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# Persistence of H5 and H7 Avian Influenza Viruses in Water

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SUMMARY. Although fecal–oral transmission of avian influenza viruses (AIV) via contaminated water represents a recognized mechanism for transmission within wild waterfowl populations, little is known about viral persistence in this medium. In order to provide initial data on persistence of H5 and H7 AIVs in water, we evaluated eight wild-type low-pathogenicity H5 and H7 AIVs isolated from species representing the two major influenza reservoirs (Anseriformes and Charadriiformes). In addition, the persistence of two highly pathogenic avian influenza (HPAI) H5N1 viruses from Asia was examined to provide some insight into the potential for these viruses to be transmitted and maintained in the environments of wild bird populations. Viruses were tested at two temperatures (17 C and 28 C) and three salinity levels (0, 15, and 30 parts per thousand sea salt). The wild-type H5 and H7 AIV persistence data to date indicate the following: 1) that H5 and H7 AIVs can persist for extended periods of time in water, with a duration of infectivity comparable to AIVs of other subtypes; 2) that the persistence of H5 and H7 AIVs is inversely proportional to temperature and salinity of water; and 3) that a significant interaction exists between the effects of temperature and salinity on the persistence of AIV, with the effect of salinity more prominent at lower temperatures. Results from the two HPAI H5N1 viruses from Asia indicate that these viruses did not persist as long as the wild-type AIVs.

RESUMEN. Persistencia de virus de influenza aviar H5 y H7 en el agua.

Aunque la transmisión oral-fecal del virus de influenza aviar por medio del agua contaminada representa un mecanismo reconocido de transmisión entre poblaciones silvestres de aves acuáticas, se conoce muy poco de la persistencia viral en este medio. Con la finalidad de proporcionar datos iniciales sobre la persistencia del virus de influenza aviar H5 y H7 en el agua, se evaluaron ocho virus silvestres de influenza aviar de baja patogenicidad H5 y H7, aislados de especies que representan los dos reservorios más grandes (Anseriformes y Charadriformes). Adicionalmente, se examinó la persistencia de dos virus de influenza aviar de alta patogenicidad H5N1 provenientes de Asia, para proporcionar alguna información sobre el potencial de estos virus de ser transmitidos y mantenidos en el ambiente de las poblaciones de aves silvestres. Los virus fueron evaluados a dos temperaturas (17 C y 28 C) y tres niveles de salinidad (0, 15 y 30 partes por mil de sal marina). Los datos de persistencia de los virus silvestres de influenza aviar H5 y H7 obtenidos hasta la fecha indican: 1) que los virus de influenza aviar H5 y H7 pueden persistir en el agua por largos períodos, con una duración de infectividad comparable a la de los virus de influenza aviar de otros subtipos; 2) que la persistencia de los virus de influenza aviar H5 y H7 es inversamente proporcional a la temperatura y salinidad del agua, y 3) que existe una interacción significativa entre los efectos de la temperatura y salinidad en la presencia de los virus de influenza aviar, con el efecto de salinidad, siendo mas prominente a bajas temperaturas. Los resultados de los dos virus de influenza aviar de alta patogenicidad H5N1 provenientes de Asia, indican que estos virus no persistieron por tanto tiempo como los virus silvestres de influenza aviar de baja patogenicidad.

Key words: avian influenza virus, environment, H5, H5N1, H7, high-pathogenicity avian influenza, persistence, water

Abbreviations: AAF = amnio-allantoic fluid; ARS = Agricultural Research Service; AIV = avian influenza virus; ANOVA = analysis of variance; BSL = biosafety level; CEF = chicken embryo fibroblast; HPAI = highly pathogenic avian influenza; LPAI = low-pathogenicity avian influenza; MEM = minimum essential medium; PID = postinoculation day; ppt = parts per thousand; SEPRL = Southeast Poultry Research Laboratory; SPF = specific-pathogen-free; $TCID_{50}$ = median tissue culture infective dose; USDA = United States Department of Agriculture

Aquatic birds in the orders Anseriformes and Charadriiformes are the natural reservoir for avian influenza viruses (AIVs) (10). Infections in these avian hosts are normally asymptomatic and characterized by preferential replication in the intestinal tract with high concentrations of virus shed in the feces (16). Viral transmission in aquatic bird populations is thought to occur through an indirect fecal–oral route involving contaminated water (4,5,16). The maintenance of AIV in these populations may also be dependent on or enhanced by environmental persistence. It is possible that virus shed by birds in the fall, prior to migration, could be preserved in the water over winter, and provide a source of infection to birds returning during the following spring (17). Despite the well-recognized role that contaminated water plays in the transmission cycle of AIVs in wild waterfowl populations, very little is known about the viral persistence in this medium.

