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Tenacity of avian influenza viruses

D.E. Stallknecht & J.D. Brown

Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, the University of Georgia, Athens, GA 30602-7388, United States of America

Summary

The goal of this review is to provide an overview of existing research on the environmental tenacity of avian influenza (AI) viruses, to identify gaps in our current understanding, and discuss how this information relates to AI control, eradication, and prevention. We are just beginning to understand the environmental factors that affect infectivity and the extent of variation in environmental tenacity that is present among these viruses. Because the environment can provide a bridge for AI virus transmission between many diverse hosts, including wild and domestic animals and man, understanding the importance of environmental transmission and identifying important points of contact are critical steps in preventing the spread of infection especially related to the introduction of these viruses to new host species.

Keywords

Avian influenza viruses – Environmental transmission – Faeces – Highly pathogenic avian influenza virus – H5N1 – Infectivity – Tenacity – Water.

Introduction

Although the transmission of avian influenza (AI) viruses within both wild and domestic avian populations can be linked to environmental sources, information on their tenacity, or the ability of these viruses to remain infective outside of the host, is limited. The goals of this short review are:

– to provide an overview of existing research on the environmental tenacity of avian influenza virus (AIV)

– to identify gaps in our current understanding of the factors potentially affecting AIV infectivity in the environment

– to discuss why this understanding is important to AI control, eradication, and prevention

– to provide insight into future research needs pertaining to AIV in the environment.

This review will not include methods for virus inactivation associated with cleaning and disinfection of AIV-infected premises as there is an excellent review of this topic currently available (3).

The role of the environment in the natural history of avian influenza

Avian influenza transmission

Wild aquatic birds in the Orders Anseriformes and Charadriiformes are the primordial reservoir for AIV (30). The transmission of AIV within these wild bird populations is dependent on faecal/oral transmission via contaminated water (11, 12, 26, 28). Replication of AIV in ducks occurs primarily in the intestinal tract, with high concentrations of infectious virus shed in faeces (13, 38). Webster *et al.* (38) reported that experimentally infected Muscovy ducks (*Cairina moschata*) shed 6.4 g of faecal material per hour, with an infectivity of $1\times10^{7.8}$ median egg infective doses (EID_{50}), and these birds excreted an estimated 1×10^{10} EID_{50} of AIV within a 24 h period. In addition to a high level of viral excretion, the duration of viral shedding in ducks can also be prolonged. Hinshaw *et al.* (12) reported that infected Pekin ducks (*Anas platyrhynchos*) were capable of shedding virus via the cloaca for more than 28 days. Avian influenza viruses have also been isolated from surface water

in Alberta (12), Minnesota (10), and Alaska (16) from aquatic habitats utilised by wild ducks. In some of these cases AIVs were isolated from the water without sample concentration.

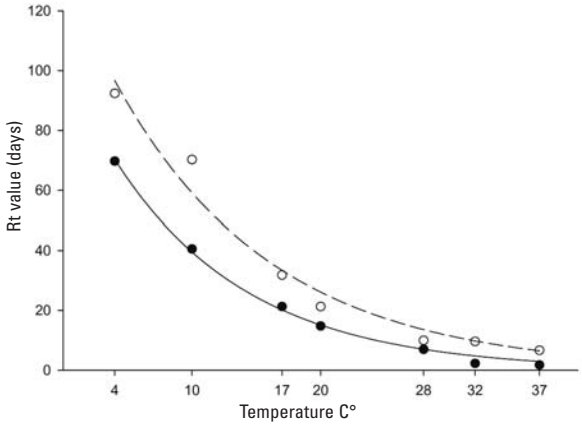
Tenacity of avian influenza viruses in water

