

## Chapter 5

# Avian Influenza Virus in Aquatic Environments: An Ecological Perspective

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## INTRODUCTION

Influenza viruses are categorized into three types: A, B, and C. While influenza types B and C primarily circulate in human hosts, type A viruses circulate in multiple species [1]. Influenza type A viruses are divided into different subtypes according to their hemagglutinin (HA) and neuraminidase (NA) envelope proteins. Currently, 16 HA proteins and 9 NA proteins have been characterized. While only subtype combinations of HA 1–3 and NA 1–2 are routinely isolated from humans, all subtypes have been isolated from aquatic or semi-aquatic birds [2–5]. The term ‘avian influenza viruses’ (AIV) generally refers to influenza A subtypes or strains that have been isolated from birds but are not commonly found in humans or other species.

Because waterfowl (Anseriformes) and shorebirds (Charadriiformes) are the animal reservoirs for AIV, the suspected natural transmission cycle of AIV is: 1) viral replication in the gastrointestinal tract of an aquatic bird, 2) high concentrations of virus shed into the aquatic environment through feces, 3) sustained AIV viability in aquatic environments, and 4) subsequent AIV infection of other animals in that same aquatic environment. Because many waterfowl and shorebirds are migratory, AIV can theoretically be carried and transmitted among aquatic habitats as well.

Two virulence phenotypes have been described for AIV based on pathogenicity in poultry: low-pathogenic AIV (LPAIV) and highly-pathogenic AIV (HPAIV; [6]). Naturally circulating AIV are generally considered to have low pathogenicity in poultry, humans, and other mammalian species. However, some AIV strains have been shown to re-assort and/or adapt to these species with potential for high pathogenicity and sometimes, in humans, at pandemic levels [3, 7]. LPAIV resident in wild birds can evolve into HPAIV once introduced into poultry [8, 9] as in the case of HPAIV Asian strain H5N1. Naturally circulating LPAIV H5N1 became highly-pathogenic only when it moved from migratory birds to domestic poultry [10]. Since then, HPAIV H5N1 has also been highly-pathogenic in a number of wild birds, humans, and other mammals [11].

Four major influenza A pandemics have occurred globally in humans during the last 100 years (1918, 1957, 1968, and 2009), resulting in over 50 million deaths [7]. In all cases, these pandemics have been genetically linked with AIV. Evidence suggests that the virus strain responsible for the 1918 influenza pandemic may have been an entirely avian-like strain that adapted to humans [12, 13] and that the 1957 and 1968 pandemics were descendants of the 1918 strain with newly acquired AIV genes [14, 15]. The most recent pandemic, H1N1 2009, surprised virologists by being a swine-derived influenza; however further genetic analysis also suggests some AIV relatedness [16]. Thus, there are potential linkages between past pandemics and wild birds, which necessitates understanding of AIV in natural systems.

Thus, the ultimate question exists, “How do AIV get into human populations?” While the modes of transmission may vary (direct transmission, re-assortment in an intermediate host, etc), we focused in this chapter on natural AIV reservoirs and their aquatic habitats. We do not provide an exhaustive review of the literature but, rather, used elements of the literature that build on previous reviews [17, 18] to link all of the major components of aquatic habitats into a conceptual model. Our hope is that understanding AIV ecology in these aquatic environments will shed light on AIV transmission, new methods for AIV surveillance, and potential risk assessments for future pandemics.

### AVIAN INFLUENZA VIRUS IN WILD BIRDS ASSOCIATED WITH AQUATIC HABITATS

AIV in wild birds appears to exhibit rapid evolutionary dynamics. The large genetic diversity in AIV can be attributed to the fact that 1) multiple viral lineages have been detected in single aquatic sites, and 2) lack of apparent species effects on infection of hosts [19]. In fact, wild waterfowl (Anseriformes) and shorebirds (Charadriiformes) have long been considered the natural reservoirs of AIV [1] and carry all 144 subtypes (HA and NA combinations) identified [2, 4, 5]. In particular, dabbling ducks show high levels of infection rates relative to other aquatic birds [5, 20, 21]. For the most part, wild birds remain asymptomatic as they carry and shed viable AIV [22, 23], although some studies argue that migratory behavior may be impaired [24].