Experimental data suggest that AIVs have evolved to persist for extended periods in aquatic habitats. The initial laboratory studies investigating the environmental stability of wild-type low-pathogenicity avian influenza (LPAI) viruses determined that virus in fecal material remained infective in nonchlorinated water for at least 30 days at 4 C and up to 7 days at 20 C (16). A validated model system using distilled water was later developed to evaluate the effects of different environmental parameters on the persistence of AIVs (9). Experimental studies using this system indicate the following: 1) wild-type AIVs can remain infective in water for an extended period, with an estimated persistence >190 days for some viruses with a starting viral concentration of $10^6$ mean tissue-culture infective dose ($TCID_{50}$)/ml; 2) the ability to persist in water differs between individual AIVs; and 3) viral persistence is markedly influenced by differences in temperature, salinity, and pH (based on limits encountered in natural field conditions). Wild-type viruses

Table 1. Linear regression models for persistence of H5 and H7 AIVs in water at 17 C.

| Viruses | Salinity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 ppt | | | 15 ppt | | | 30 ppt | | |
| | LRM[A] | $R^2$ | Estimated persistence[B] | LRM | $R^2$ | Estimated persistence | LRM | $R^2$ | Estimated persistence |
| LPAI viruses | | | | | | | | | |
| MN/98 (H5N2) | $y = 5.375 - 0.014x$ | 0.28 | 429 (71)[C] | $y = 4.695 - 0.047x$ | 0.71 | 128 (21) | $y = 5.057 - 0.095x$ | 0.86 | 63 (11) |
| MN/00 (H5N3) | $y = 5.318 - 0.019x$ | 0.41 | 316 (53) | $y = 5.549 - 0.018x$ | 0.53 | 333 (56) | $y = 6.000 - 0.053x$ | 0.85 | 113 (19) |
| NJ/01 (H5N7) | $y = 6.265 - 0.026x$ | 0.57 | 231 (38) | $y = 5.049 - 0.016x$ | 0.61 | 375 (63) | $y = 5.495 - 0.071x$ | 0.84 | 85 (14) |
| NJ/01 (H5N8) | $y = 4.029 - 0.021x$ | 0.12 | 286 (48) | $y = 3.815 - 0.048x$ | 0.62 | 125 (21) | $y = 4.135 - 0.103x$ | 0.89 | 58 (10) |
| MN/98 (H7N3) | $y = 6.075 - 0.028x$ | 0.70 | 214 (36) | $y = 6.474 - 0.045x$ | 0.64 | 133 (22) | $y = 6.427 - 0.065x$ | 0.90 | 92 (15) |
| TX/02 (H7N4) | $y = 6.517 - 0.034x$ | 0.65 | 176 (29) | $y = 5.973 - 0.045x$ | 0.80 | 133 (22) | $y = 6.282 - 0.056x$ | 0.97 | 107 (18) |
| DE/00 (H7N3) | $y = 5.469 - 0.009x$ | 0.15 | 667 (111) | $y = 5.619 - 0.042x$ | 0.75 | 143 (24) | $y = 5.926 - 0.057x$ | 0.91 | 105 (18) |
| DE/02 (H7N3) | $y = 5.109 - 0.031x$ | 0.71 | 194 (32) | $y = 4.680 - 0.043x$ | 0.81 | 140 (23) | $y = 5.370 - 0.035x$ | 0.75 | 171 (29) |
| HPAI H5N1 viruses | | | | | | | | | |
| Mongolia/05 | $y = 4.787 - 0.038x$ | 0.77 | 158 (26) | $y = 4.869 - 0.072x$ | 0.88 | 83 (14) | $y = 4.033 - 0.073x$ | 0.77 | 82 (14) |
| Anyang/01 | $y = 4.950 - 0.064x$ | 0.94 | 94 (16) | $y = 4.445 - 0.033x$ | 0.59 | 182 (30) | $y = 4.724 - 0.052x$ | 0.79 | 115 (19) |

