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FAILURE OF TRANSMISSION OF LOW-PATHOGENIC AVIAN INFLUENZA VIRUS BETWEEN MALLARDS AND FRESHWATER SNAILS: AN EXPERIMENTAL EVALUATION

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ABSTRACT: In aquatic bird populations, the ability of avian influenza (AI) viruses to remain infectious in water for extended periods provides a mechanism that allows viral transmission to occur long after shedding birds have left the area. However, this also exposes other aquatic organisms, including freshwater invertebrates, to AI viruses. Previous researchers found that AI viral RNA can be sequestered in snail tissues. Using an experimental approach, we determined whether freshwater snails (*Physa acuta* and *Physa gyrina*) can infect waterfowl with AI viruses by serving as a means of transmission between infected and naïve waterfowl via ingestion. In our first experiment, we exposed 20 *Physa* spp. snails to an AI virus (H3N8) and inoculated embryonated specific pathogen–free (SPF) chicken eggs with the homogenized snail tissues. Sequestered AI viruses remain infectious in snail tissues; 10% of the exposed snail tissues infected SPF eggs. In a second experiment, we exposed snails to water contaminated with feces of AI virus–inoculated Mallards (*Anas platyrhynchos*) to evaluate whether ingestion of exposed freshwater snails was an alternate route of AI virus transmission to waterfowl. None of the immunologically naïve Mallards developed an infection, indicating that transmission via ingestion likely did not occur. Our results suggest that this particular trophic interaction may not play an important role in the transmission of AI viruses in aquatic habitats.

Key words: *Anas platyrhynchos*, avian influenza, Mallards, *Physa* snails, transmission, environmental persistence, water.

INTRODUCTION

Wild birds, especially species in the orders Anseriformes (waterfowl such as ducks and geese) and Charadriiformes (gulls and terns), are considered the natural reservoirs for avian influenza (AI) viruses (Stallknecht and Shane, 1988; Webster et al., 1992). Avian influenza (family *Orthomyxoviridae*, genus *Influenzavirus A*) viruses are transmitted among aquatic birds primarily through an indirect fecal-oral route involving contaminated water in aquatic habitats (Webster et al., 1992). Avian influenza virus infections in these birds are generally subclinical, but the birds can shed infectious virus into the environment for several weeks (Webster et al., 1978; Hinshaw et al., 1980; Alexander, 1993).

Avian influenza viruses can remain infectious in water for weeks to months; viral persistence in water is influenced by temperature, pH, salinity, and AI virus subtype (Stallknecht et al., 1990a, b; Brown et al., 2007). In addition, AI viruses have an affinity for suspended solids in the aquatic environment, and viruses that bind to these solids can remain viable longer and may accumulate in the sediment (Bitton, 1980). This environmental persistence of AI viruses in water allows transmission among waterfowl without direct contact (VanDalen et al., 2010; Lebarbenchon et al., 2011), but it also exposes other animals that share the aquatic environment to AI viruses.

Bioaccumulation refers to the accumulation of substances (e.g., viruses, bacteria, toxicants) in an organism through any exposure route, including respiration, ingestion, or absorption (Farris and Van Hassel, 2007). Freshwater aquatic invertebrates are likely exposed to infectious AI viruses, not only while waterfowl are

shedding, but as long as the virus remains viable in the environment. Bioaccumulation of virus by aquatic invertebrates may impact the ecology of AI viruses by creating a "reservoir" for the virus, thereby extending the infectious period beyond the seasonal shedding period by waterfowl. Zebra mussels (*Dreissena polymorpha*) bioaccumulated and maintained infectious AI virus (low pathogenic H5N1) in their tissues for 14 days (Stumpf et al., 2010). Similarly, Asiatic clams (*Corbicula fluminea*) bioaccumulated LPAI viruses in their tissues (Faust et al., 2009; Huyvaert et al., 2012), but ingestion of the tissues failed to transmit AI virus to Wood Ducks (*Aix sponsa*; Faust et al., 2009). No investigators have examined the potential role of aquatic snails in AI virus persistence and transmission via ingestion of infected invertebrates.