Despite the recognised importance of faecal/oral and water-borne transmission of these viruses in bird populations, existing data on AIV persistence in faeces, water, environmental surfaces, and carcasses are limited. Environmental persistence of AIV was initially investigated by Webster *et al.* (38) using A/Duck/Memphis/546/74 (H3N2) in both faecal material and non-chlorinated river water. An initial dose of $10^{6.8}$ EID_{50} in faeces, and $10^{8.1}$ EID_{50} in water remained infective for at least 32 days (when the experiment ended), suggesting that contaminated aquatic environments could serve as a source of infection. Subsequently, AIV persistence was evaluated in faeces (2, 23) and allantoic fluid (23). Other than the original work (38), only four studies (4, 5, 31, 32) have evaluated the persistence of low pathogenic AIV (LPAIV) isolated from wild ducks in water using an experimental system (32). Collectively, these experimental studies demonstrated that these naturally occurring AIVs can persist for months in water at 4°C, 17°C, and 28°C. The duration of infectivity was inversely related to water temperature, and temperature-related variation was extreme, as some viruses remained infective well over a year at 4°C, but only days at 37°C (5). These studies also determined that AIV infectivity is dependent on basic water chemistry (pH and salinity) at values within ranges normally encountered in surface water in the field. Individual AIVs demonstrate phenotypic variation in their ability to remain infective under variable pH and salinity conditions, and an interactive effect between salinity and pH has been reported (31). These laboratory data are relevant to field conditions, and results obtained with a distilled water system paralleled results obtained using surface water collected from duck habitats in Louisiana (31).

The effects of pH, salinity, and temperature as described for AIVs representing 12 haemagglutinin (HA) subtypes (5) can be summarised as follows:

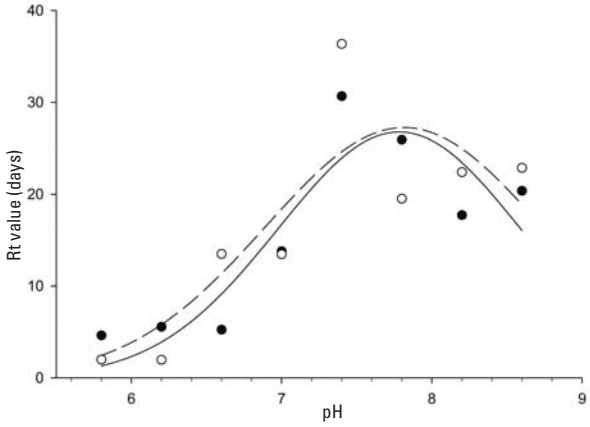
– temperature greatly influences the duration of viral infectivity and the temperature/infectivity relationship can be described with an exponential decay function; variation between viruses is most evident under cold water (4°C) conditions, with little variation observed at temperatures >28°C (Fig. 1)

– pH greatly affects infectivity, with a rapid loss of infectivity below pH 6.5; all viruses were most stable between pH 7.4 and pH 8.2, but variation in pH tolerance was observed between individual viruses (Fig. 2)



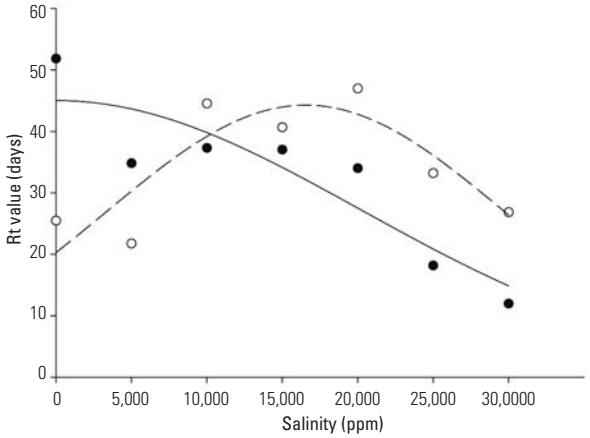
Rt : time in days for 90% reduction in viral titre ($CCID_{50}$/ml)
Filled circles and solid line: A/Mallard/MN/355788/00 (H12N5)
Open circles and dashed line: A/Northern Pintail/Tx/421716/01 (H8N4)

Fig. 1
Effects of temperature on avian influenza virus inactivation in water (5)



Rt: time in days for 90% reduction in viral titre ($CCID_{50}$/ml)
Filled circles and solid line: A/Mallard/MN/355788/00 (H12N5)
Open circles and dashed line: A/Northern Pintail/Tx/421716/01 (H8N4)