[A]LRM = linear regression model, $y = \log_{10}$ $TCID_{50}$/ml, x = persistence in days.
[B]Estimated persistence (days) for a starting viral concentration of $1 \times 10^6$ $TCID_{50}$/ml water.
[C]Time (days) required to reduce the starting viral concentration by 90% (1 $\log_{10}$).

persist longest in cold freshwater (17 C, 0 parts per thousand [ppt]), with a slightly elevated pH (8.2) (9,11).

To date, evaluations of environmental persistence of wild-type AIVs have not included H5 or H7 AIVs. Understanding the potential for environmental persistence of these viruses is important because H5 and H7 AIVs can become highly pathogenic in domestic poultry, causing up to 100% mortality and substantial financial losses (15), and because some AIVs of the H5 and H7 subtypes have the ability to transmit directly from the avian host to humans (3,6,14).

AIV infections in wild birds are rarely associated with morbidity or mortality. In 2002, this paradigm was challenged when highly pathogenic avian influenza (HPAI) H5N1 viruses caused mortality in wild and captive birds in two waterfowl parks in Hong Kong (2,12). Though HPAI H5N1 viruses have been isolated from wild species of waterfowl throughout Eurasia since this initial outbreak, it is unknown if the viruses can be maintained in these avian populations. Information on the environmental persistence of HPAI H5N1 viruses would greatly improve our understanding of the natural transmission cycle of the viruses and the potential risk for maintenance of these viruses in wild waterfowl populations. Currently, there is limited information on persistence of any HPAI virus in aquatic habitats, and to our knowledge, this is the first study to evaluate environmental stability of HPAI H5N1 viruses circulating in Eurasia.

The goals of this study are as follows: 1) to experimentally determine the length of time that wild-type H5 and H7 AIVs and HPAI H5N1 viruses can remain infective in water; 2) to evaluate the effects of temperature and salinity on the duration of infectivity of AIVs; and 3) to compare the environmental persistence of HPAI H5N1 viruses recently isolated from Asia with wild-type H5 AIVs.

## MATERIALS AND METHODS

**Viruses.** The eight LPAI viruses used in this study were isolated from wild avian species within the orders Anseriformes and Charadriiformes. Specific viruses included: A/Laughing Gull/DE/AI00-2455 (H7N3), A/Mallard/MN/182761/98 (H7N3), A/Blue-winged Teal/TX/578597/02 (H7N4), A/Ruddy Turnstone/DE/650635/02 (H7N3), A/Ruddy Turnstone/NJ/828227/01 (H5N8), A/Mallard/MN/182742/98 (H5N2), A/Mallard/MN/355790/00 (H5N3), and A/Ruddy Turnstone/NJ/828219/01 (H5N7). The viruses were propagated in 9- to 11-day-old specific-pathogen-free (SPF) embryonated chicken eggs and infective amnio-allantoic fluid (AAF) was harvested after 96 hr postinoculation. The AAF was stored at −70 C. All trials were performed with low-passage isolates (second, third, or fourth passage). These experiments were conducted under biosafety level (BSL) 2 facilities at the College of Veterinary Medicine, The University of Georgia, Athens, GA.

The two HPAI H5N1 viruses evaluated in this study were obtained from the Southeast Poultry Research Laboratory (SEPRL), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), Athens, GA. Individual stocks of A/Whooper Swan/Mongolia/244/05 (H5N1) (Mongolia/05) and A/Duck Meat/Anyang/01 (H5N1) (Anyang/01) AIVs were prepared by second passage in embryonated chicken eggs as described above. Stock AAF of Mongolia/05 virus was diluted 1:20 in uninfected SPF AAF to approximate the Anyang/01 stock titer. All experiments with HPAI viruses were conducted under a USDA-certified BSL 3-Ag facility at SEPRL (1).