Tadpole snails (*Physa acuta* and *Physa gyrina*) are found throughout North America in virtually all freshwater habitats (Dillon et al., 2005; Turner and Montgomery, 2009). These snails feed exclusively on algae and detritus on the sediment (Dillon, 2000; Vaughn, 2009), and accumulate hepatotoxins through ingestion of cyanobacteria, which they likely transmit to predators (Zurawell et al., 1999). *Physa* spp. are prey for several aquatic bird species, including Black-bellied Tree Ducks (*Dendrocygna autumnalis*) and Mottled Ducks (*Anas fulvigula*; Bolen and Forsyth, 1967; Bielefeld et al., 2010). In addition, at least 51 species of aquatic bird, including Mallards (*Anas platyrhynchos*), Northern Shovelers (*Anas clypeata*), Ruddy Ducks (*Oxyura jamaicensis*), Red Knots (*Calidris canutus*), Herring Gulls (*Larus smithsonianus*), Great Black-backed Gulls (*Larus marinus*), and Glaucous Gulls (*Larus hyperboreus*) consume gastropods (Tinbergen, 1961; Ingolfsson, 1976; Siegfried, 1976; Swanson et al., 1985; Thompson et al., 1992; Cornell Laboratory of Ornithology, 2004).

Physa spp. share the aquatic environment with waterfowl, and it is likely that these snails are exposed to AI viruses that have been shed into the water by infected birds. Preliminary studies demonstrated that *Physa* spp. snails have sialic acid receptors capable of binding AI viruses on the intestinal epithelium, and that these snails can bioaccumulate AI viral RNA (Oesterle, 2011). We exposed tadpole snails (*P. acuta* and *P. gyrina*) to an AI virus to determine whether 1) AI viruses remain infectious after bioaccumulation in snail tissues and 2) AI viruses can be transmitted from infected Mallards to naïve Mallards via ingestion of snails harboring infectious virus.

MATERIALS AND METHODS

Experiment preparation

Snail collections: Tadpole snails (*P. acuta* and *P. gyrina*, $n=300$) were collected by hand from a private lake in Loveland, Colorado, USA on two occasions during August 2010. Snails were placed in 20-L buckets with lake water, transported to the National Wildlife Research Center in Fort Collins, Colorado, USA, and transferred to four 50-L aquaria (Marineland, Cincinnati, Ohio, USA). The snails were maintained for 8 mo and fed algae wafers (Hikari, Himeji, Japan) and leafy greens (lettuce or spinach) supplied ad libitum. Adult snails in the colony repeatedly laid eggs and numerous eggs hatched. Subsequently, the offspring of captive snails were retained and we used these offspring in our experiments.

Virus preparation: An AI virus isolate (A/H3N8/mallard/C0/2008) from a cloacal swab of an experimentally infected Mallard (originally collected from wild bird feces A/H3N8/mallard/CA/187718/2008) was propagated in embryonated specific pathogen–free (SPF) chicken eggs, resulting in a viral stock of 10^7 median egg infectious dose (EID_{50})/mL. The inocula used in the following experiments were made by diluting the viral stock in BA-1 (M199-Hanks' salts, 0.05 M Tris, pH 7.6, 1% bovine serum albumin, 0.35 g/L sodium bicarbonate, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 μg/mL amphotericin B) to a final concentration of 10^6 EID_{50}/mL.

Snail experiment

Preinoculation: One day prior to inoculation, 25 large (shells 9–11 mm in length) adult snails were transferred to three 4-L glass beakers

(two exposure beakers, $n=10$ snails/beaker, and $n=5$ snails in a single control beaker) and these were partially filled with 2 L of well water (collected from Colorado State University Foothills Fisheries Laboratory, Fort Collins, Colorado, USA). A spinach leaf and algae wafers were provided as food. The beakers were moved into a biosafety cabinet, and the snails were allowed to acclimate to this new environment for 24 hr.