Although considerable cloacal sampling of wild birds has been conducted, little work has been done on quantifying virus in wild bird feces, the most probable source of water contamination with AIV [1, 25, 26]. VanDalen et al. [27] found that peak AIV concentrations in the feces of experimentally-infected mallards (*Anas platyrhynchos*) averaged  $10^{4.4}$  PCR EID<sub>50</sub> equivalents/mL while Webster et al. [26] found viral concentrations of  $10^{6.8}$ – $10^{8.8}$  EID<sub>50</sub>/mL in fresh feces of experimentally-infected mallards. In addition, AIV persisted and remained infective in feces over time, depending on the storage temperature. We analyzed the data from Table 3 in [26] using generalized linear models [28] in an information-theoretic approach [29] and developed the following equation for infectivity over time by temperature:

$$\text{Log}_{10} \text{ Virus Concentration} = \hat{\beta}_0 + \hat{\beta}_1(\text{Day}) + \hat{\beta}_1(\text{Temperature } ^\circ\text{C}) + \hat{\beta}_3(\text{Day} \times \text{Temperature}),$$

where  $\hat{\beta}_0$  = the starting virus concentration ( $\text{log}_{10}$  EID<sub>50</sub>/mL),  $\hat{\beta}_1 = -0.0405$  (SE = 0.0566),  $\hat{\beta}_2 = -0.0626$  (SE = 0.0584), and  $\hat{\beta}_3 = -0.0240$  (SE = 0.0093). The presence of an interac-

tion suggested that the rate of decline in AIV persistence in waterfowl feces differed according to temperature (Figure 1A) and this model explained 63.4% of the variation in the data. If the starting virus concentration of  $10^{4.4}$  PCR EID<sub>50</sub> equivalents/mL in feces from [27] was used, then virus would persist for 7 days at 17 °C and for 3 days at 28 °C, indicating relatively long survival of AIV in feces exposed to the environment. In per-

