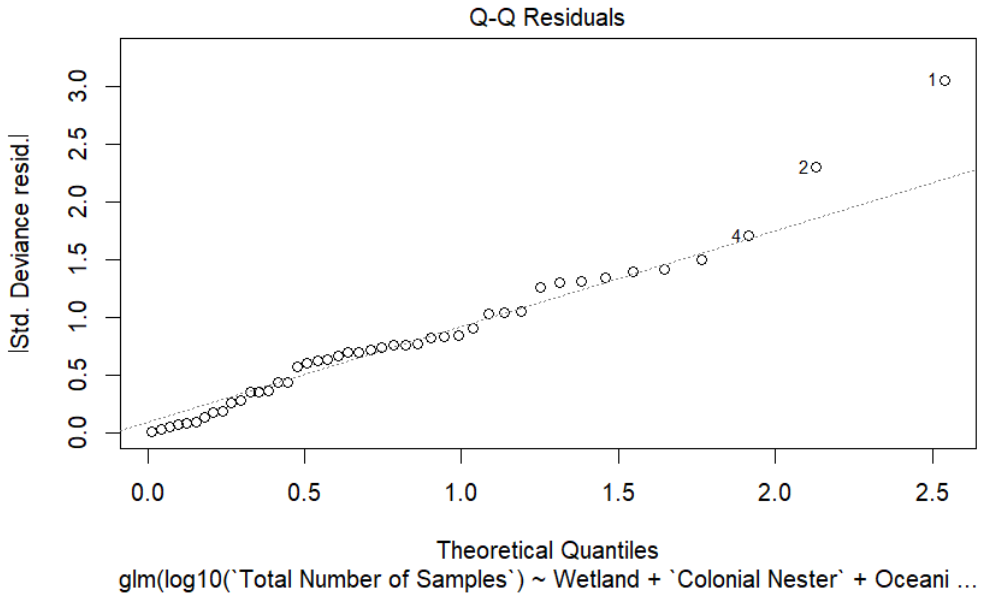


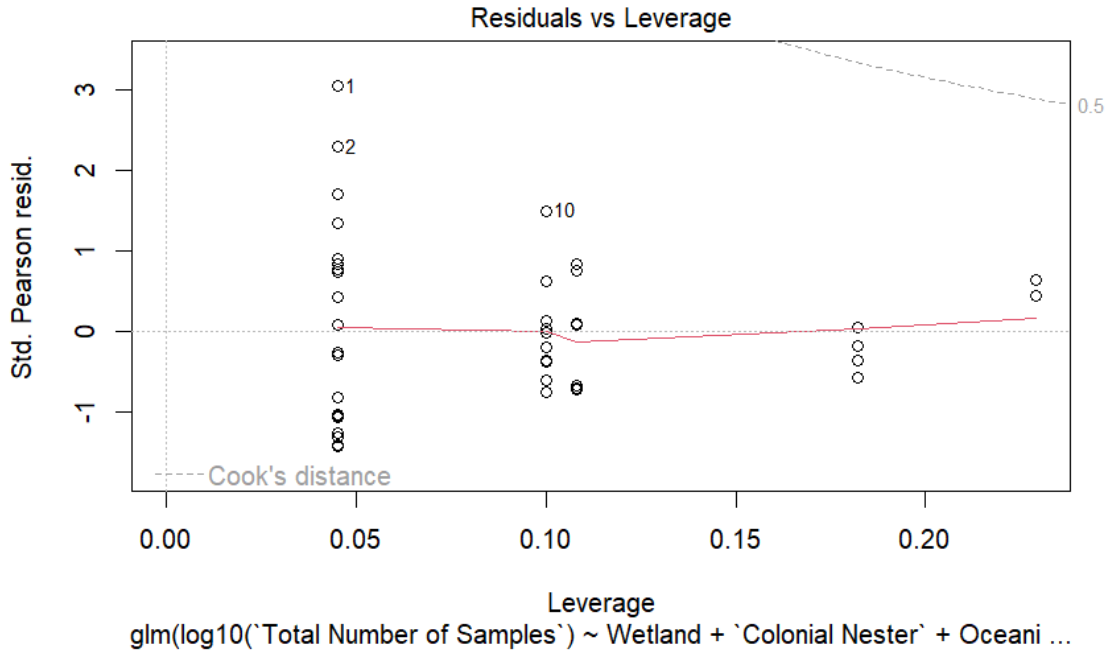


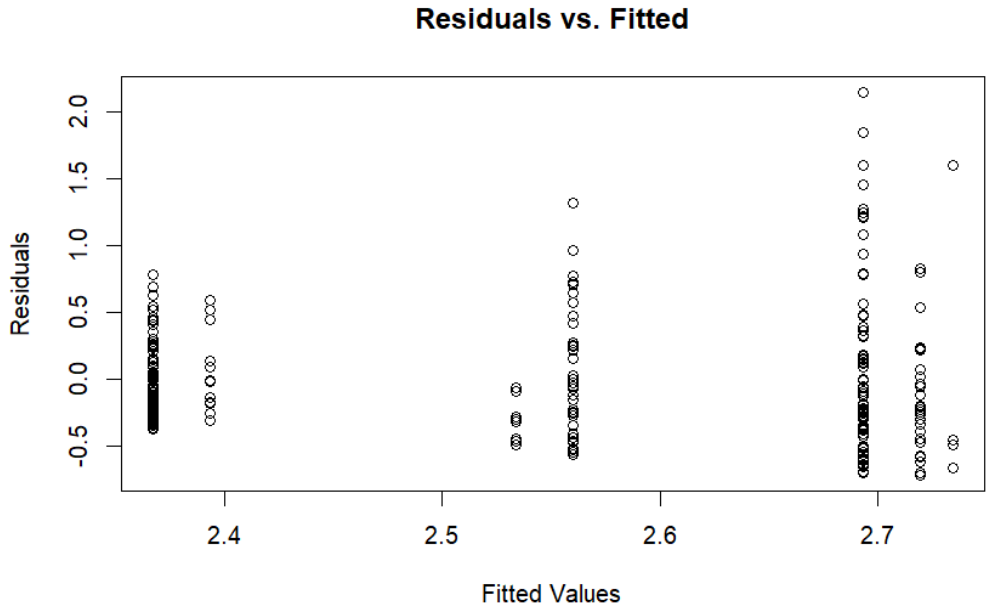


The ratio of Deviance to Degrees of Freedom= 0.22 (no overdispersion)