Exposure and depuration: The two exposure beakers were inoculated with 2 mL of diluted AI virus stock (10^6 EID_{50}/mL) such that the beaker water had a final virus concentration of approximately 10^3 EID_{50}/mL (based on AI viral concentrations found in water of experimentally infected Mallards; VanDalen et al., 2010) and the control beaker was inoculated with 2 mL of BA-1 to serve as a negative control. The water in all three beakers was thoroughly stirred to create a homogeneous mixture.

Snails were maintained in these beakers for 72 hr. After this exposure period, individual snails were removed from the aquaria, rinsed by pipetting 10 mL fresh well water over them with an automatic pipette, and transferred to three clean glass beakers with 2 L of fresh well water and new food items. The transferred snails were allowed to depurate for 24 hr.

Sample collection: Water samples (two 1-mL samples/beaker) were collected at three time points: immediately after inoculation ("start-exposure water"), at the end of the exposure period (72 hr; "end-exposure water"), and at the end of the depuration period ("depuration water"). Additionally, snail tissues were harvested, and any egg sacs laid during depuration were opportunistically collected. The start-exposure and end-exposure water samples were stored at −80 C until testing and the depuration water, snail tissues, and egg sacs were stored at −20 C for 24 hr before testing.

Snails collected at the end of the depuration period were removed from the shell by cutting along the inner spiral of the shell until the body was freed from the shell. The shell-less snail tissue was placed in a microcentrifuge tube (Fisher Scientific, Pittsburgh, Pennsylvania, USA) with 1 mL BA-1 and a single 4-mm stainless steel ball bearing (Grainger, Fort Collins, Colorado, USA). The samples were stored on wet ice until processing. Once in the lab, tissues were placed in chilled racks (TissueLyser Adapter Set, Qiagen, Valencia, California, USA) and agitated for 10 min at 25 Hertz using a Mixer Mill homogenizer (MM301, Retsch, Newton, Pennsylvania, USA), followed by centrifugation at 10,000 × G for 3 min. The supernatant was then transferred to cryovials.

Sample testing: Samples ($n=37$) from the snail experiment, including aquarium water (start- and end-exposure [$n=1$/beaker]), depuration water ($n=2$/exposure beaker, $n=1$ negative control), snail tissues ($n=10$/exposure beaker, and $n=5$ negative control) and egg sac ($n=1$ from an exposure beaker) were tested for exposure to AI viruses. Assays (described below) included virus isolation (VI) in chicken eggs, hemagglutination assay (HA) on harvested allantoic fluid, and quantitative reverse transcriptase PCR (qRT-PCR).

Mallard experiment

Thirty day-old Mallard ducklings were purchased in April 2011 (Stomberg's Chicks and Game Birds, Pine River, Minnesota, USA). The birds were raised indoors for 4–8 wk (room size 3.8×3.7×2.6 m), fed commercially available chick starter feed, and provided with water enhanced with an electrolyte/vitamin powder (Durvet, Blue Springs, Missouri, USA) ad libitum in poultry waterers; pools for bathing were added to the pens when Mallards were 10 days old for enrichment. A pre-experiment blood sample (0.6 mL) and cloacal swabs (placed in 1.0 mL BA-1) were collected from each individual when the ducklings were 20 days old.

Inoculated group: Twelve Mallards were randomly selected to serve as the inoculation group. This cohort was divided into four subgroups of three birds each (three inoculated subgroups and one negative-control subgroup) and housed in four separate pens (2.6×2.2×2.1 m). A temporary wall (Zipwall®, Arlington, Massachusetts) was used to partition a portion of the room to house the negative-control birds. Each pen included a 60-L stock tank, a poultry waterer, and food bowls.

Simultaneously, four 45-L aquaria (200–300 snails/aquarium) were placed adjacent to the treatment pens, and the entire volume of water in each snail aquarium was replaced with fresh well water and food items. In addition, each pen/aquarium combination had a 200-L storage tank filled with fresh well water to be used to replenish ponds (Fig. 1). To facilitate water transfer from the ponds to the snail aquaria and from the storage tanks to the ponds, 12-volt pumps (LVM, Hoddesdon, Herts, UK) were placed in each Mallard pond and water storage tank.