Fig. 2
Effects of pH on avian influenza virus inactivation in water (5)



Rt: time in days for 90% reduction in viral titre ($CCID_{50}$/ml)
Filled circles and solid line: A/Green-winged Teal/LA/213GW/87 (H1N1)
Open circles and dashed line: A/Mallardl/MN/199057/99 (H4N6)

Fig. 3
Effects of salinity on avian influenza virus inactivation in water (5)

– viruses were most stable at 0 ppm (fresh water) or 15,000 ppm (brackish water) rather than 30,000 ppm (ocean water) sodium chloride; individual viruses differed with some more stable at 0 ppm and some more stable at 15,000 ppm (Fig. 3).

Within-subtype variation has been investigated using the laboratory model system with LPAI viruses of the H5 and H7 subtypes (4). As in the previous studies, the duration of viral persistence decreased with increasing temperature and a variable response was observed with salinity; some viruses persisted longer at 0 ppm while others persisted longer at 15,000 ppm. Observed variation within subtype suggests that environmental tenacity is not dependent on an HA subtype. However, these results are limited to two subtypes (H5 and H7) and the effects of pH were not evaluated in this study.

These findings suggest that even minor fluctuations in temperature, pH, and salinity at levels normally encountered in natural aquatic habitats may enhance or diminish environmental persistence and potential transmission of AIV. In addition, variation in the response of AIVs to these variables may indicate that environmental selective pressures impact on virus maintenance and transmission in the field and the ability of an AIV to transmit within and between wildlife reservoirs and domestic animal systems.

Environmental conditions may also influence the potential for aerosol transmission. With human influenza viruses evaluated in a guinea pig model, it was demonstrated that transmission was enhanced under both cold and dry conditions (22). Low relative humidity can affect dispersal factors that enhance virus availability, and in aerosols virus infectivity is maximised at low relative humidity (27). The environmental conditions that potentially affect aerosol transmission of AIV have not been evaluated but may have important implications for transmission within domestic avian populations, especially those kept under confinement or artificially modified conditions, such as feral waterfowl populations or sanctuaries.

Detection of avian influenza viruses from environmental samples

Relatively few studies have aimed at isolating AIVs directly from surface waters, but the presence of these viruses in environmental samples has been repeatedly documented (10, 12, 16, 19). The methodology used in these studies varied and at present there is no single recommended method for recovering or detecting these viruses from environmental samples. To date, AIV has been successfully demonstrated in water samples through direct culture (9) and through virus concentration with formalin fixed chicken erythrocytes (16, 18). Using polymerase chain reaction (PCR), AIV ribonucleic acid (RNA) has also been detected in sediment samples (19). Long-term detection of AIV from aquatic habitats following the departure of waterfowl has been reported in two studies in Alaska, and in both cases a relatively high prevalence of infected water (1% to 7% by virus isolation) (16) or sediment (56% PCR positive) samples were reported (19).

Avian influenza tenacity in man-made environments

The environment and transmission within poultry populations

With regard to viral transmission from wild to domestic fowl, contaminated surface and ground water has been suggested as a long- and short-term source of AIV for domestic turkeys (10). In addition, contaminated fomites have long been recognised as an important factor in AIV transmission between widely separated poultry flocks (34). Domestic chickens and turkeys can shed large quantities of virus for extended periods of time, as long as 36 days for chickens (14) and 72 days for turkeys (35). Few specifics are known regarding the contribution of environmental transmission to maintaining infections within domesticated avian flocks, but in captive studies (33) and in live-bird markets (21) influenza viruses were isolated from water sources. In fact, the surveillance method used to detect AIV of subtype H9 in water provided to caged birds in live bird markets in Hong Kong has been recommended as a very efficient surveillance methodology that may be more efficient than isolation from the cloacal or faecal samples (21).