**Infectivity assays.** Infectivity of AIV in water samples was quantified using a microtiter endpoint titration as previously described (11). In this protocol, a 0.5-ml sample of AIV-inoculated water was diluted 1:2 by addition of 0.5 ml of 2× serum-free Eagle's minimum essential medium (MEM). Tenfold dilutions ($10^{-1}$ to $10^{-8}$) were then made in MEM supplemented with antibiotics (100 U penicillin G with 100 µg streptomycin sulfate/ml). Each well of a 96-well microtiter plate received 100 µl of cell suspension and 50 µl of the appropriate virus dilution. Cell suspensions were prepared as primary cultures of chicken embryo fibroblasts (CEFs) from 9- to 11-day-old SPF chicken embryos. Final cell suspensions consisted of $3 \times 10^6$ CEF/ml suspended in serum-free MEM supplemented with antibiotics. For LPAI virus trials, each well was then supplemented with 50 µl of MEM containing 2.8 µg/ml of highly purified trypsin (final concentration per well of 0.7 µg/ml). Preliminary studies conducted at SERPL determined that supplemental trypsin did not affect the titer of HPAI viruses using this assay (data not shown) and was therefore not required for HPAI virus trials.

For both LPAI and HPAI experiments, plates were covered and incubated at 37 C under 5% $CO_2$ for 96 hr. Examination for cytopathic effects was performed with light microscopy and, to confirm results, plates were stained with 1% crystal violet in 10% neutral buffered formalin (to inactivate virus) for further examination. Endpoints were recorded as 100% monolayer destruction with $TCID_{50}$ calculated as previously described (7). The minimal detectable limit of this assay is 2.2621 $TCID_{50}$/ml.

**Experimental procedures.** Infective AAF was diluted 1:50 in sterile glass-filtered distilled water that was previously adjusted to the following conditions. Three salinities (0, 15, 30 ppt) were evaluated, corresponding to fresh, brackish, and salt water, respectively (9). Adjustments in salinity were made with commercially available sea salt. The pH of all three water treatments was adjusted to 7.4 with a sterile 1 N solution of NaOH and stabilized with 2 mM HEPES buffer. The pH

Table 2. Linear regression models for persistence of H5 and H7 AIVs in water at 28 C.

| Viruses | Salinity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 ppt | | | 15 ppt | | | 30 ppt | | |
| | LRM[A] | $R^2$ | Estimated persistence[B] | LRM | $R^2$ | Estimated persistence | LRM | $R^2$ | Estimated persistence |
| LPAI viruses | | | | | | | | | |
| MN/98 (H5N2) | $y = 5.278 - 0.051x$ | 0.56 | 118 (20)[C] | $y = 4.691 - 0.098x$ | 0.78 | 61 (10) | $y = 5.310 - 0.214x$ | 0.96 | 28 (5) |
| MN/00 (H5N3) | $y = 4.767 - 0.071x$ | 0.60 | 85 (14) | $y = 5.650 - 0.108x$ | 0.90 | 56 (9) | $y = 6.184 - 0.279x$ | 0.93 | 22 (4) |
| NJ/01 (H5N7) | $y = 6.619 - 0.114x$ | 0.90 | 53 (9) | $y = 5.199 - 0.128x$ | 0.99 | 47 (8) | $y = 5.364 - 0.230x$ | 0.84 | 26 (4) |
| NJ/01 (H5N8) | $y = 4.379 - 0.167x$ | 0.75 | 36 (6) | $y = 4.352 - 0.143x$ | 0.99 | 42 (7) | $y = 4.071 - 0.143x$ | 0.75 | 42 (7) |
| MN/98 (H7N3) | $y = 5.490 - 0.086x$ | 0.81 | 74 (12) | $y = 6.409 - 0.205x$ | 0.92 | 29 (5) | $y = 6.306 - 0.188x$ | 0.84 | 32 (5) |
| TX/02 (H7N4) | $y = 6.054 - 0.100x$ | 0.82 | 60 (10) | $y = 6.006 - 0.320x$ | 0.83 | 19 (3) | $y = 5.934 - 0.209x$ | 0.89 | 29 (5) |
| DE/00 (H7N3) | $y = 5.668 - 0.090x$ | 0.89 | 67 (11) | $y = 5.744 - 0.233x$ | 0.97 | 26 (4) | $y = 6.258 - 0.269x$ | 0.97 | 22 (4) |
| DE/02 (H7N3) | $y = 5.611 - 0.252x$ | 0.99 | 24 (4) | $y = 5.153 - 0.242x$ | 0.89 | 25 (4) | $y = 5.080 - 0.231x$ | 0.93 | 26 (4) |
| HPAI H5N1 viruses | | | | | | | | | |
| Mongolia/05 | $y = 5.270 - 0.228x$ | 0.95 | 26 (4) | $y = 5.071 - 0.216x$ | 0.93 | 28 (5) | $y = 4.595 - 0.333x$ | 1[D] | 18 (3) |
| Anyang/01 | $y = 4.921 - 0.203x$ | 0.94 | 30 (5) | $y = 5.071 - 0.216x$ | 0.93 | 28 (5) | $y = 5.212 - 0.228x$ | 0.92 | 17 (3) |