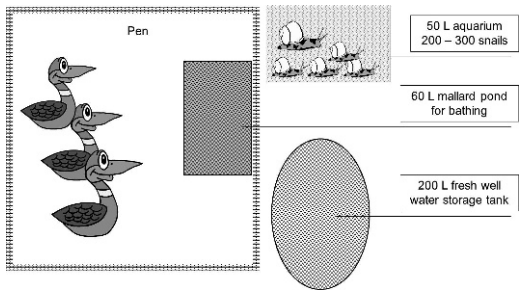

FIGURE 1. Mallard pen configuration with aquarium, duck pond, and water storage tank. After Mallards (*Anas platyrhynchos*) were inoculated with avian influenza virus, a portion of aquarium water was drained, pond water was pumped into the aquarium to replace the drained water, and water was pumped from the storage tank into the Mallard pond (2–7 days postexposure).

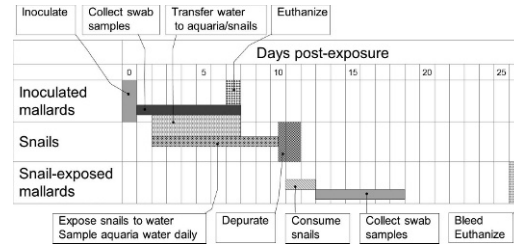

FIGURE 2. Experimental design of a Mallard (*Anas platyrhynchos*) snail-exposure experiment to test for transmission of avian influenza virus between Mallards via ingestion of *Physa* spp. snails. Timelines are for both groups of Mallards and snails.

Two days after being moved into the building, the treatment Mallards ($n=9$ in three pens) were inoculated with 1 mL of approximately 10^6 EID_{50}/mL of AI virus inoculum. The inoculum for each bird was divided between routes of exposure: each bird received a portion of the inoculum orally (0.6 mL), intranasally (0.2 mL), and intraocularly (0.2 mL). The three Mallards serving as negative controls were sham inoculated with 1 mL of BA-1 (Fig. 2).

Oropharyngeal and cloacal swabs were collected from all 12 Mallards on days postexposure (DPE) 2–7; swabs were placed in BA-1 and stored at −80 C. Water samples (1 mL) were collected from the Mallard ponds and aquaria daily during this period. After sample collection on DPE 7, all 12 Mallards were euthanized with an intravenous injection of Beuthanasia®-D Special (1 mL/kg; Schering-Plough, Summit, New Jersey, USA). The duck pens were then disinfected and remained empty for 4 days.

Snail exposure to virus: During the period the inoculated Mallards were being sampled, the snails were exposed to water from the Mallard ponds. On the Mallards' 2nd DPE, approximately 40 L of water was siphoned out of each aquarium. The aquaria were refilled with water pumped directly from the Mallard ponds. The Mallard ponds were refilled with clean well water from the storage tank. A similar process was repeated daily on DPE 3–7 except the water that was transferred into the aquaria was a 5:1 mixture of fresh well water and Mallard pond water. This adjustment was made because snails were observed moving out of the aquarium water to the rim of the aquaria, which was likely caused by the high concentration of nitrogen compounds in the Mallard pond effluent.

Snails were exposed in this way to the Mallard pond water for 8–9 days and then removed from the aquaria and rinsed with fresh well water. The food items were removed and the aquaria were disinfected with a 10% bleach solution, rinsed thoroughly, and refilled with fresh well water. The snails were placed back into the aquaria, fresh food was added, and the snails were allowed to depurate 24–36 hr.

Snail-exposure mallards: Twelve more Mallards (6 wk old) were randomly selected to serve as the snail-exposed group. As with the first cohort, these Mallards were separated into four sets of three animals each (three inoculated sets and one negative-control set) and housed in the same pens as the inoculated ducks.

Twelve hours after these Mallards were placed in the new pens, each was caught, restrained, hand fed 1 g of virus-exposed snails, and released back into the pen. The following day, 6–7 g of virus-exposed snails was offered in a bowl in each pen and the Mallards were allowed to free feed until the snails were consumed. At the time of feeding, 10 snails from each aquarium were retained to assess tissue concentrations of virus in the snails offered to Mallards. These snails were processed as described earlier.