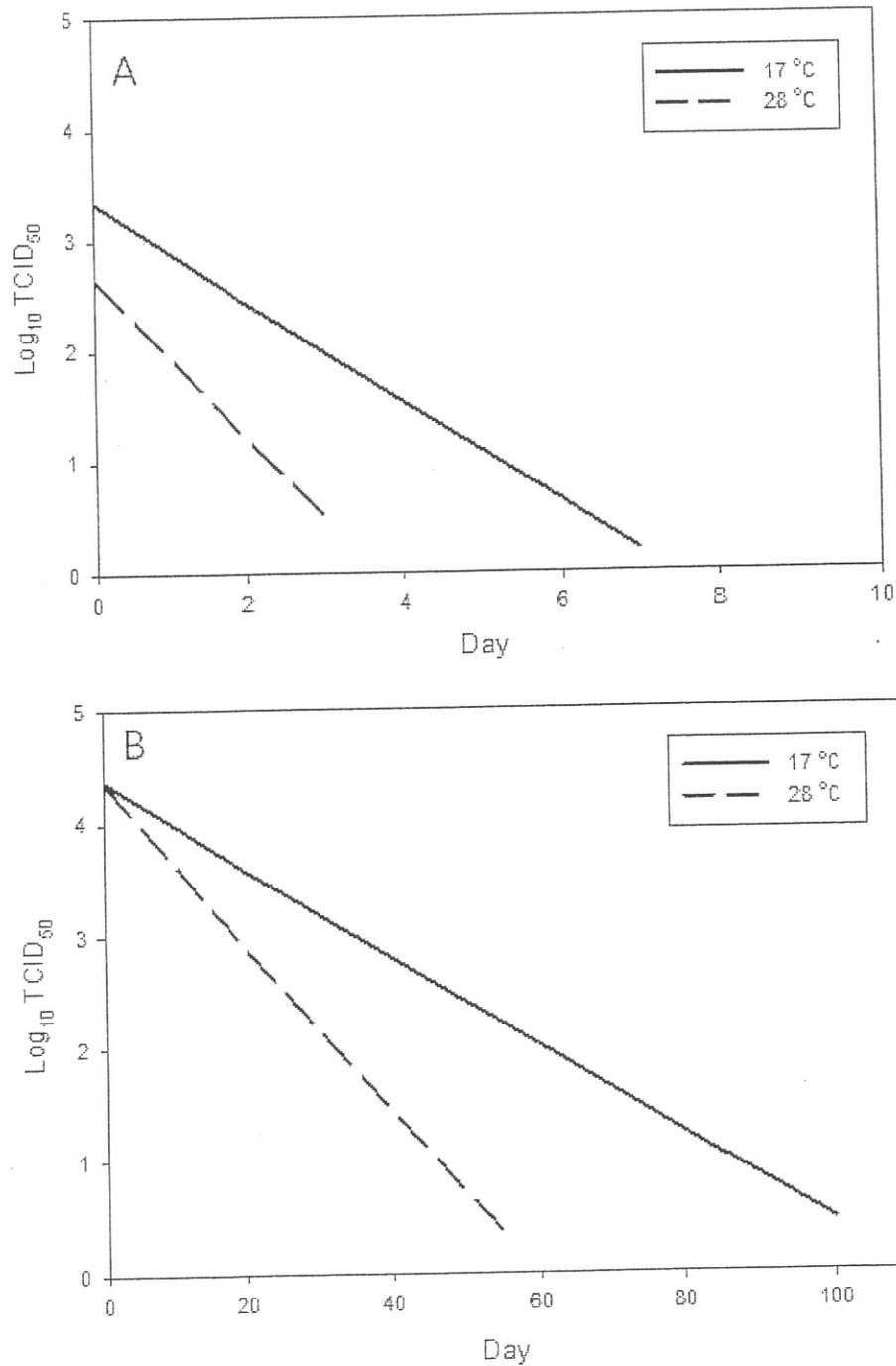


Figure 1: Persistence of avian influenza virus within the range of waterfowl fecal shedding rates and infectious doses from water in (A) feces based on analysis of data in [26] and (B) water based on data re-analyzed from [44, 45].

forming this, and subsequent analyses, we assumed that trends estimated with response variables in  $TCID_{50}$  could be extrapolated to  $EID_{50}$ .

The question of whether waterfowl can be long-distance transmitters of AIV continues to be debated [30–32]. In experiments, mallards exhibited minimal physiological responses to infection with LPAIV, with only slight body temperature increases in some birds over 1–2 days [22]. However, observational data are contradictory. One study suggested that LPAIV infected swans had delayed migration, travelled shorter distances and fed at reduced rates [24]. However, this study was hampered by limited sample size ( $n = 2$  infected individuals). Long distance transmission of AIV may depend even more on infection history of a given migratory waterbird. Upon AIV infection, mallards develop degrees of homo- and heterosubtypic immunity, resulting in substantially reduced AIV shedding [22, 33]. In addition, younger birds shed more virus [34] and have higher prevalence in surveys of wild waterfowl [35] perhaps because of differences in immunity or lack of exposure to AIV. This line of evidence suggests that naïve (e.g., younger) individuals will be the primary transmitters while older birds exposed to a wide variety of AIV subtypes will be poor transmitters of AIV over longer distances.

A water-borne transmission route of AIV to wild water birds has long been hypothesized and evidence for such a route has increased over the years [18]. Evidence to support this hypothesis was initially based on similar subtypes being found in wild bird feces and lake water where the feces were collected [26, 36, 37] but has since been supported by research on AIV persistence in water and prevalence of AIV in waterfowl feces (see next section). However, one needs to understand how ducks forage to put this into perspective.

Dabbling ducks feed in water at depths up to 50 cm and use different foraging strategies at different levels within the water column [38–40]. Food intake for mallards can be 1.5 times higher in shallow (<5 cm) than in deep (>35 cm) water, especially in the winter [41]. Dabbling ducks, such as green-winged teal (*Anas carolinensis*) and mallards, also consume seeds and invertebrates from sediments at the bottom of the water column [39, 42] while density of northern pintails (*Anas acuta*) has been positively correlated with polychaetes in wetland sediments where they forage [38]. In addition, prevalence of AIV in wild dabbling ducks has been correlated with their morphological adaptations for feeding. Some species (e.g., northern shovelers [*Anas clypeata*]) that have finely spaced lamellae to enhance water filtration for intake of small invertebrates also had the highest AIV prevalence [43]. This supports the water-borne AIV infection of dabbling ducks, which tend to forage in shallow water and bottom sediments, have morphological features which expose them to AIV in water and sediment, and also exhibit the highest AIV prevalence.