[A] LRM = Linear regression model, $y = \log_{10}$ $TCID_{50}$/ml, x = persistence in days.
[B] Estimated persistence (days) for a starting viral concentration of $1 \times 10^6$ $TCID_{50}$/ml water.
[C] Time (days) required to reduce the starting viral concentration by 90% (1 $\log_{10}$).
[D] An $R^2$ value of 1 indicates models from trials in which virus was present above the minimal detectable limit on only two sequential assays.

of the water measured at the end of the trial did not vary by more than 0.2 for all evaluated water treatments. We previously determined that the inoculation of AAF into this system at a dilution of 1:50 or greater did not affect the duration of virus infectivity (data not shown). Aliquots of inoculated water (2.0 ml) were transferred to 2.5-ml glass tubes. The samples were then evenly divided and placed in environmental chambers at 17 C and 28 C. These values represent the winter and summer temperatures, respectively, for coastal marshland in Louisiana and were chosen to allow accurate comparison of data from this study to previous trials with non-H5 or -H7 subtypes (9). Starting at postinoculation day (PID) 0, aliquots were removed weekly, and titrations were conducted as described above, over a 60-day trial period. For three of the viruses (A/Mallard/MN/98 [H5N2]; A/Mallard/MN/98 [H7N3]; and A/Laughing Gull/DE/00 [H7N3]), water samples were also titrated on PID 120 and 180 to confirm long-term persistence of AIVs in this model system and to determine whether linear regression models continued to accurately describe the data. Weekly titrations for each virus/salinity/temperature treatment group were terminated when virus was not detected in the water on two consecutive assays. To account for the minimal detectable limit of this assay, a value of 2.26 $TCID_{50}$/ml was used for the first weekly titration point that virus was not detected in each trial.

**Statistical analysis.** Sequential data for each virus/salinity/temperature trial were $\log_{10}$ transformed and subjected to linear regression analysis using Microsoft Excel (Microsoft Office Excel 2003; Microsoft, Redmond, WA). The resulting models were used to determine the estimated persistence (days) for each virus/salinity/temperature trial with a starting viral concentration of $10^6$ $TCID_{50}$/ml. In addition, the time required for each virus to reduce infectivity by 90%, as evidenced by a decrease in titer by 1 $\log_{10}$/ml, was calculated for all salinity and temperature combinations. The persistence of virus in water was evaluated using analysis of variance (ANOVA) with a full factorial design including the fixed effects of temperature (17 C and 28 C), salinity (0, 15, and 30 ppt), and pathogenicity (high pathogenicity or low pathogenicity). The above analyses on the estimated persistence of virus were performed using the general linear models procedure of SAS® (SAS Inc., Cary, NC) (8).

## RESULTS

The linear regression models, $R^2$, and estimated persistence for each of the virus/salinity/temperature trials are summarized in Tables 1 and 2. Persistence was highly variable between viruses within the same subtype and between individual HPAI H5N1 viruses. Although ANOVA results indicated that the persistence of virus in water was reduced by both salinity ($F_{2,48} = 9.16$; $P = 0.0004$) and temperature ($F_{1,48} = 52.37$; $P < 0.0001$), a significant interaction existed between the effects of salinity and temperature ($F_{2,48} = 4.48$; $P = 0.0165$). Fig. 1 characterizes the nature of this interaction in which the effect of salinity on viral persistence is less at 28 C than at 17 C. The HPAI H5N1 viruses examined in this study did not persist as long as the wild-type AIV viruses ($F_{1,48} = 4.09$; $P = 0.0488$).