Oropharyngeal and cloacal swabs were collected from all 12 Mallards on DPE 2–7; swabs were placed in BA-1 and stored at −80 C. At 14 DPE, blood was collected from all 12 Mallards (0.6 mL) and they were euthanized with an intravenous injection of Beuthanasia-D Special (1 mL/kg).

Sample testing: Oral and cloacal swabs, water samples, and snail tissues were temporarily

stored on wet ice and then frozen at −80 C prior to testing. These samples were tested by qRT-PCR for the influenza A virus matrix gene. To conserve resources, inoculated Mallard swab samples ($n=9$) were tested on alternating days; swab samples collected from four Mallards on DPE 2, 4, and 6 were tested, and swab samples collected from the remaining five Mallards on DPE 3, 5, and 7 were tested. All swabs collected from the snail-exposed Mallards, water samples from both Mallard cohorts, and snail tissue samples were tested by qRT-PCR. Blood samples (prescreen $n=30$, snail-exposed $n=12$) were centrifuged (10,000 × G for 5 min), and stored at −20 C for 24 hr until testing. Sera were evaluated for influenza A virus antibodies using a commercial blocking enzyme-linked immunosorbent assay (bELISA, FlockCheck AI MultiS-Screen antibody test kit, IDEXX Laboratories, Westbrook, Maine, USA)

Assays

We used published protocols (Szretter et al., 2006) for VI. In brief, embryonated SPF chicken eggs were incubated 10 days. Water and snail samples were diluted 1:1 in TBTB-33T (tris-buffered tryptose broth with antibiotics [kanamycin, gentamicin, nystatin, penicillin, and streptomycin]) and inoculated into eggs (100 μL/egg) in replicate (five eggs/sample). Positive (inoculate diluted 1:1 in TBTB-33T) and negative (TBTB-33T) controls were also inoculated into eggs. The eggs were incubated for an additional 5 days. Each day, eggs were inspected for signs of infection such as unresponsive embryo or degraded blood vessels. Allantoic fluid was harvested from dead or dying eggs 2–4 days postinoculation (DPI), and all eggs on 5 DPI (eggs that were dead by 1 DPI were discarded). The allantoic fluid was tested by HA using chicken blood (Rockland Immunochemicals, Gilbertsville, Pennsylvania, USA) and by qRT-PCR for AI virus RNA.

We used a qRT-PCR protocol developed at the National Veterinary Services Laboratories (United States Department of Agriculture, Ames, Iowa, USA). All samples were tested in duplicate. RNA was extracted using the MagMAX-96 AI/ND Viral RNA Isolation Kit (Ambion, Austin, Texas, USA). Primers and probe specific for the influenza type A matrix gene developed by Spackman et al. (2003) were used in conjunction with the ABI One-Step RT-PCR master mix and run on an ABI 7900 Real Time PCR Thermocycler (Life Technologies Corp., Carlsbad, California, USA) with thermocycler conditions developed by Agüero et al. (2007). Calibrated controls with known viral titers (10^2–10^5 EID_{50}/mL, or 0.5, 5, 50, and 500 EID_{50} per RT-PCR reaction) were included on each plate to construct four-point standard curves. Sample viral RNA quantities were interpolated from the standard curves and presented as PCR EID_{50} equivalents/mL. Samples were considered positive if both replicate results were $>10^1$ PCR EID_{50} equivalents.

The bELISA was performed according to the manufacturer's instructions. The absorbance values for the bELISA were read with a Benchmark Microplate Reader (Bio-Rad Laboratories, Hercules, California, USA) at 650 nm. The sample result to negative control (S/N) values were calculated for each sample; samples with S/N values ≥0.50 were considered negative for antibodies to AI virus, and samples with S/N values <0.50 were considered positive. The bELISA is designed so the color development is inversely proportional to the anti-AI antibody titer in the sample and an S/N value that is close to 1 will be most similar to the negative control.