## AVIAN INFLUENZA VIRUS IN WATER

AIV persists for relatively long periods of time in water, depending on environmental conditions, such as temperature, pH, and salinity. The bulk of the pioneering work done on AIV in water has been conducted by David Stallknecht and his group [17, 18, 44–47]. We re-analyzed some of the data from published sources [44, 45] to develop a general equation for examining persistence of AIV in water under different conditions in the wild and to determine whether there were generalizations across subtypes. We used the same approach as in the previous analysis on AIV in feces. Based on this analysis, we found that four subtypes (H3N8, H4N6, H10N7, and H6N2) had similar rates of persistence in fresh

water at neutral pH because subtype was an additive effect with no support for interactions with subtypes. From this model, we used the following equation (eliminating the empirical subtype effect):

$\text{Log}_{10}$  Virus Concentration =

$$\beta_0 + \hat{\beta}_1(\text{Day}) + \hat{\beta}_2(\text{Water Temperature } ^\circ\text{C}) + \hat{\beta}_3(\text{Day} \times \text{Water Temperature}),$$

where  $\beta_0$  = the starting virus concentration ( $\log_{10}\text{EID}_{50}/\text{mL}$ ),  $\hat{\beta}_1 = 0.0127$  (SE = 0.0096),  $\hat{\beta}_2 = -0.0019$  (SE = 0.0101), and  $\hat{\beta}_3 = -0.0031$  (SE = 0.0004). This model explained 86.7% of the variation in the data. Peak AIV concentrations in the feces of infected mallards averaged  $10^{4.4}$  PCR  $\text{EID}_{50}$  equivalents/mL and viral concentrations as low as  $10^{2.8}$  PCR  $\text{EID}_{50}$  equivalents/mL in water have been documented to infect naïve mallards [27]. If infected waterfowl on a natural body of fresh water shed similar levels of virus into the water, AIV would persist for 41 days (95% CI = 32, 49 days) at 17 °C and for 21 days (95% CI = 32, 49) at 28 °C until it declined to where the infectious dose of  $10^{2.8}$  PCR  $\text{EID}_{50}$  equivalents/mL was reached (Figure 1B). However, other environmental conditions, such as pH and salinity, departing from neutral freshwater have negative effects on persistence time of AIV in water [44].

In a similar manner, we also examined the effects of these conditions on AIV persistence by re-analyzing data obtained from [44] for naturally occurring subtypes in the wild (H4N6, H6N2, and H10N7). Again, we found only additive subtype effects, indicating that rates of persistence would be similar for the subtypes examined; the intercepts would only shift up or down depending on the subtype. This model explained 83.2% of the variation in the data. We found that the effects of pH, salinity, and water temperature for the three subtypes (without the specific subtype effects) could be explained by:

$$\begin{aligned} \text{Log}_{10} \text{Virus Concentration} = & \hat{\beta}_0 + \hat{\beta}_1(\text{pH}) + \hat{\beta}_2(\text{pH}^2) + \hat{\beta}_3(\text{Salinity}) \\ & + \hat{\beta}_4(\text{Water Temperature } ^\circ\text{C}) + \hat{\beta}_5(\text{pH} \times \text{Salinity}) + \hat{\beta}_6(\text{pH}^2 \times \text{Salinity}), \end{aligned}$$

where  $\hat{\beta}_0 = -14.5579$  (SE = 6.2925),  $\hat{\beta}_1 = 5.1295$  (SE = 1.8152),  $\hat{\beta}_2 = -0.3300$  (SE = 0.1295),  $\hat{\beta}_3 = -0.0078$  (SE = 0.2913),  $\hat{\beta}_4 = -0.0496$  (SE = 0.0091),  $\hat{\beta}_5 = 0.0194$  (SE = 0.0841), and  $\hat{\beta}_6 = -0.0031$  (SE = 0.0060). As concluded by the original study, all three factors affected AIV persistence with optimal AIV persistence occurring in water with colder temperatures, lower salinity, and closer to neutral pH (Figure 2).

In addition to water, AIV has been found in the sediments of both frozen and unfrozen ponds and in lake ice [48, 49] and in aquatic plants [49]. Four AIV HA subtypes were identified in the pond sediment in Alaska, with H3 subtypes being the most common, followed by H12, H11, and H8 [48]. Interestingly, the H11 subtypes found in sediments were genetically linked primarily to subtypes found in shorebirds while the H3 subtypes were genetically linked to those primarily found in ducks. Some of the ponds sampled for sediment in [48] were frozen and AIV H1 subtypes (the only subtype examined) have been found in Siberian lake ice [50], at times when no wild birds were present, suggesting that AIV may have been able to overwinter in aquatic environments. In both cases, presence and number of positive samples were roughly correlated with use of the water sources by wild water-