>300 samples species GLM

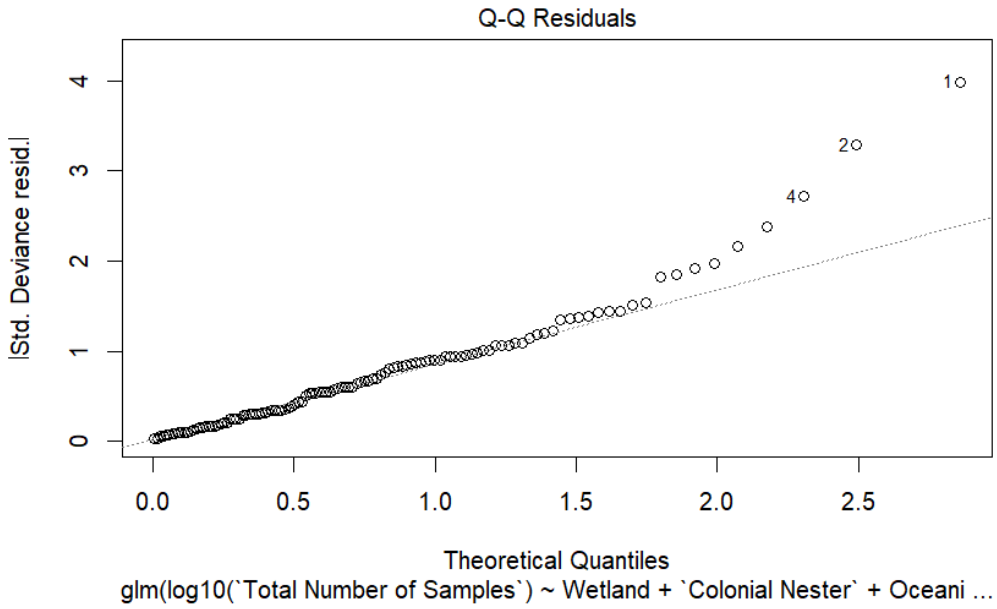


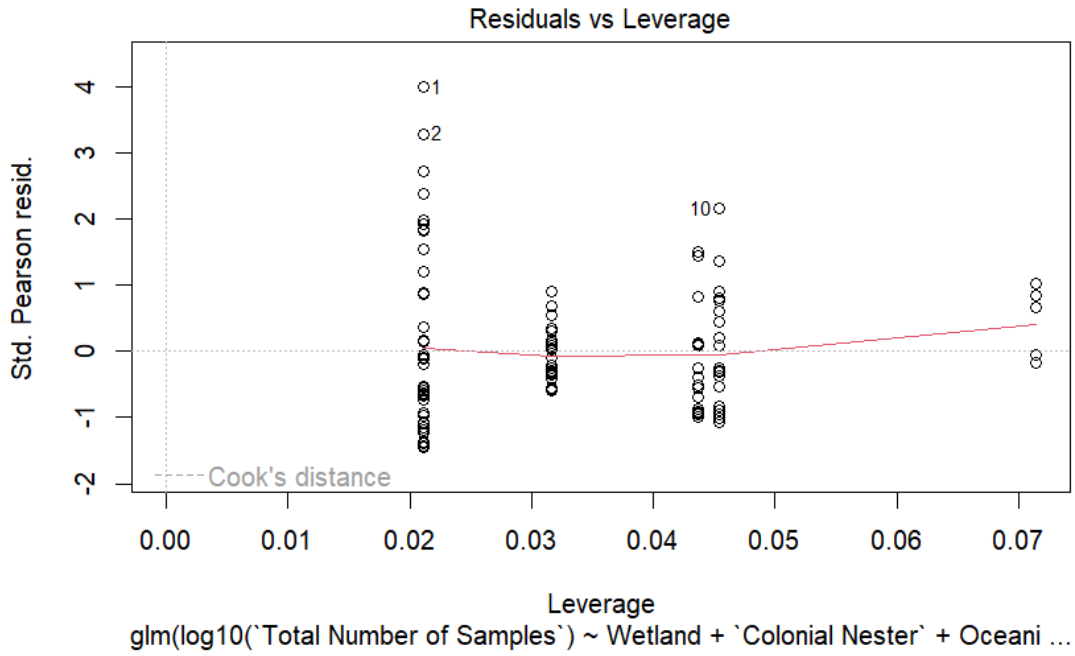


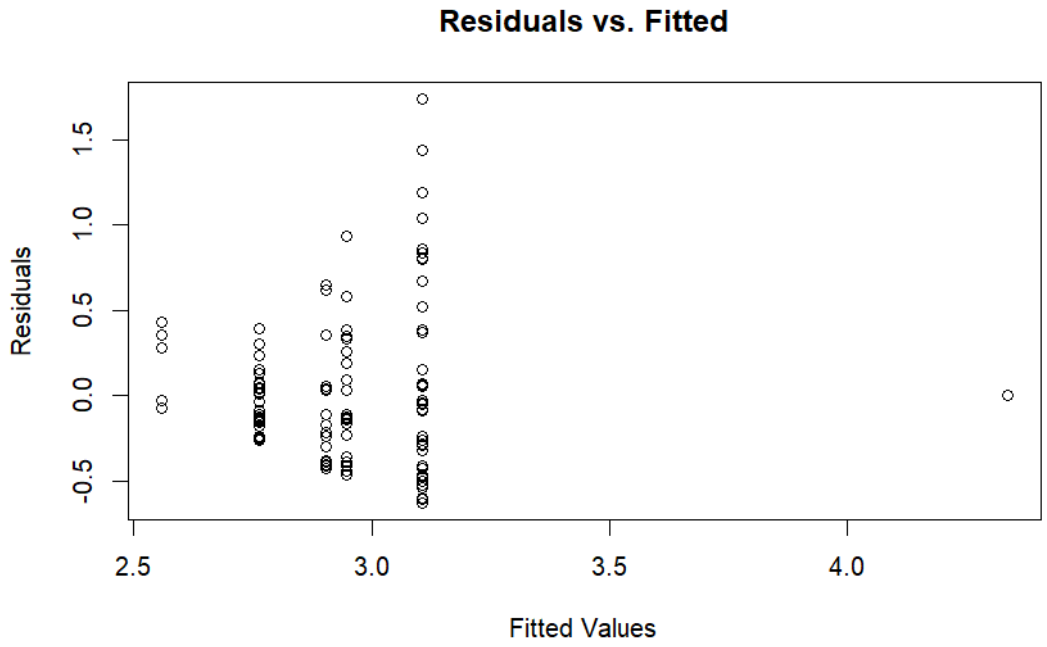


The ratio of Deviance to Degrees of Freedom= 0.22 (no overdispersion)