Environmental transmission may be extremely important within domestic duck populations. However, to date, few research efforts have been directed toward this topic. In a study in Hong Kong, an H3N2 AIV was isolated from faeces and pond water every month during a one year period, and the maintenance of this virus was proposed to be dependent on environmental persistence and the continued introduction of susceptible ducklings (25). Domestic ducks are recognised as an important reservoir for the Asian lineage highly pathogenic H5N1 AIV (15), but the extent and significance of environmental contamination in this reservoir is undefined.

The environment and transmission from poultry

Information related to the transmission of AIV from infected poultry flocks to other animals or humans via

environmental sources is lacking. This is an area that deserves attention, especially in those cases where AIVs are present in free-ranging domestic flocks or under confinement conditions where faeces or other effluent are deposited into the environment. In chicken faeces, inactivation of AIV can be rapid at high temperatures (above 25°C) (6) but is prolonged at low temperatures; for example, at 20°C AIV can remain infectious in chicken faeces for 7 days (23), but at 4°C the virus can remain infectious for as long as 30 days (2). However, these studies evaluating AIV tenacity in chicken faeces included a very limited number of viruses and the extent of variation related to virus strains and subtypes is currently unknown. The host may also be important as longer persistence of AIV in domestic duck faeces (4 to 6 days) has been reported (36). Although not identified as a risk factor in a case-control study of LPAIV H7N2 in domestic poultry in the United States of America (24), contaminated poultry litter has a potential role in AIV transmission, to date, however, this role remains undefined. Another source of environmental contamination involves infected bird carcasses, which have been implicated in the transmission of highly pathogenic avian influenza virus (HPAIV) H5N1 to carnivores (17). Although there has been significant work related to inactivation of AIV in poultry products (34), information about the duration of AI infectivity in wild and domestic bird carcasses under applicable field conditions is essentially lacking.

Highly pathogenic H5N1 viruses

Although data from environmental surveillance conducted during an outbreak of HPAI H5N1 viruses suggests a potential role for environmental transmission (36), information related to the ability of these viruses to persist in the environment is very limited, as summarised by Algers (1). Brown *et al.* (4) evaluated the duration of infectivity of two HPAI H5N1 viruses (A/Whooper Swan/Mongolia/244/05 and A/Duck Meat/Anyang/AVL-1/01). The evaluation showed that the duration of infectivity of these viruses was shorter than those of LPAIV H5 and H7 derived from ducks and shorebirds. While these initial results indicated that these two HPAI viruses were not as environmentally fit as LPAIV, subsequent evaluations of additional and more recent HPAI H5N1 viruses from Asia indicate much more variation. Some of the HPAI H5N1 viruses are very stable in water, with the duration of infectivity similar to naturally occurring LPAIVs (J.D. Brown, unpublished data). This scenario is consistent with other phenotypic examinations of HPAI H5N1 viruses, which indicate that they have evolved over 10 years into a group of AIVs that exhibit highly variable biological properties.

Unknowns and possibilities related to avian influenza in the environment

The role of the biotic community in influenza inactivation or concentration

There is limited information on the environmental tenacity of these viruses in intact biological systems. Biological components, such as bacteria (8, 37), biofilms (29), or feeding bivalves (20) have all been associated with loss of infectivity, removal, or in some cases concentration of many viruses. In a recent study of AIV in surface water samples from the Black Sea, Zarchov *et al.* demonstrated a loss in AIV infectivity related to increasing concentrations of normally occurring microorganisms (39). There are no published reports of AIV uptake by feeding bivalves or other filter feeding organisms, nor any reports related to possible associations with biofilms. To date, the potential effects of the biotic community on AIV infectivity in environmental sources has been largely ignored.

Other abiotic factors that may reduce infectivity in the environment

To date, abiotic factors that have been evaluated include temperature, pH, salinity, and in the case of human influenza viruses, relative humidity. From this limited data it is obvious that much additional work remains to be done to evaluate the importance of, for example, desiccation (tissue and faeces), other water quality parameters (metal oxides, dissolved oxygen and fluctuations in temperature or salt levels) and ultra violet (UV) irradiation. No effect was observed when AIVs in faeces were subjected to UV irradiation and this was attributed to the inability of UV irradiation to penetrate the faecal samples (6). This reinforces the need to fully understand the distribution of these viruses in the environment, as this could greatly influence how potential biotic and abiotic components affect AIV persistence. Due to the complexity of these environments and the large number of potential interacting variables that can affect infectivity, it is essential that these questions be addressed using both controlled laboratory experiments and well designed field studies.