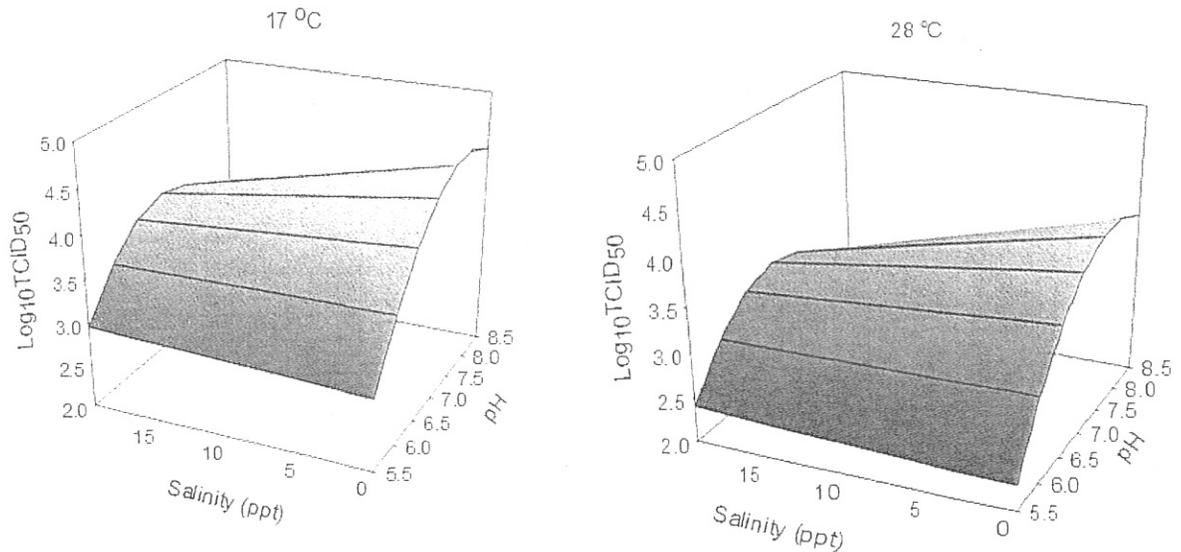


Figure 2: Effects of salinity (ppt), pH, and temperature on persistence of avian influenza virus in water, based on data re-analyzed from [44].

fowl and shorebirds [48, 50]. However, in all these studies, only viral RNA was detected with no supporting evidence as to whether the virus was still infective.

This line of evidence, coupled with the feeding ecology of dabbling ducks in the preceding section, suggests that transmission among waterfowl is likely not a direct fecal-oral route but includes the intermediate step of water, where transmission from water can take place weeks, months or even the next season with no infected birds in the immediate vicinity as infection occurs.

### AVIAN INFLUENZA VIRUS IN MAMMALS ASSOCIATED WITH AQUATIC HABITATS

It is unclear what role wild mammals play in the perpetuation and transmission of AIV. However, compelling evidence exists that certain species of wild mammals can acquire AIV, remain asymptomatic, and shed AIV. AIV has been documented in a wide range of mammals in the families Phocidae, Delphinidae, Mustelidae, Procyonidae, Sciuridae, Viverridae, Canidae, Felidae, and Suidae [51]. Some of these mammals are purely aquatic and occupy only marine habitats, such as the harbor seal (*Phoca vitulina*), and some are mostly terrestrial and spend minimal time in aquatic habitats, such as Owston's civet (*Chrotogale owstoni*). While these animals may be susceptible to AIV and possibly contribute to AIV transmission, their habitat may limit them from being direct links between waterfowl habitats and humans. Here, we discuss three semi-aquatic mammalian species and their potential to bring AIV from aquatic to terrestrial environments, especially those encompassing agricultural, suburban, and/or urban areas. These potential AIV hosts include mink (*Mustela vison*), raccoon (*Procyon lotor*) and feral swine (*Sus scrofa*).

Mink are susceptible to avian-, human-, swine-, and equine-derived viruses [52–54]. Experimental infections have been performed on mink since researchers discovered antibodies to AIV and human-derived influenza A viruses in field investigations. Experimentally, mink have been susceptible to AIV strains representing subtypes H3N8, H5N3,

H7N2, H7N7, H8N4, and H11N4 via intranasal inoculation and contact transmission [52–54]. Both routes of infection yielded infectious virus for up to 11 days post-inoculation. The susceptibility of mink to AIV was also demonstrated in a large outbreak of H10N4 within several mink farms in southern Sweden. Presumably, 100% of mink died from the infection resulting in approximately 3000 deaths [55, 56].