>783 samples species GLM

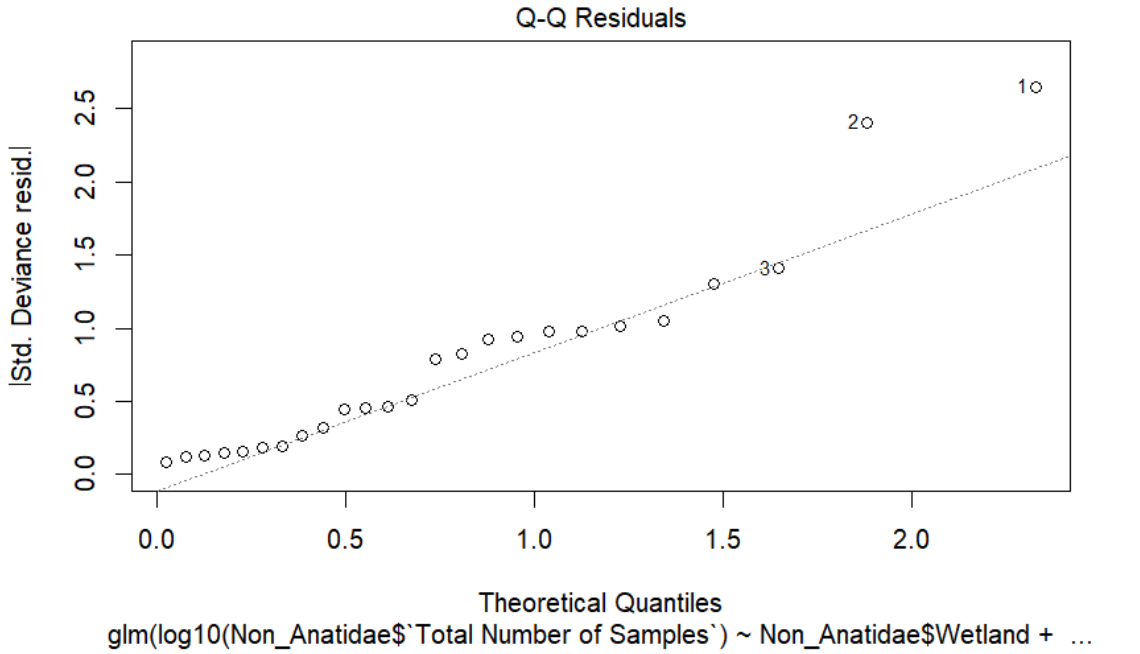


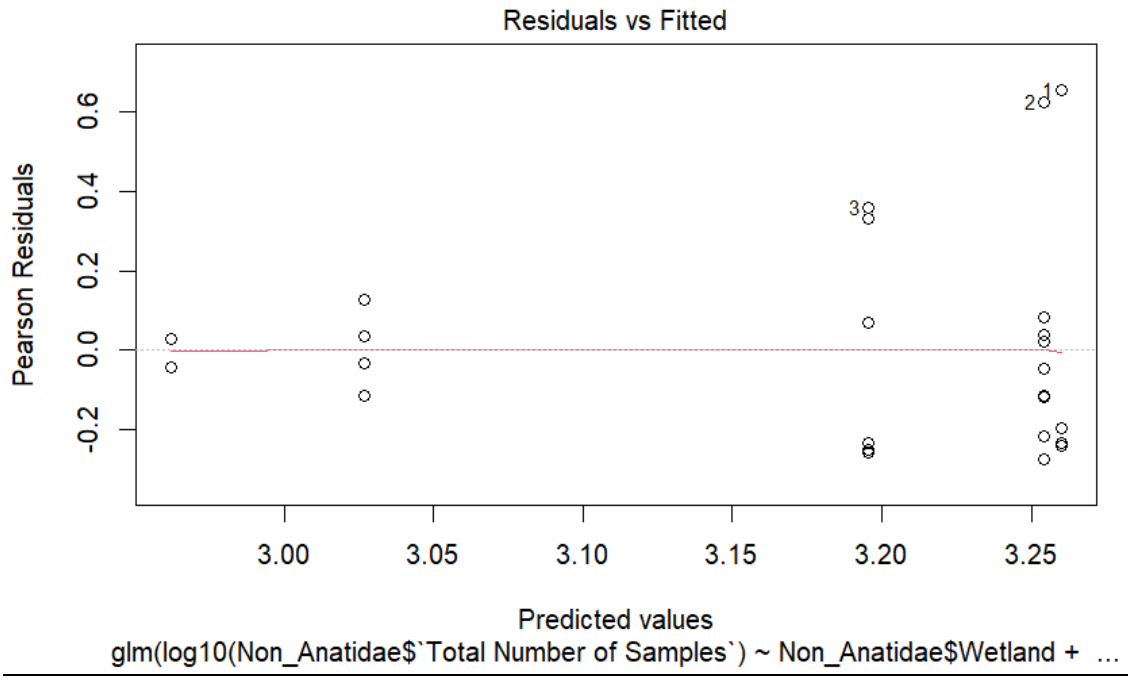


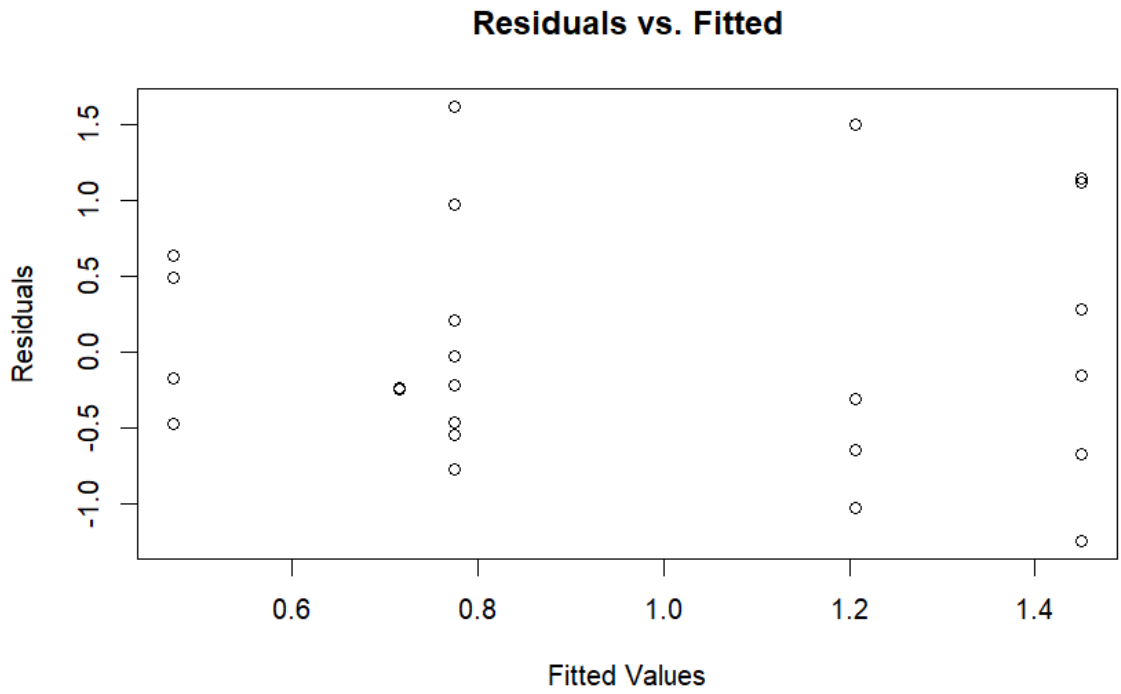


The ratio of Deviance to Degrees of Freedom= 0.19 (no overdispersion)