Linear regression models continued to appropriately describe the data from the three viruses that were assayed at 120 and 180 days (Fig. 2, Table 3). The coefficients of determination for the 6-mo data were higher for all three viruses in these long-term persistence models. In addition, the slopes from the 2-mo data and the 6-mo data did not vary by more than 0.01 for any of three viruses.

## DISCUSSION

Wild-type AIVs in ducks are thought to have evolved over time into the perfect host–parasite relationship (16). Within this reservoir, viruses are transmitted between ducks through water contaminated with feces. Prolonged infectivity of a virus in water would potentially enhance its transmissibility and, in such an indirect transmission cycle, it is conceivable that AIVs would adapt not only to the host but also to the aquatic environment.

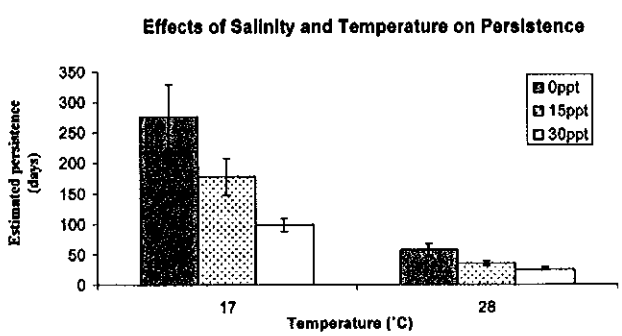


Fig. 1. The plotted interaction between the effects of salinity and temperature on estimated persistence.

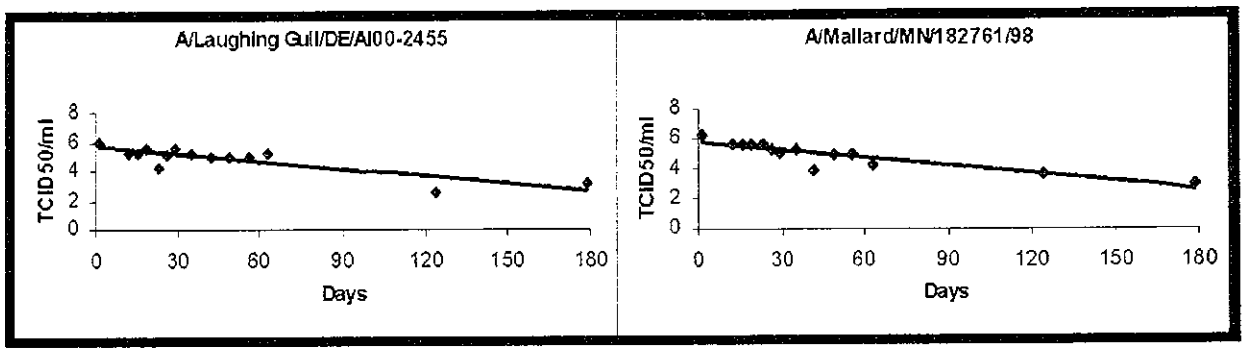


Fig. 2. Linear regression models for persistence of three wild-type AIVs in water (0 ppt; pH 7.4; 17 C).

The results of the water trials with wild-type H5 and H7 AIVs were consistent with previous studies on AIVs using the same model system (9,11). Though variability in duration of infectivity existed between H5 and H7 viruses of the same subtype, the estimated persistence values were comparable to results for other AIVs. As in previous studies, environmental persistence was inversely proportional to both temperature and salinity. Persistence was influenced by the interaction among the two effects as well, although the mechanism of this interaction is currently unknown. However, temperature strongly impacts many physiologic processes through its effects on enzyme activation and catalytic rates (18), and these temperature-dependent changes in enzyme kinetics may account for the observed interaction in our study.

The HPAI H5N1 viruses had a shorter persistence of infectivity in the environment than the wild-type AIVs. In general, the wild-type AIVs persisted longer than Mongolia/05 at all salinity and temperature combinations. However, the earlier H5N1 isolate (Anyang/01), persisted longer than six of the eight wild-type viruses at 15 ppt, and seven of the eight at 30 ppt. It is currently unknown why this virus has a prolonged infectivity at higher salinities, but this finding emphasizes the need to evaluate environmental persistence of any AIV (LPAI and HPAI viruses) under a variety of conditions.