RESULTS

Snail experiment

Tissue from 10% (2/20) of the virus-exposed snail samples caused egg death, and these tissues were positive by HA and by qRT-PCR (Table 1). All of the start-exposure (2/2) and end-exposure (2/2) water samples from both exposed beakers were positive by both VI and qRT-PCR. The mean titer of the start-exposure water was $10^{3.0}$ PCR EID_{50}/mL equivalents, and declined to $10^{2.0}$ PCR EID_{50}/mL equivalents in the second water samples. Only 50% (2/4; one sample from each exposed beaker) of the depuration water samples were VI and HA positive and none were qRT-PCR positive, suggesting the snails were possibly defecating AI virus at titers detectable by VI, but undetectable by qRT-PCR. All of the negative control samples were negative by VI and by qRT-PCR.

Mallard experiment

Prior to the experiment, all 30 Mallards were negative for AI virus antibodies by bELISA. All 12 inoculated Mallards became infected with AI virus as indicated by oral and cloacal shedding. Oral and cloacal swabs from the inoculated Mallards (2 and 7 DPE)

TABLE 1. Results of samples tested for two experiments. In the snail experiment, *Physa* spp. snails were directly expose to avian influenza (AI) virus. In the Mallard experiment, the inoculated Mallards were exposed directly to AI virus, the snails were exposed to the virus shed into the Mallard pond water, and the snail-exposed Mallards were fed the exposed snails.[a]

Sample type	No.		
	Tested	qRT-PCR+	VI+
Snail experiment			
Snail tissue	20	2	2
Aquaria water			
Start-exposure	2	2	2
End-exposure	2	2	2
Depuration	4	0	2
Mallard experiment			
Inoculated mallards			
Oropharyngeal swabs	27	27	NT
Cloacal swabs	27	27	NT
Pond water	18	18	NT
Aquaria water	30	30	NT
Snail tissue	30	4	NT
Snail-exposed mallards			
Oropharyngeal swabs	54	0	NT
Cloacal swabs	54	0	NT

[a] Negative control samples were excluded from this table. qRT-PCR = quantitative reverse transcriptase PCR; VI = virus isolation; NT = not tested.

had high titers of influenza A virus by qRT-PCR (Fig. 3 and Table 1). All pond water samples taken from pens of inoculated Mallards were positive for AI virus by qRT-PCR. Aquarium water samples were positive for AI virus RNA for all days between 2 DPE and 11 DPE. In addition, 13% (4/30) of the snail tissues exposed to the duck pond water were positive for AI viral RNA by qRT-PCR.

The swabs collected from snail-exposed Mallards were all negative by qRT-PCR and blood samples from these birds were negative by bELISA. However, two Mallards had an S/N change >0.25 between the pre- and postexposure blood samples, suggesting possible serologic activity in response to AI virus infection in these snail-exposed Mallards. The negative control Mallards had changes in S/N ratios of <0.05.

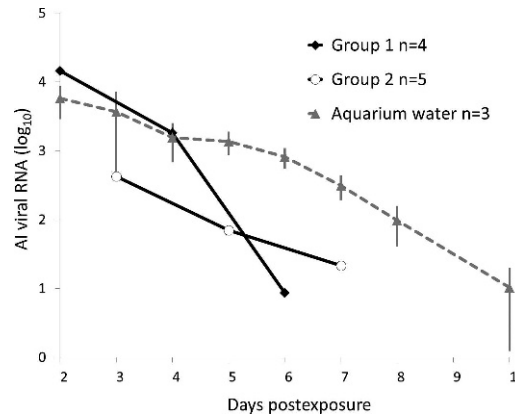

FIGURE 3. Avian influenza (AI) viral RNA detected in cloacal swabs from Mallards (*Anas platyrhynchos*) inoculated with AI virus via oropharyngeal cavity and in aquarium water transferred from the exposed Mallard ponds. Samples from two groups of Mallards were tested for AI virus on alternating days (group 1 on odd days and group 2 on even days, 95% confidence interval [mean±1.96SE] shown for aquarium water).