Non Anatidae GLM

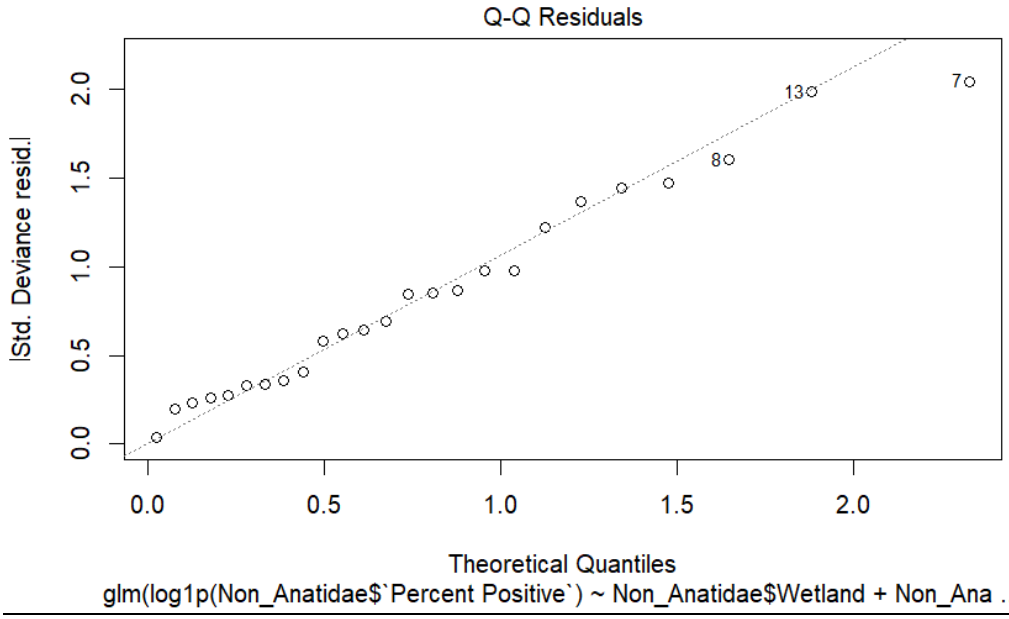


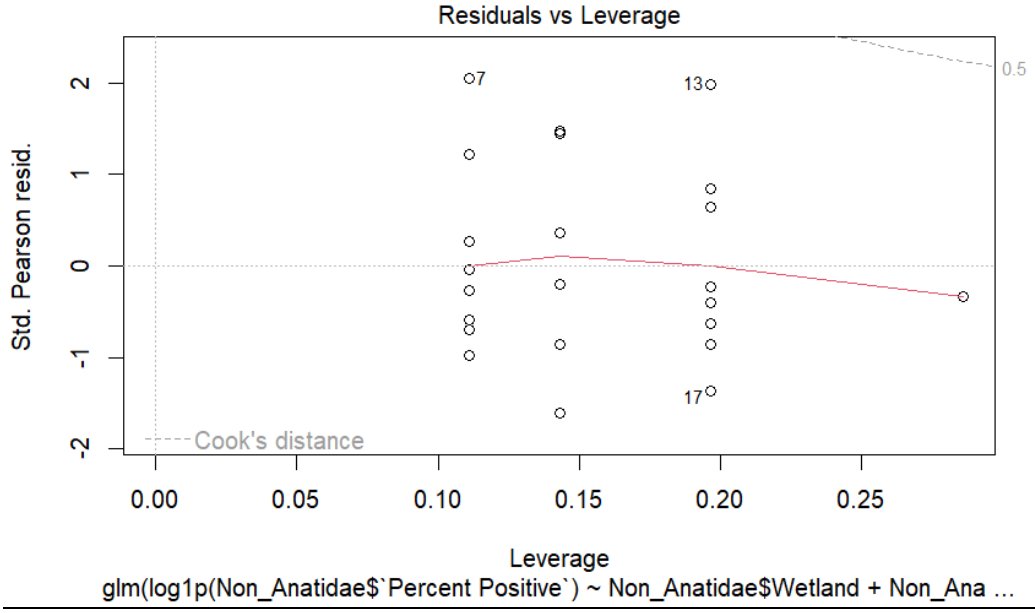


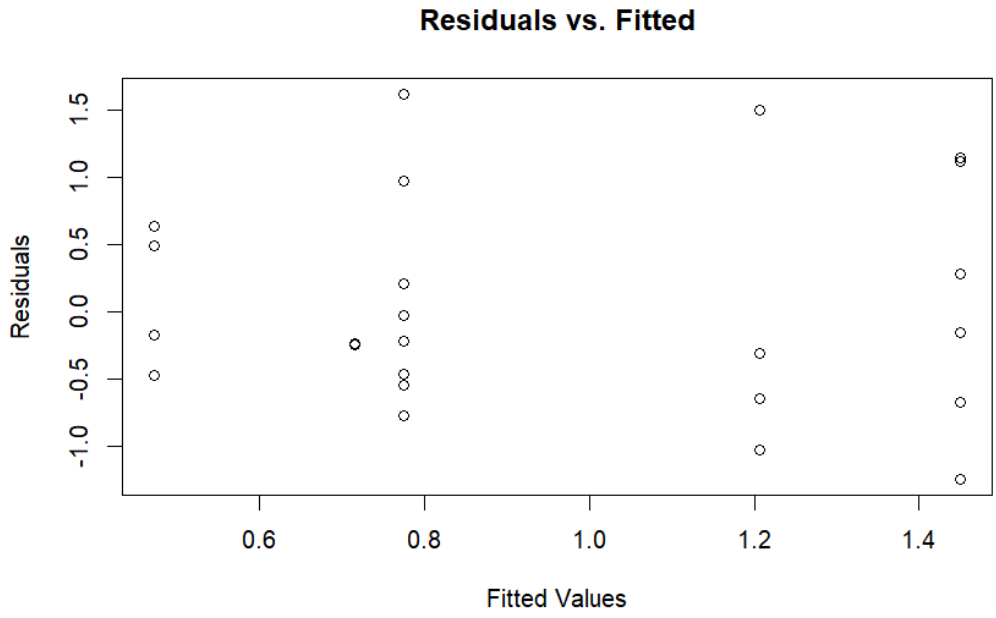


The ratio of Deviance to Degrees of Freedom= 0.08 (no overdispersion)

Non-Anatidae AIV Prvelance GLM







The ratio of Deviance to Degrees of Freedom= 0.08 (no overdispersion)

Chapter 2 Appendix 5: Table of species sampled for live H5 strain avian influenza from systematic review dataset.

Family	***Species***	**Number of H5 positives**	**Number of papers with positives**	**Strains identified**	**Number of countries where a positive sample was taken**	**Countries providing a positive sample**
Accipitridae	*Haliaeetus leucocephalus*	2	2	H5N8	1	USA
Accipitridae	*Buteo buteo*	28	3	H5N6, H5N8	6	Denmark, Ireland, Sweden, Netherlands, UK, Germany

Accipitridae	*Accipiter cooperii*	4	2	H5N2	1	USA
Accipitridae	*Circus aeruginosus*	1	1	H5N6	1	Netherlands
Accipitridae	*Accipiter nisus*	1	1	H5N8	1	Germany
Accipitridae	*Accipiter gentilis*	3	3	H5N6, H5N8	3	Sweden, UK, Germany
Accipitridae	*Buteo jamaicensis*	3	2	H5N2	1	USA
Accipitridae	*Buteo lagopus*	1	1	H5N8	1	Germany
Accipitridae	*Haliaeetus albicilla*	39	3	H5N6, H5N8	5	Denmark, Finland, Ireland, Sweden, Germany
Alaudidae	*Eremoptera leucotis*	1	1		1	Zimbabwe
Alcedinidae	*Coryornis cristata*	1	1		1	South Africa
Alcidae	*Uria lomvia*	1	1	H5N1	1	Greenland
Anatidae	*Anas rubripes*	3	2	H5N2	2	Canada, USA
Anatidae	*Mareca americana*	52	4	H5N8, H5N1	1	USA
Anatidae	*Branta leucopsis*	5	2	H5N8	2	Germany, Netherlands
Anatidae	*Anser fabalis*	1	1	H5N8	1	Germany

Anatidae	*Cygnus atratus*	1	1	H5N8	1	Germany
Anatidae	*Spatula discors*	6	3	H5N3	3	USA, Canada, Guatemala
Anatidae	*Branta bernicla*	1	1	H5N8	1	Germany
Anatidae	*Branta canadensis*	5	3	H5N8	2	Germany, USA
Anatidae	*Bucephala clangula*	1	1	H5N8	1	Germany
Anatidae	*Aythya ferina*	3	3	H5N8, H5N1	3	Germany, Netherlands, China
Anatidae	*Anas zonorhynca*	1	1	H5N8	1	China
Anatidae	*Anas crecca*	34	9	H5N6, H5N2, H5N8, H5N1	4	Netherlands, Canada, USA, Ukraine
Anatidae	*Mareca strepera*	5	3	H5N8, H5N2	3	USA, Germany, Netherlands
Anatidae	*Aythya marila*	2	2	H5N6, H5N8	2	Netherlands, Germany
Anatidae	*Anser albifrons*	1	1	H5N8	1	Germany
Anatidae	*Anser anser*	9	5	H5N6, H5N8	5	Denmark, UK, India, Germany, Netherlands

Anatidae	*Anser erythropus*	1	1	H5N8	1	Germany
Anatidae	*Anas platyrhynchos*	431	18	H5N2, H5N6, H5N8, H5N3	9	Spain, Denmark, Netherlands, Germany, USA, Canada, Japan, Italy, China
Anatidae	*Cygnus olor*	21	4	H5N6, H5N8	4	Denmark, UK, Serbia, Germany
Anatidae	*Anas acuta*	26	4	H5N8, H5N2	3	Germany, Canada, USA
Anatidae	*Spatula clypeata*	5	2		1	USA
Anatidae	*Anser brachyrhynchus*	1	1	H5N8	1	Germany
Anatidae	*Branta ruficollis*	1	1	H5N8	1	Germany
Anatidae	*Aythya americana*	6	1		1	Canada
Anatidae	*Oxyura jamaicensis*	2	2	H5N2, H5N8	2	USA, Germany
Anatidae	*Aythya fuligula*	2	2	H5N8	2	Germany, Netherlands
Anatidae	*Cygnus cygnus*	2	2	H5N8, H5N1	2	Germany, China

Anatidae	*Aix sponsa*	9	3	H5N8, H5N2	3	Germany, Canada, USA
Anatidae	*Mareca falcata*	2	1	H5N8	1	China
Anatidae	*Mareca penelope*	69	4	H5N8	2	Germany, Netherlands
Anatidae	*Melanitta nigra*	1	1	H5N8	1	Germany
Anatidae	*Mergus merganser*	1	1	H5N8	1	Germany
Anatidae	*Netta rufina*	1	1	H5N8	1	Germany
Anatidae	*Somateria mollissima*	3	3	H5N6, H5N8	3	Denmark, Germany, Netherlands
Anatidae	*Unidentified*	3	2	H5N6, H5N8	2	China, South Korea
Anatidae	*Tadorna tadorna*	1	1	H5N8	1	Germany
Strigidae	*Strix aluco*	1	1	H5N8	1	Germany
Ardeidae	*Ardea cinerea*	2	2	H5N8	2	Germany, Mongolia
Ardeidae	*Ardea alba*	1	1	H5N8	1	Germany
Ardeidae	*Unidentified*	2	1	H5N2	1	China
Ciconiidae	*Ciconia ciconia*	2	2	H5N6, H5N8	1	Germany
Columbidae	*Columba livia*	1	1	H5N1	1	Norway
Corvidae	*Corvus corone*	3	2	H5N6, H5N8	2	Denmark, Germany