Distribution in aquatic environments

Most of the isolations of AIV from wild bird habitats have been associated with faeces and water. Although it is well established that these viruses can be isolated from avian faeces (38), there is little information available related to their tenacity in wild bird faeces. With water, a different deficiency in our understanding exists. Although there are

numerous reports of AIV isolation from surface waters, there have been no evaluations to determine the distribution of this virus in an aquatic ecosystem. It has only recently been demonstrated that AIV RNA can be detected for long periods of time in sediments of habitats utilised by waterfowl (19), but infectious virus has not been detected. It would appear unlikely that AIV is distributed equally throughout the water column, and in fact, this may not present the best opportunity for birds that feed at the sediment interface (e.g. dabbling ducks such as mallard) to be infected. It is possible, and likely, that virus is present in the environment associated with faeces or other organics within or at the sediment surface, and if so, this association could greatly influence the potential for transmission to host species using or having contact with a contaminated environment.

The short- and long-term significance of environmental tenacity

The ability of these viruses to remain infectious for extended periods in the environment could greatly influence transmission and it is possible that this is critical to AIV maintenance within the primordial wild bird reservoir. It has been suggested that AIV preserved in ice associated with waterfowl habitats may represent a long-term (years) environmental reservoir (40). The detection of AIV RNA in sediment samples in Alaska waterfowl habitats supports this (19), but the concept has not been confirmed through virus isolation in any study to date. Survival in water has also been suggested as a source of groundwater contamination, which could represent a source of virus to domestic poultry (9), but this also remains to be verified by virus isolation.

The environmental tenacity and subsequent transmission of viruses through contact with infected environments have potential short-term significance, because environmental tenacity and transmission provide mechanisms for:

– sustaining annual epidemics in migratory wild bird populations that utilise the same habitats but are temporally disconnected

– connecting avian species that are spatially disconnected (utilising different components of the same habitats)

– enhancing transmission on wintering grounds.

On the wintering grounds, birds are more dispersed, population immunity is increased (fewer susceptible birds) and a low prevalence of AIV is observed (30). An environmental source of virus on wintering ground habitats may be critical for these viruses to persist in wild bird populations during this season.

Potential selection due to environmental fitness

Existing studies (4, 5, 31, 32) demonstrate that individual AIVs differ greatly in their ability to remain infectious under environmental stressors such as pH, temperature, and salinity. Although these variables represent only a small portion of the potential environmental variables that could enhance or diminish viral infectivity in water, it is clear that some viruses may be more environmentally fit than others. Considering the potential transmission advantages associated with remaining infectious in the environment for extended periods of time, environmental tenacity may be a prerequisite for the survival of specific AIV strains, subtypes or genotypes. Although these viruses are genetically and phenotypically diverse and continually changing as a result of genetic drift and shift, they are not equally represented in wild bird populations. For example, isolates from ducks are dominated by H3, H4, and H6 viruses, while H13 and H16 viruses are primarily associated with gulls (30). This non-random pattern may be driven by environmental as well as host-related factors, and with wild birds that occupy very different habitats (gulls versus ducks), host and environmental factors may be linked. Additional work is needed in this area as an understanding of 'environmental fitness' may be critical to assessing the potential of viruses like HPAIV H5N1 to spill over from domestic to wild birds and subsequently be transmitted or maintained in these wild populations.