Raccoons are also susceptible to both avian- and human-derived influenza A viruses. Experimentally, raccoons have been susceptible to AIV strains representing the subtypes H4N8 and H1N1 and a human strain representing the subtype H3N2 [57, 58]. A concurrent field study also detected antibodies to influenza A viruses in wild-caught raccoons in the states of Maryland, Colorado, and Wyoming. Most of these positive raccoons had antibodies specific for H4 proteins (82%), which suggest an avian-derived infection [58].

Feral swine populations are increasing and expanding their geographic range throughout much of the world. These feral mammals frequent aquatic habitats as well as farms where they can have contact with domestic swine and humans [59]. While AIV has yet to be reported in feral swine, susceptibility of domestic swine to AIV has been documented [60–62]. In fact 29 AIV isolates have been shown to successfully replicate in domestic swine, often resulting in high titers of nasal shedding ( $>10^4$  EID<sub>50</sub>/mL; [63]). Because feral swine and domestic swine are essentially the same species, it seems probable that feral swine would also be susceptible to the same AIV strains.

Numerous species of mammals are susceptible to AIV and may be capable of transmitting AIV to humans. Especially interesting are the wild mammals, which frequent both wild waterfowl habitats and terrestrial habitats within agricultural and urban landscapes. Mink, raccoons, and feral swine are only a few animals that may directly link aquatic environments and humans. Striped skunks (*Mephitis mephitis*), red foxes (*Vulpes vulpes*), and a variety of felids are also AIV-susceptible mammals that may encounter wild waterfowl and humans [57, 64–69]. The expansion of farms and cities has decreased the distance between these wild animals and humans and these overlapping habitats may be just one way that AIV can be transmitted from aquatic environments to human populations, via wild and feral mammals.

## AVIAN INFLUENZA VIRUS IN AQUATIC INVERTEBRATES

While AIV can persist in water for relatively long periods of time (see above), and a good deal is known about the abiotic factors affecting persistence of AIV in water, less well-known is the degree to which biotic factors, such as aquatic invertebrates, contribute to the persistence of AIV in water.

Aquatic invertebrates could have three possible functions in the ecology of AIV transmission in aquatic habitats. Filter-feeding invertebrates, such as many bivalves, may remove infectious AIV from the water column. During filter feeding, water is passed across the gills to remove particles from the water column, and, in the process, animals may filter out and bioconcentrate pollutants, protozoal parasites, and viruses. This process of bioconcentration by bivalves has been harnessed to track contamination of water with a number of pollutants [70] as well as water-borne parasites [71], and viruses such as Norwalk virus [72]. In addition, shellfish (bivalves including oysters, mussels, clams) have been implicated in the transmission of a number of viruses pathogenic to humans that are transmitted via the fecal-oral route [73]. The role of filter-feeders in AIV ecology has only recently been studied in the laboratory. Filter-feeding bivalves may reduce infectivity of

AIV-contaminated water by actually removing the virus from the water as has been shown with the freshwater Asiatic clam (*Corbicula fluminea*) [74].

Aquatic invertebrates may function to transmit AIV to predators through their consumption if virus remains infective within the tissue of the prey item. Ample evidence exists for the transmission of enteric viruses to humans through the consumption of whole, often raw, filter-feeding shellfish [73]. However, very little is known about the role of aquatic biota in AIV ecology [18]. Faust et al. [74] fed the tissue of a single HP AIV-exposed clam to wood ducks ( $n = 3$ ) and found that none of the treated animals displayed signs of infection such as morbidity, mortality, antibody development or viral shedding suggesting that bivalves may not function to transmit AIV. Nevertheless, bivalves, and perhaps univalves such as snails that many waterfowl eat, might act as reservoirs of AIV in wild systems because viral concentrations may be higher in invertebrates exposed to virus repeatedly deposited through fecal deposits of infected birds. Faust et al. [74] also found that the pH of water in which a clam was housed increased more than in water without a clam, though these changes were small with limited sample size. The pH in wild, aquatic ecosystems likely does not change as abruptly in the presence of invertebrates because of the size of the body of water.