Corvidae	*Corvus macrorhynchus*	8	1	H5N1	1	Bangladesh
Corvidae	*Pica pica*	1	1	H5N8	1	Germany
Falconidae	*Falco peregrinus*	6	3	H5N6, H5N8	3	Netherlands, Germany, USA
Falconidae	*Falco rusticolus*	2	1		1	USA
Gruidae	*Unidentified*	5	1	H5N8	1	Japan
Gruidae	*Antigone vipeo*	1	1	H5N8	1	Japan
Gruidae	*Grus monarcha*	4	1	H5N8	1	Japan
Laridae	*Larus argentatus*	4	3	H5N6, H5N8	3	Denmark, Germany, Netherlands
Laridae	*Larus canus*	2	2	H5N8	2	Germany, Netherlands
Laridae	*Larus domincanus*	1	1	H5N9	1	Chile
Laridae	*Larus fuscus*	2	2	H5N8	2	Germany, Netherlands
Laridae	*Larus glaucescens*	3	1	H5N2	1	USA
Laridae	*Sterna hirundo*	1	1	H5N8	1	Mongolia
Laridae	*Larus marinus*	6	2	H5N8	2	Germany, Netherlands
Laridae	*Hydrocoloeus minutus*	1	1	H5N8	1	Germany

Laridae	*Chroicocephalus ridibundus*	8	3	H5N6, H5N8	4	Denmark, Slovakia, Germany, Netherlands
Pelecanidae	*Pelecanus crispus*	21	1	H5N1	1	Bulgaria
Pelecanidae	*Pelecanus philippensis*	2	1	H5N8	1	India
Phalacrocoracidae	*Phalacrocorax carbo*	7	3	H5N6, H5N8, H5N1	3	Denmark, Germany, Ukraine
Phasianidae	*Phasianus colchicus*	7	2	H5N6	2	Denmark, UK
Phasianidae	*Perdix perdix*	2	1	H5N3	1	Portugal
Phoenicopteridae	*Unidentified*	1	1	H5N2	1	Portugal
Podicepidae	*Podiceps cristatus*	6	4	H5N8, H5N1	4	Germany, Netherlands, Mongolia, Ukraine
Podicepidae	*Podiceps grisegena*	1	1	H5N8	1	Germany
Podicepidae	*Tachybaptus ruficollis*	1	1	H5N8	1	Germany
Pycnonotidae	*Pycnonotus nigricans*	1	1		1	South Africa
Rallidae	*Fulica atra*	1	1	H5N8	1	Germany

Scolopacidae	*Numenius arquata*	7	1	H5N8	1	China
Scolopacidae	*Arenaria interpres*	2	2	H5N4, H5N1	2	USA, Iceland
Scolopacidae	*Tringa totanus*	1	1	H5N8	1	Germany
Strigidae	*Asio otus*	1	1	H5N8	1	Germany
Strigidae	*Bubo virginianus*	2	2	H5N2	1	USA
Strigidae	*Bubo scandiacus*	2	2	H5N8	1	USA
Unidentified	*Unidentified*	4	1	H5N6, H5N3	1	China

Chapter 2 Appendix 6: Table of species sampled for antibodies against H5 strain avian influenza from systematic review dataset.

Family	**Species**	**Number of H5 positives**	**Number of papers with positives**	**Strains identified**	**Number of countries where a positive sample was taken**	**Countries providing a positive sample**
Laridae	*Chroicocephalus ridibundus*	1	1		1	Croatia
Anatidae	*Apolochen aegyptiaca*	1	1	H5N8	1	Netherlands
Rallidae	*Fulica atra*	4	1	H5N8	1	Netherlands
Anatidae	*Anser erythropus*	1	1	H5N8	1	Netherlands
Ardeidae	*Egretta garzetta*	18	1		1	China

Anatidae	*Cygnus olor*	41	1	H5N8	1	Netherlands
Anatidae	*Mareca penelope*	16	1	H5N8	1	Netherlands
Ardeidae	*Nycticorax nycticorax*	1	17		1	China

Chapter 4 Appendix 1: Exposure score tables for all calculated models.

Mean Exposure Scores and interquartile range for species present within the bio-secure-fenced areas of surveyed poultry holding sites.

Species	Species Code	Mean	IQR 1	IQR3
Pied Wagtail	**PW**	**0.758519**	**0.44934**	**1.010265**
Yellowhammer	**Y.**	**0.239021**	**0.083798**	**0.381129**
Tree Sparrow	**TS**	**0.143415**	**0.082946**	**0.189459**
Dunnock	**D.**	**0.07631**	**0.052287**	**0.095417**
Blue Tit	**BT**	**0.06248**	**0.046277**	**0.075287**
Wren	**WR**	**0.046959**	**0.036772**	**0.0572**
Carrion Crow	**C.**	**0.040348**	**0.023803**	**0.053768**
Blackbird	**B.**	**0.033481**	**0.02477**	**0.041828**
Red-legged Partridge	**RL**	**0.026507**	**0.014763**	**0.038057**
Common Woodpigeon	**WP**	**0.014285**	**0.005807**	**0.023203**
Great Tit	**GT**	**0.014285**	**0.005807**	**0.023203**
Little Owl	**LO**	**0.006766**	**0.002087**	**0.011354**

Chaffinch	**CH**	**0.006766**	**0.002087**	**0.011354**
Robin	**R.**	**0.006766**	**0.002087**	**0.011354**
Common Gull	**CG**	**0.006766**	**0.002087**	**0.011354**
Magpie	**MP**	**0.006766**	**0.002087**	**0.011354**
Ring-necked Pheasant	**PH**	**0.006766**	**0.002087**	**0.011354**
Western Barn Owl	**BO**	**0.006766**	**0.002087**	**0.011354**

Mean Exposure Scores and interquartile range for species present inside the bio-secure-fenced areas and within 50m of surveyed poultry holding sites.

Species	Species Code	Mean	IQR 1	IQR3
Red-legged Partridge	**RL**	**2.714707**	**0.681486**	**4.185718**
Tree Sparrow	**TS**	**0.339126**	**0.195529**	**0.446339**
Pied Wagtail	**PW**	**0.257429**	**0.103166**	**0.372632**
Carrion Crow	**C.**	**0.160161**	**0.027295**	**0.257409**
Magpie	**MP**	**1.54x10^1**	**0.047758**	**0.249867**
Ring-necked Pheasant	**PH**	**1.11x10^1**	**0.034545**	**0.178451**
Common Gull	**CG**	**4.92x10^2**	**0.012653**	**0.078938**
Western Barn Owl	**BO**	**1.31x10^2**	**-0.00046**	**0.024175**
Blue Tit	**BT**	**0.013004**	**0.007573**	**0.017315**

Dunnock	**D.**	**0.01299**	**0.003402**	**0.020218**
Robin	**R.**	**9.75x10^4**	**9.58E-05**	**0.001628**
Chaffinch	**CH**	**1.99x10^6**	**-9.3E-05**	**0.000103**
Great Tit	**GT**	**-8.30x10^6**	**-0.0004**	**0.000364**
Common Woodpigeon	**WP**	**-2.3x10^5**	**-0.00552**	**0.005856**
Wren	**WR**	**-4.9x10^5**	**-0.00167**	**0.001621**
Blackbird	**B.**	**-0.00015**	**-0.00503**	**0.004435**
Little Owl	**LO**	**-6.37x10^4**	**-0.02974**	**0.026036**
Yellowhammer	**Y.**	**-0.00971**	**-0.10327**	**0.078657**

Mean Exposure Scores and interquartile range for species recorded flying over poultry holding sites.