The potential role of environmental fitness and adaptation to new host systems: the bridge

An environment contaminated with AIV would potentially provide a connection between wild and domestic avifauna and mammals utilising a specific area (7). The potential for this exchange was recently demonstrated in a study in Cambodia (36), where AIV was detected by PCR in 35% of environmental samples (mud, pond water, water plants, and water swabs) associated with backyard poultry. Environmental contamination offers a two-way street to connect all potential hosts and this connection needs to be considered when designing and implementing preventive measures or when interpreting field data. For example, the isolation of HPAIV H5N1 from wild birds in the presence of infected domestic birds (as currently occurring in Asia) does not indicate a wildlife reservoir, as the virus could potentially originate from an environment contaminated by a domestic bird source. This is especially relevant when considering the impact of domestic ducks, which are considered the most important reservoir for these HPAI H5N1 viruses (15). Likewise, it is possible that an infected wild bird could effectively disseminate these viruses if its movements are associated with habitats shared with other wild or domestic birds.

Environmental fitness may also affect the ability of a given virus to effectively adapt to changing environments associated with new host systems and it is important to consider that the significance of environmental tenacity may change as these adaptations occur. For example, an AIV might be dependent on environmental (water) persistence to be maintained in a wild or domestic waterfowl population, but this may be irrelevant when a virus spreads to poultry in confinement. In this case, direct contact may drive transmission, or transmission may be more dependent on environmental factors affecting aerosols. These connections and possibilities, at present, are largely circumstantial and speculative, but need to be understood and considered if we are to gain a clearer understanding of AIV natural history or the epidemiology of new viruses such as HPAIV H5N1.

Conclusion

Although current research has detected AIV in environmental sources, and experiments have demonstrated the potential for these viruses to remain infectious for long periods of time under conditions that would be encountered in the field, the significance and extent of AIV environmental contamination is not well defined. These gaps in our knowledge impact our ability to fully understand the transmission and maintenance of these viruses within and between wild and domestic animal reservoirs, and they represent a major impediment to designing and implementing effective AI prevention, control, or eradication strategies.

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La stabilité des virus de l'influenza aviaire

D.E. Stallknecht & J.D. Brown

Résumé

Après avoir fait le point sur les travaux de recherche dédiés à la capacité des virus de l'influenza aviaire de perdurer dans l'environnement, les auteurs indiquent les lacunes de connaissance subsistant dans ce domaine et expliquent l'intérêt de ces informations pour le contrôle, l'éradication et la prévention de l'influenza aviaire. L'importance des facteurs environnementaux pour l'infectiosité des virus de l'influenza et les variations de la stabilité environnementale constatées chez ces virus commencent seulement à être élucidées. L'environnement intervenant comme pont de transmission des virus de l'influenza aviaire entre une grande diversité d'hôtes, dont les animaux domestiques et sauvages et l'homme, il est primordial de bien comprendre le rôle de la transmission par l'environnement et d'identifier les principaux points critiques de contact afin de prévenir la propagation de l'infection et plus particulièrement d'empêcher que ces virus puissent investir de nouvelles espèces hôtes.

Mots-clés

Eau – Fèces – Infectiosité – Sous-type H5N1 – Stabilité – Transmission par l'environnement – Virus de l'influenza aviaire – Virus de l'influenza aviaire hautement pathogène.

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Persistencia de los virus de la influenza aviar

D.E. Stallknecht & J.D. Brown

Resumen
En este artículo se presenta una reseña general de los estudios realizados sobre la persistencia de los virus de la influenza aviar en la naturaleza, las lagunas de los conocimientos actuales sobre el tema y sus consecuencias en el control, erradicación y prevención de la enfermedad. Apenas se han comenzado a comprender los factores medioambientales que influyen en la infecciosidad de esos virus y las variaciones de su supervivencia en la naturaleza. El medio ambiente es una vía potencial de transmisión de los virus de la influenza aviar entre muchos hospedadores distintos, comprendidos los animales silvestres y domésticos, así como los seres humanos. Por consiguiente, es preciso comprender su diseminación por conducto de la naturaleza y determinar los principales puntos de contacto para impedir su propagación, en particular a nuevas especies hospedadoras.

Palabras clave
Agua – Excremento – H5N1 – Infecciosidad – Persistencia – Transmisión medioambiental – Virus de la influenza aviar – Virus de la influenza aviar altamente patógena.

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