Even less is known about whether aquatic invertebrates can retain AIV as they overwinter in an aquatic environment that waterbirds, mammals, or other potential hosts use. Because filtration rates of bivalves drop as water temperatures decrease (see [75]), particles such as viruses that are not metabolized as food might be retained within the filter feeder's body cavity or tissues until they are depredated by a returning avian or mammal predator. A critical component to this idea is whether virus remains viable in the invertebrate's tissue; our limited evidence from [74] suggests that this may not be the case. Nevertheless, AIV might persist in its infective state within a freshwater invertebrate, an idea that would be important to test in the laboratory or in the wild.

### A CONCEPTUAL MODEL OF AVIAN INFLUENZA VIRUS CYCLING IN AQUATIC SYSTEMS

Water has long been considered the mechanism by which AIV is transmitted among wild birds. However, most research has focused on waterfowl and shorebirds while other potential organisms as reservoirs and hosts have not been well-studied, especially in terms of transmission and spread among wild populations and from wild populations to domestic animals and humans. Most of the information we presented in this chapter can be condensed into a conceptual model of how AIV could be maintained, transmitted, and spread from an aquatic system, such as a shallow, freshwater wetland (Figure 3).

Under this model, infected water birds shed virus into the water through their feces; waterfowl have the capability to shed sufficient AIV concentrations to contaminate at least low volume water sources where naïve individuals can become infected. Once deposited, AIV can persist for months and infect other avian species visiting the aquatic source. In addition, AIV can be sequestered in sediments and bioconcentrated in at least some invertebrates, which dabbling and diving ducks will encounter during foraging. These same invertebrates can also be consumed by wild mammals using aquatic environments and humans. Wild mammals using these systems can also become infected by ingesting water and scavenging or preying on infected waterbirds. AIV infections can then be spread to

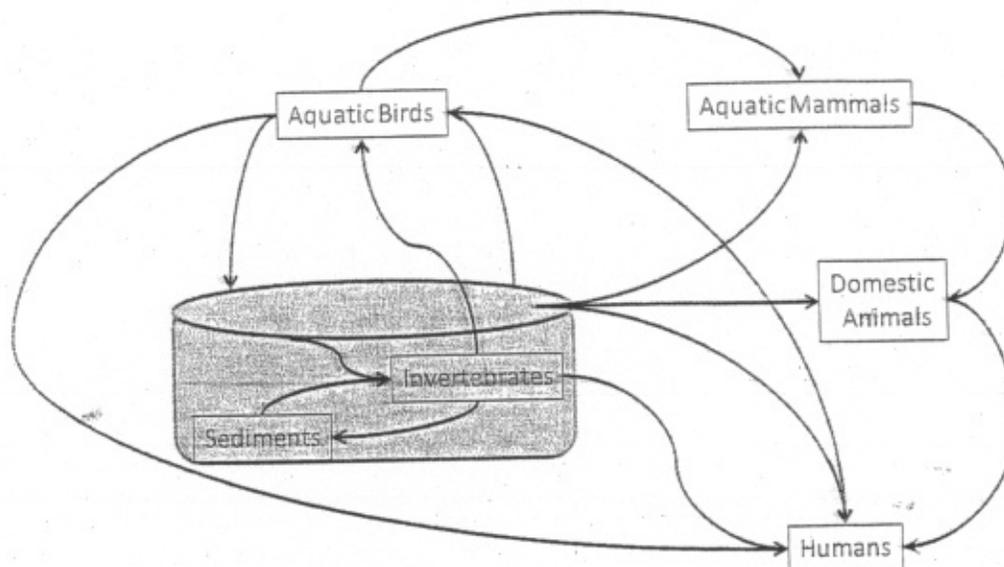


Figure 3: Conceptual model for introduction, maintenance, transmission, and spread of avian influenza viruses in aquatic systems.

domestic animals by interactions with wild mammals that use both aquatic systems and that visit agricultural facilities (e.g., raccoons), which in turn can infect humans. Domestic animals and humans can also become infected by using untreated water from water sources contaminated with AIV. This latter mechanism has been implicated in outbreaks of AIV in poultry (e.g., [76]) and is a plausible mechanism where humans use untreated water sources, such as in most developing countries.

We used this conceptual model to integrate the information we reviewed as a starting point to synthesize existing research. Such a conceptual model is a collection of hypotheses that require further testing and evaluation through laboratory and field experiments coupled with well-designed observational studies.