Species	Species Code	Mean	IQR 1	IQR3
Common Woodpigeon	**WP**	**8.463246**	**3.766542**	**12.40305**
European Starling	**SG**	**3.228145**	**1.531548**	**4.490719**
Common Blackbird	**B.**	**2.79336**	**1.78247**	**3.663684**
Eurasian Blue Tit	**BT**	**2.136669**	**1.418618**	**2.716861**

Eurasian Tree Sparrow	**TS**	**1.992299**	**1.187419**	**2.635942**
Common Chaffinch	**CH**	**1.742576**	**1.223576**	**2.199523**
Feral Pigeon	**FP**	**1.631118**	**0.619154**	**2.33119**
Fieldfare	**FF**	**1.564012**	**0.932548**	**2.115139**
Pied Wagtail	**PW**	**1.455355**	**0.583441**	**2.119705**
Common Gull	**CM**	**1.374565**	**0.828147**	**1.844893**
European Robin	**R.**	**1.31538**	**0.940833**	**1.630979**
Dunnock	**D.**	**1.235952**	**0.827717**	**1.580022**
Great Tit	**GT**	**0.998037**	**0.66696**	**1.273283**
Eurasian Wren	**WR**	**0.989461**	**0.748051**	**1.211721**
Yellowhammer	**Y.**	**0.955018**	**0.49477**	**1.314731**
Redwing	**RE**	**0.82494**	**0.423444**	**1.118754**
Goldfinch	**GO**	**0.587242**	**0.346544**	**0.773308**
Carrion Crow	**C.**	**0.565822**	**0.355488**	**0.739186**
House Sparrow	**HS**	**0.557128**	**0.306843**	**0.728102**
Eurasian Skylark	**S.**	**0.350314**	**0.244982**	**0.435675**
Coal Tit	**CT**	**0.269414**	**0.184157**	**0.331844**
Eurasian Magpie	**MG**	**0.259185**	**0.179122**	**0.322729**
Ring-necked Pheasant	**PH**	**0.226208**	**0.167424**	**0.272129**

Song Thrush	**ST**	**0.181596**	**0.149131**	**0.209325**
Mistle Thrush	**M.**	**0.150939**	**0.100601**	**0.187462**
Collared Dove	**CD**	**0.122612**	**0.089477**	**0.150018**
Greenfinch	**GF**	**0.121236**	**0.068293**	**0.160144**
Red-legged Partridge	**RL**	**0.113519**	**0.064749**	**0.151439**
Long-tailed Tit	**LT**	**0.093107**	**0.057947**	**0.119355**
Eurasian Bullfinch	**BF**	**0.090508**	**0.070506**	**0.107836**
Stock Dove	**SD**	**0.078991**	**0.059355**	**0.096206**
Black-headed Gull	**BH**	**0.065392**	**0.03469**	**0.093792**
Common Reed Bunting	**RB**	**0.064482**	**0.048998**	**0.078903**
Goldcrest	**GC**	**0.063197**	**0.046212**	**0.078204**
Common Kestrel	**KE**	**0.047041**	**0.036973**	**0.056554**
Western Jackdaw	**JD**	**0.044898**	**0.031884**	**0.056544**
Grey Partridge	**P.**	**0.04099**	**0.026038**	**0.054369**
Linnet	**LI**	**0.034004**	**0.021992**	**0.045414**
Great Spotted Woodpecker	**GS**	**0.033339**	**0.0249**	**0.041799**
Rook	**RO**	**0.027456**	**0.017109**	**0.037135**
Marsh Tit	**MT**	**0.026302**	**0.016136**	**0.035088**

Common Buzzard	**BZ**	**0.020466**	**0.011824**	**0.028858**
Western Barn Owl	**BO**	**0.020261**	**0.013488**	**0.02718**
Common Chiffchaff	**CC**	**0.013855**	**0.00795**	**0.019668**
European Herring Gull	**HG**	**0.013513**	**0.007944**	**0.019182**
Northern Lapwing	**L.**	**0.01334**	**0.007878**	**0.018832**
Eurasian Woodcock	**WK**	**0.006771**	**0.002301**	**0.011272**
Eurasian Treecreeper	**TC**	**0.006643**	**0.002093**	**0.010978**
Grey Wagtail	**GL**	**0.006561**	**0.002089**	**0.011049**
Eurasian Nuthatch	**NH**	**0.006513**	**0.001949**	**0.010657**

Mean Avian Influenza specific Exposure Scores and interquartile range for species present within the bio-secure-fenced areas of surveyed poultry holding sites.

Species	**Species Code**	**Mean**	**IQR 1**	**IQR3**
Red-legged Partridge	**RL**	**12.26618**	**0.885147**	**21.01792**
Feral Pigeon	**FP**	**11.87479**	**4.515012**	**16.77555**
Common Gull	**CM**	**9.967581**	**5.114894**	**13.40282**
Eurasian Magpie	**MG**	**5.842314**	**3.858557**	**7.359539**
Eurasian Tree Sparrow	**TS**	**4.671381**	**2.778998**	**6.22842**
Ring-necked Pheasant	**PH**	**3.68167**	**2.499637**	**4.595052**

Black-headed Gull	**BH**	**2.245157**	**1.207311**	**3.238271**
Carrion Crow	**C.**	**2.077849**	**0.491017**	**3.215981**
European Starling	**SG**	**1.728309**	**0.507659**	**2.492972**
Common Buzzard	**BZ**	**0.991249**	**0.527414**	**1.398115**
European Herring Gull	**HG**	**0.79176**	**0.417211**	**1.119827**
Pied Wagtail	**PW**	**0.498911**	**0.11818**	**0.707506**
Eurasian Blue Tit	**BT**	**0.454225**	**0.252224**	**0.603118**
Dunnock	**D.**	**0.206614**	**0.055699**	**0.330466**
European Robin	**R.**	**0.191946**	**0.093397**	**0.266324**
Collared Dove	**CD**	**0.178213**	**0.084034**	**0.248627**
Stock Dove	**SD**	**0.130749**	**-1.20082**	**1.472768**
Grey Partridge	**P.**	**0.108335**	**0.0032**	**0.194323**
House Sparrow	**HS**	**0.093931**	**0.042834**	**0.12841**
Greenfinch	**GF**	**0.066429**	**-0.00934**	**0.121191**
Common Reed Bunting	**RB**	**0.040765**	**0.027377**	**0.051296**
Western Barn Owl	**BO**	**0.037608**	**-0.00813**	**0.082279**
Northern Lapwing	**L.**	**0.023659**	**-0.14928**	**0.201498**
Coal Tit	**CT**	**0.013749**	**-0.0864**	**0.120072**
Redwing	**RE**	**0.010091**	**-0.28259**	**0.285793**

Common Chiffchaff	**CC**	**0.003899**	**0.001406**	**0.005799**
Eurasian Skylark	**S.**	**0.00317**	**-0.11934**	**0.130992**
Common Blackbird	**B.**	**0.002788**	**-0.3945**	**0.403582**
Marsh Tit	**MT**	**0.002029**	**-0.0291**	**0.031721**
Song Thrush	**ST**	**0.000598**	**-0.00972**	**0.010994**
Grey Wagtail	**GL**	**0.000203**	**-0.00162**	**0.001994**
Linnet	**LI**	**0.000171**	**-0.01761**	**0.018004**
Long-tailed Tit	**LT**	**9.39x10^5**	**-0.00578**	**0.006842**
Goldcrest	**GC**	**-1.4x10^5**	**-0.00096**	**0.000937**
Eurasian Nuthatch	**NH**	**-0.00012**	**-0.0272**	**0.027868**
Great Tit	**GT**	**-0.00031**	**-0.03249**	**0.028968**
Common Kestrel	**KE**	**-0.00069**	**-0.02036**	**0.017252**
Eurasian Treecreeper	**TC**	**-0.0007**	**-0.00751**	**0.005628**
Rook	**RO**	**-0.001**	**-0.03333**	**0.03093**
Mistle Thrush	**M.**	**-0.00105**	**-1.46858**	**1.453795**
Eurasian Bullfinch	**BF**	**-0.00111**	**-0.02748**	**0.024504**
Eurasian Wren	**WR**	**-0.00149**	**-0.03487**	**0.032105**
Great Spotted Woodpecker	**GS**	**-0.00192**	**-0.07934**	**0.070867**

Western Jackdaw	**JD**	**-0.00193**	**-0.06973**	**0.074519**
Common Chaffinch	**CH**	**-0.00281**	**-0.03181**	**0.025777**
Goldfinch	**GO**	**-0.00297**	**-0.0701**	**0.061204**
Eurasian Woodcock	**WK**	**-0.00581**	**-0.53602**	**0.491819**
Yellowhammer	**Y.**	**-0.01381**	**-0.37075**	**0.339159**
Fieldfare	**FF**	**-0.03043**	**-0.6394**	**0.578661**
Common Woodpigeon	**WP**	**-0.11279**	**-2.91396**	**3.087368**

Mean Avian Influenza specific Exposure Scores and interquartile range for species present outside the bio-secure-fenced areas and within 50m of surveyed poultry holding sites.