The HPAI H5N1 viruses circulating in Eurasia exhibit varying biologic characteristics, including antigenic patterns, pathogenicity, and variation in the extent and duration of viral shedding (13). In experimental trials it has been demonstrated that unlike wild-type AIVs, replication of the HPAI H5N1 Asian strains is primarily associated with the respiratory tract. However, fecal shedding does occur and contact transmission has been demonstrated under experimental conditions (13). The extent and duration of fecal shedding to date suggests that it is less than would be expected with ducks infected with wild-type AIVs (16). The results of this study indicate that HPAI H5N1 virus isolates also differ in their environmental persistence profiles. Taken together, these results suggest that compared to wild-type AIVs, ducks infected with HPAI H5N1 viruses would shed less virus into the environment and that the viruses remain infective in those environments for a shorter duration of time. This may imply that the HPAI H5N1 viruses may not be as fit as the wild-type viruses to persist and transmit within wild bird populations. However the variation in salinity tolerance between the two H5N1 viruses indicates that much more information is needed before we can accurately predict the extent of environmental fitness within the diversity of aquatic habitats utilized by wild birds.

The coefficients of determination for some models in the 17 C trials (Table 1) were much lower than those obtained in previous studies using linear models to describe AIV persistence in water (9,11). Possible factors contributing to the reduced fit of these models include a small sample size, spurious laboratory results, or changes in the slope of viral persistence during the trial. As previously discussed, minimal variation was present between the slopes of viral trials examined at 2 mo and 6 mo, indicating that changes in slope are an unlikely cause for the low coefficient of determination. Spurious laboratory results could produce outliers in the data that would reduce the coefficient of determination. For example, the linear model for the persistence of MN/98 (H5N2) in 0 ppt water at 17 C has a coefficient of determination of 0.28. However, if one influential data point is omitted, the coefficient of determination increases to 0.72. The effect of outliers on the coefficient of determination is stronger in experiments with a small sample size, so including additional assays in future trials should improve the ability of the linear models to explain the data. This could be accomplished by performing dual titrations at each assay (11), sampling more frequently, or extending the trial period.

The results of this study indicate the following: 1) H5 and H7 AIVs have the ability to persist in water for extended periods of time; 2) persistence is highly variable between viruses within the same subtype and between individual HPAI H5N1 viruses; 3) an interaction exists between the effects of salinity and temperature; and 4) the two HPAI H5N1 viruses examined in this study do not persist

Table 3. Linear regression models for persistence of three wild-type AIVs in water (0 ppt; pH 7.4; 17 C) based on data collected for 2 mo and 6 mo.

| | 2-mo data | | | 6-mo data | | |
|---|---|---|---|---|---|---|
| Viruses | LRM[A] | $R^2$ | Estimated persistence[B] | LRM | $R^2$ | Estimated persistence |
| MN/98 (H5N2) | $y = 5.375 - 0.014x$ | 0.28 | 429 (71)[C] | $y = 5.228 - 0.010x$ | 0.55 | 600 (100) |
| DE/00 (H7N3) | $y = 5.469 - 0.009x$ | 0.15 | 667 (111) | $y = 5.663 - 0.017x$ | 0.73 | 353 (59) |
| MN/98 (H7N3) | $y = 6.075 - 0.028x$ | 0.70 | 214 (36) | $y = 5.757 - 0.017x$ | 0.83 | 353 (59) |

[A]LRM = linear regression model, $y = \log_{10}$ $TCID_{50}$/ml, x = persistence in days.
[B]Estimated persistence (days) for a starting viral concentration of $1 \times 10^6$ $TCID_{50}$/ml water.
[C]Time (days) required to reduce the starting viral concentration by 90% (1 $\log_{10}$).

as long as the wild-type viruses under conditions of low salinity (0 ppt). This is an important observation because freshwater habitats are of major importance to the biology of wild waterfowl, a recognized reservoir of AIVs. The ecology of AIV in the environment remains largely unknown, including the mechanism and molecular determinants of viral persistence. Though the environmental persistence of AIV has been largely overlooked up to this point, we feel it is vital to understanding the epidemiology of AIV and has important implications to human, wild animal, and domestic animal health.

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