DISCUSSION

The prevalence of AI virus infection in North American waterfowl often follows

an annual cycle, peaking in late summer and declining throughout migration (Hinshaw et al., 1985; Stallknecht et al., 1990c). It is likely that multiple factors influence this cyclical pattern, including the differences in timing of migration among waterfowl species (Stallknecht and Brown, 2007). Early-migrating species, such as Blue-winged Teal (*Anas discors*), may help perpetuate transmission across seasons because they migrate prior to peak AI virus prevalence, leaving a significant portion of the Blue-winged Teal population unexposed and therefore susceptible to AI viruses during their northward migration (Stallknecht et al., 1990c; Hanson et al., 2005). Environmental persistence of the virus may allow transmission to these immunologically naïve waterfowl (Stallknecht et al., 1990b). However, other factors, such as the persistence of virus in aquatic invertebrates, may enhance environmental persistence, thereby contributing to the seasonal prevalence patterns of AI virus infection.

For aquatic invertebrates to play a role in AI virus transmission, these organisms need to retain infectious virus through bioaccumulation. The preliminary event in AI virus infection of a susceptible host is the binding of the virus to the host's cellular surface (Suarez, 2008); the hemagglutinin of AI viruses binds with cellular surface glycoproteins known as α2,3 sialic acid receptors (Wan and Perez, 2006). Previous research has demonstrated that *Physa* spp. snails have these sialic acid receptors, suggesting that *Physa* spp. snails are physiologically capable of bioaccumulating AI viruses (Oesterle, 2011).

Avian influenza virus persistence in water likely enhances viral transmission among waterfowl, but it also exposes other animals to the virus, including aquatic invertebrates. Bioaccumulation of AI viruses by these invertebrates could provide an additional route of exposure, particularly through ingestion of them as prey. Preliminary experiments showed that *Physa* spp. snails are capable of bioaccumulating AI viruses and maintaining infectious virus at low titers for at least 48 hr (Oesterle, 2011). Work reported here provides additional data suggesting that bioaccumulated AI viruses remain infectious in snail tissues and that Mallards will readily eat these snails. However, the quantity of snails fed to the Mallards may have been too low to produce viral shedding or a significant antibody response.

Mallards are important AI virus reservoirs and likely have a significant impact on the seasonal variation of AI virus prevalence in aquatic habitats like marshaling and breeding areas (Stallknecht and Shane, 1988). Mallards consume snails throughout the year, but snail consumption increases in spring, prior to the breeding season; 25% of the female Mallard diet consists of snails during this period (Swanson et al., 1985). The quantity of snails we fed to the Mallards was likely smaller than what some waterfowl consume regularly. Although no published data exist describing the volume of food consumed by wild Mallards, the recommended volume of feed for captive Mallards is 120 g/day (Ash, 1969). Assuming that wild Mallards eat as much food as captive Mallards, they may consume as much as 30 g of snails/day. The consumption of a larger quantity of virus-exposed snails, even at low viral titers, may increase the likelihood of transmission of AI virus among Mallards via ingestion of snails.

Although transmission via ingestion of AI-exposed snails did not occur in this study, two Mallards that fed on exposed snails demonstrated apparent serologic activity, whereas the Mallards fed on negative-control snails did not. All serum samples were considered negative for antibodies to influenza A virus, but the difference in the change of S/N value for two of the fed Mallards suggests that the concept of AI virus transmission by consumption of freshwater snails may be worth exploring further.

These experiments demonstrated that infectious AI virus is maintained briefly in

snail tissues, long enough for tissue samples to be infectious to embryonated eggs. However, transmission of an AI virus (H3N8) to Mallards through ingestion failed to occur. This study, along with previous experiments, demonstrates that, although snails may be capable of serving as a transmission conduit of AI viruses between infected and naïve waterfowl, the window of opportunity is likely short (Oesterle, 2011). This brief period of infectiousness would not likely have a substantial impact on transmission or maintenance of AI viruses. Nevertheless, snails may play a minor role in the maintenance of AI virus infections in wild waterfowl through waterfowl ingestion of large quantities of exposed snails. These questions and others regarding the optimal environmental conditions for virus persistence and transmission in aquatic environments warrant additional careful study under both laboratory and natural conditions.

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