Species	Species Code	Mean	IQR 1	IQR3
Common Woodpigeon	**WP**	**40.80261**	**17.69129**	**60.05038**
European Starling	**SG**	**28.22753**	**10.9302**	**43.24821**
European Herring Gull	**HG**	**20.01936**	**8.29383**	**29.66473**
Western Jackdaw	**JD**	**5.173721**	**2.334876**	**7.296437**
Common Gull	**CM**	**2.547112**	**1.187942**	**3.674547**
Redwing	**RE**	**1.597045**	**0.929522**	**2.148337**
Carrion Crow	**C.**	**1.461338**	**0.957231**	**1.896783**
Black-headed Gull	**BH**	**1.392872**	**0.715482**	**1.944058**

Rook	**RO**	**1.215401**	**0.57844**	**1.71844**
Goldfinch	**GO**	**1.087726**	**0.658317**	**1.43524**
Feral Pigeon	**FP**	**0.798858**	**0.481289**	**1.057523**
Common Chaffinch	**CH**	**0.74271**	**0.397071**	**1.034795**
Great Cormorant	**CA**	**0.720795**	**0.233605**	**1.041213**
Fieldfare	**FF**	**0.593591**	**0.378127**	**0.777858**
Pied Wagtail	**PW**	**0.565715**	**0.393658**	**0.711999**
European Golden Plover	**GP**	**0.474419**	**0.263331**	**0.636477**
Linnet	**LI**	**0.466199**	**0.255322**	**0.632191**
Whooper Swan	**WS**	**0.370559**	**0.145573**	**0.602255**
Eurasian Tree Sparrow	**TS**	**0.195378**	**0.136861**	**0.241144**
Greylag Goose	**GJ**	**0.14436**	**0.085398**	**0.184267**
Yellowhammer	**Y.**	**0.13478**	**0.102884**	**0.162176**
Stock Dove	**SD**	**0.118279**	**0.077013**	**0.149532**
Northern Lapwing	**L.**	**0.094973**	**0.046416**	**0.137502**
Mallard	**MA**	**0.080283**	**0.057135**	**0.098812**
Common Kestrel	**KE**	**0.054098**	**0.039773**	**0.067689**
Greenfinch	**GF**	**0.053229**	**0.04137**	**0.064505**

Collared Dove	**CD**	**0.046977**	**0.036306**	**0.057256**
Eurasian Magpie	**MG**	**0.045595**	**0.028099**	**0.058264**
Common Blackbird	**B.**	**0.04044**	**0.031231**	**0.049788**
Common Reed Bunting	**RB**	**0.034503**	**0.026015**	**0.043391**
Great Black-backed Gull	**GB**	**0.033621**	**0.02043**	**0.044145**
Eurasian Skylark	**S.**	**0.027579**	**0.019346**	**0.036191**
Pink-footed Goose	**PG**	**0.027524**	**0.014455**	**0.038734**
Eurasian Siskin	**SK**	**0.027007**	**0.016912**	**0.036156**
Great Spotted Woodpecker	**GS**	**0.026625**	**0.018989**	**0.034279**
Song Thrush	**ST**	**0.020516**	**0.007597**	**0.033495**
Eurasian Sparrowhawk	**SH**	**0.020491**	**0.011121**	**0.029397**
Common Buzzard	**BZ**	**0.020466**	**0.011824**	**0.028858**
Grey Wagtail	**GL**	**0.020334**	**0.013372**	**0.026942**
Eurasian Bullfinch	**BF**	**0.013629**	**0.00789**	**0.019506**
Red-legged Partridge	**RL**	**0.013491**	**0.005355**	**0.021923**
Lesser Redpoll	**LR**	**0.01335**	**0.004808**	**0.021391**
Mistle Thrush	**M.**	**0.013231**	**0.007509**	**0.018927**
Red Kite	**KT**	**0.006914**	**0.00275**	**0.011357**

European Robin	**R.**	**0.006907**	**0.00272**	**0.011462**
Little Egret	**ET**	**0.006751**	**0.002402**	**0.011194**
Peregrine	**PE**	**0.006698**	**0.002415**	**0.011185**
Brambling	**BL**	**0.006555**	**0.002145**	**0.011081**
Merlin	**ML**	**0.006535**	**0.002197**	**0.010829**
Lesser Black-backed Gull	**LB**	**0.006504**	**0.00181**	**0.011123**

Mean Avian Influenza specific Exposure Scores and interquartile range for species recorded flying over poultry holding sites.

Species	Species Code	Mean	IQR 1	IQR3
Whooper Swan	**WS**	**1435.79**	**547.7662**	**2304.84**
European Herring Gull	**HG**	**1183.247**	**471.2785**	**1720.434**
Black-headed Gull	**BH**	**47.85165**	**24.47678**	**66.40001**
Common Gull	**CM**	**18.50041**	**7.33672**	**26.03538**
Great Cormorant	**CA**	**16.89881**	**5.457246**	**24.18712**
European Starling	**SG**	**15.09184**	**3.292759**	**22.17951**
Mallard	**MA**	**14.61625**	**10.38163**	**18.0252**
Greylag Goose	**GJ**	**13.00797**	**7.536322**	**16.7974**

Red-legged Partridge	**MS**	**6.339086**	**2.42318**	**10.15969**
Feral Pigeon	**FP**	**5.804473**	**3.466199**	**7.624718**
Carrion Crow	**C.**	**5.373985**	**1.31573**	**8.404839**
Great Black-backed Gull	**GB**	**3.738285**	**2.169328**	**4.852635**
Eurasian Magpie	**MG**	**1.027404**	**0.615376**	**1.322957**
Common Buzzard	**BZ**	**0.991249**	**0.527414**	**1.398115**
Pink-footed Goose	**PG**	**0.822103**	**0.243234**	**1.21188**
Eurasian Tree Sparrow	**TS**	**0.458667**	**0.314864**	**0.575819**
European Golden Plover	**GP**	**0.200521**	**0.066534**	**0.294873**
Stock Dove	**SD**	**0.199331**	**-1.72924**	**2.125691**
Lesser Black-backed Gull	**LB**	**0.197711**	**0.046343**	**0.322151**
Pied Wagtail	**PW**	**0.192171**	**0.094602**	**0.267288**
Northern Lapwing	**L.**	**0.158851**	**-1.0616**	**1.325529**
Peregrine	**PE**	**0.123642**	**0.043655**	**0.198793**
Collared Dove	**CD**	**0.068238**	**0.033117**	**0.099274**
Eurasian Sparrowhawk	**SH**	**0.028851**	**0.002345**	**0.046878**
Greenfinch	**GF**	**0.02851**	**-0.00512**	**0.058925**