## CONCLUSIONS

Aquatic systems may serve as reservoirs and sources of infection for both wild birds and mammals, where AIV can be infectious during use by migratory waterfowl and shorebirds, and AIV can even overwinter in those habitats [48, 50]. Based on simulation models and published evidence, Rohani et al [46] proposed that aquatic virus reservoirs give rise to indirect transmission, which would alter the transmission dynamics beyond just direct interactions between infectious and susceptible individuals. Although they considered only the abiotic element of aquatic systems, one could extend this concept to certain biotic elements, such as aquatic plankton and invertebrates, such that the biotic and abiotic components of aquatic systems serve as the primary reservoir of AIV (*sensu* [77]) in nature and waterfowl hosts are secondary. That is, the primary source of infection at a large scale is the aquatic system, where a system can be wetlands, ponds, lakes, etc.

The models developed by [46] predicted a relatively high probability of secondary AIV outbreaks in wild birds on small lakes, even when infected birds were absent, and a low probability for large lakes because of higher dilution of AIV in water. Based on this and

optimal water conditions for AIV persistence, aquatic environments that are likely important reservoirs include shallow, freshwater systems with slow water movement, such as wetlands and ponds, where AIV could concentrate at sufficient levels to infect aquatic wildlife and where wildlife could contact infected invertebrates and sediments. Aquatic systems deviating from this norm in terms of water chemistry and hydrologic structure will probably function less optimally as AIV reservoirs. Thus, we argue that aquatic systems are likely reservoirs but that there will be a gradation in aquatic reservoir quality, similar to the concept of habitat quality in wildlife ecology [78].

This concept of aquatic systems as reservoirs is somewhat consistent with the "sit-and-wait" pathogen strategy ascribed to influenza A viruses in humans [79], which predicts that pathogen virulence should be positively correlated with pathogen survival in the wild. However, the inclusion of virulence in this theoretical concept may not apply to AIV under natural circumstances. Higher virulence only seems to be expressed in AIV when it passes from its natural environment to human-dominated environments and then spills back into wild environments and host populations. Therefore, we believe that AIV is an ecological "sit-and-wait" pathogen because of its ability to persist for long periods in the environment before infecting animal hosts, even though virulence may be episodic in humans.

Transmission of AIV among reservoirs is probably at two scales, a large scale connecting different aquatic systems and a smaller, more local scale that links aquatic and terrestrial systems. At a large scale, linkages may be predominantly migratory waterfowl and shorebirds while at smaller, local scales both waterbirds and wild mammals may transmit AIV among adjacent aquatic systems and to adjacent or nearby terrestrial systems, such as agricultural operations and humans.

At the larger scale, arguments about whether migratory birds can fly significant distances while shedding AIV, including highly-pathogenic AIV [80], may become moot when the migratory behavior of waterfowl is considered. The use of stopovers to migrate between breeding and wintering grounds shortens the distance infected individuals need to move. Even if an animal dies before reaching the migratory endpoint, they may have infected multiple aquatic sites within relatively short distances. Thus, infected waterfowl do not need to carry AIV long distances but only need to carry it to migratory stopover areas where they can infect the area, which in turn will infect other waterfowl using and foraging in that area. For example, northern pintails wintering in California use a variety of strategies when migrating north to the breeding grounds, ranging from indirect migration using multiple stopovers to direct migration without stopping [81]. When stopovers were used, individuals typically spent an average of 7–10 days (range = 3–38 days) at stopover sites.

Of concern is the ability of AIV to undergo rapid evolutionary changes through antigenic shift [82]. As AIV is transmitted through different organisms in aquatic environments, where different subtypes may be present in a single location, the potential for antigenic shift increases. The fact that influenza A viruses that cause pandemics have arisen from strains found in wild birds underscores the importance of understanding these relationships.

When dealing with large-scale surveillance of AIV, it may be more efficient to monitor water and sediments in aquatic habitats in key migratory areas, rather than individual migratory birds. This is especially true when trying to detect introduction of HPAIV strains into an area. Surprisingly, most large-scale surveillance programs for AIV still focus almost exclusively on sampling wild birds (e.g., [20]), rather than aquatic systems, despite

the evidence that aquatic systems may be a critical component to the perpetuation and persistence of AIV in the wild. Along with [18], we argue that surveillance of aquatic systems is a useful component for surveillance of AIV, which could be most efficiently accomplished by (1) identifying key waterfowl stopover and use areas in the region of interest, (2) identifying the above with respect to water chemistry and hydrological structure that provides optimal conditions for AIV persistence, and (3) sampling key elements of the system during critical movement periods of waterfowl over years.

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