Common Reed Bunting	**RB**	**0.021814**	**0.014546**	**0.027674**
Little Egret	**ET**	**0.018937**	**0.002245**	**0.030945**
Redwing	**RE**	**0.01883**	**-0.57991**	**0.597692**
Merlin	**ML**	**0.009708**	**-0.05582**	**0.072798**
European Robin	**R.**	**0.00105**	**0.000138**	**0.001685**
Eurasian Skylark	**S.**	**0.000416**	**-0.00917**	**0.00997**
Common Blackbird	**B.**	**0.000265**	**-0.0055**	**0.006312**
Song Thrush	**ST**	**6.32x10^5**	**-0.00099**	**0.00102**
Eurasian Siskin	**SK**	**2.64x10^5**	**-0.00222**	**0.002479**
Brambling	**BL**	**4.42x10^6**	**-0.00028**	**0.000254**
Lesser Redpoll	**LR**	**-9.3x10^6**	**-0.0002**	**0.000175**
Eurasian Bullfinch	**BF**	**-0.00016**	**-0.00326**	**0.002989**
Grey Wagtail	**GL**	**-0.0004**	**-0.00616**	**0.005226**
Common Kestrel	**KE**	**-0.00065**	**-0.02286**	**0.01868**
Common Chaffinch	**CH**	**-0.00125**	**-0.01171**	**0.009172**
ChapterMistle Thrush	**M.**	**-0.0015**	**-0.12004**	**0.118042**
Great Spotted Woodpecker	**GS**	**-0.0016**	**-0.06313**	**0.056553**
Yellowhammer	**Y.**	**-0.00206**	**-0.06083**	**0.056798**

Linnet	**LI**	**-0.00215**	**-0.22606**	**0.235004**
Red Kite	**KT**	**-0.00338**	**-0.04576**	**0.04168**
Goldfinch	**GO**	**-0.00529**	**-0.13185**	**0.118057**
Fieldfare	**FF**	**-0.01034**	**-0.25431**	**0.216014**
Rook	**RO**	**-0.11188**	**-1.40672**	**1.187126**
Western Jackdaw	**JD**	**-0.27912**	**-6.56811**	**7.332676**
Common Woodpigeon	**WP**	**-0.6046**	**-13.286**	**14.42282**

Chapter 4 Appendix 2: Avian Influenza prevalence data and sample size for species detected at poultry holding surveys.

Family	Species	Total number of tested samples.	AI Prevalence (%)	Lower Credible Interval (Jeffreys)	Upper Credible Interval (Jeffreys)
Anatidae	Greylag Goose	885	2.71	1.635019374	3.784980626
	Pink-footed Goose	175	1.14	-0.546779552	2.826779552
	Mute Swan	1487	4.37	3.329549921	5.410450079
	Whooper Swan	29	41.38	24.16117618	58.59882382
	Mallard	68936	16.83	16.550716	17.109284
Numididae	Helmeted Guineafowl	25	4	-4.389128057	12.38912806
Phasianidae	Grey Partridge	290	0.69	-0.335340156	1.715340156
	Ring-necked Pheasant	687	1.46	0.550740444	2.369259556
	Red-legged Partridge	5	20	-10.30568173	50.30568173
	Red Junglefowl	18	0	-6.426565435	6.426565435
Columbidae	Feral Pigeon/Rock Dove	1422	2.04	1.302014351	2.777985649
	Stock Dove	4	0	-22.23236424	22.23236424
	Common Woodpigeon	382	0	-0.327431669	0.327431669
	Eurasian Collared Dove	202	0.99	-0.47576816	2.45576816
Charadriidae	Northern Lapwing	8	0	-13.10788792	13.10788792
	European Golden Plover	1044	0.19	-0.095659904	0.475659904
Scolopacidae	Eurasian Woodcock	0	0	-49.84586669	49.84586669
Laridae	Black-headed Gull	3361	12.14	11.03598464	13.24401536
	Common Gull	279	1.79	0.192864353	3.387135647
	Great Black-backed Gull	280	6.79	3.83586712	9.74413288
	European Herring Gull	642	5.45	3.690529522	7.209470478
	Lesser Black-backed Gull	217	3.69	1.150389202	6.229610798
Phalacrocoracidae	Great Cormorant	7570	1.11	0.873582855	1.346417145
Ardeidae	Little Egret	214	0.93	-0.451816167	2.311816167
Accipitridae	Eurasian Sparrowhawk	166	0.6	-0.755850222	1.955850222
	Red Kite	46	0	-2.642554394	2.642554394
	Common Buzzard	635	5.04	3.334334802	6.745665198
Tytonidae	Western Barn Owl	175	0.57	-0.71766206	1.85766206
Strigidae	Little Owl	10	0	-10.85741885	10.85741885
Picidae	Great-spotted Woodpecker	22	0	-5.335882529	5.335882529
Falconidae	Common Kestrel	166	0	-0.749642941	0.749642941
	Merlin	4	0	-22.23236424	22.23236424
	Peregrine	334	2.4	0.733636218	4.066363782
Corvidae	Eurasian Magpie	173	10.98	6.326495268	15.63350473
	Western Jackdaw	98	0	-1.261878649	1.261878649
	Rook	240	0	-0.51995052	0.51995052
	Carrion Crow	152	0.66	-0.822781927	2.142781927
Paridae	Coal Tit	6	0	-16.51552047	16.51552047
	Marsh Tit	4	0	-22.23236424	22.23236424
	Eurasian Blue Tit	201	1.99	-0.003144972	3.983144972
	Great Tit	191	0	-0.652293339	0.652293339
Alaudidae	Eurasian Skylark	31	0	-3.860716159	3.860716159
Aegithalidae	Long-tailed Tit	68	0	-1.806406222	1.806406222
Phylloscopidae	Common Chiffchaff	325	3.38	1.396446623	5.363553377
Regulidae	Goldcrest	122	0	-1.016689964	1.016689964
Trogolodytidae	Eurasian Wren	96	0	-1.287758338	1.287758338
Sittidae	Eurasian Nuthatch	1	0	-42.64340514	42.64340514
Certhiidae	Eurasian Treecreeper	3	0	-26.77163035	26.77163035
Sturnidae	Common Starling	978	0.61	0.109496072	1.110503928
Turdidae	Song Thrush	412	0	-0.30367658	0.30367658
	Mistle Thrush	2	0	-33.3302423	33.3302423
	Redwing	50	0	-2.437434389	2.437434389
	Common Blackbird	264	0	-0.472951304	0.472951304
	Fieldfare	164	0	-0.758701324	0.758701324
Muscicapidae	European Robin	241	0.83	-0.401325965	2.061325965
Passeridae	Eurasian Tree Sparrow	307	10.75	7.286998244	14.21300176
	House Sparrow	828	0.6	0.057752746	1.142247254
Prunellidae	Dunnock	127	0.79	-0.979827987	2.559827987
Motacillidae	Grey Wagtail	38	0	-3.177270891	3.177270891
	Pied Wagtail	124	1.61	-0.756359161	3.976359161
	Meadow Pipit	149	0	-0.834312339	0.834312339
Fringillidae	Common Chaffinch	419	0	-0.298621432	0.298621432
	Brambling	164	0	-0.758701324	0.758701324
	Eurasian Bullfinch	0	0	-49.84586669	49.84586669
	European Greenfinch	50	2	-2.375692844	6.375692844
	Common Linnet	21	0	-5.572360528	5.572360528
	Lesser Redpoll	259	0	-0.482028684	0.482028684
	European Goldfinch	41	0	-2.953190317	2.953190317
	Eurasian Siskin	47	0	-2.588105405	2.588105405
Emberizidae	Yellowhammer	20	0	-5.830736821	5.830736821
	Common Reed Bunting	294	3.4	1.306647171	5.493352829

Chapter 5 Appendix 1: Locations of eBird complete lists downloaded for use in exposure risk modelling at waterbodies

Location	**Number of Complete Lists**
Kilnsea Wetlands	78
Beacon Ponds	6
Canal Scrape	15
Welwick Marsh	8
Thornwick Pools	11
Blacktoft Sands	2
North Cave Wetlands YWT NR	55
Beverley Beck	6
Swinemoor	25
Blacktoft Sands RSPB Reserve	55
Wheldrake Ings YWT NR	18
Tophill Low Nature Reserve	10
Brandesburton Ponds	1
Southfield Reservoirs	3
Hornsea Mere	12

Blacktoft Sands RSPB Reserve--Howden Dyke Is (Hook Is)	21
Welton Waters and Riverside	14
Hessle Foreshore	4
Brough Haven	2
Lower Derwent Valley NNR	1
Stone Creek	6
Sugar Mill Ponds	2
Humber Flats off Kilnsea	6
Skeffling Clays	11
Easington Lagoons	3
North Marsh	1
Total	**376**