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NO EVIDENCE OF INFECTION OR EXPOSURE TO HIGHLY PATHOGENIC AVIAN INFLUENZAS IN PERIDOMESTIC WILDLIFE ON AN AFFECTED POULTRY FACILITY

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ABSTRACT: We evaluated the potential transmission of avian influenza viruses (AIV) in wildlife species in three settings in association with an outbreak at a poultry facility: 1) small birds and small mammals on a poultry facility that was affected with highly pathogenic AIV (HPAIV) in April 2015; 2) small birds and small mammals on a nearby poultry facility that was unaffected by HPAIV; and 3) small birds, small mammals, and waterfowl in a nearby natural area. We live-captured small birds and small mammals and collected samples from hunter-harvested waterfowl to test for active viral shedding and evidence of exposure (serum antibody) to AIV and the H5N2 HPAIV that affected the poultry facility. We detected no evidence of shedding or specific antibody to AIV in small mammals and small birds 5 mo after depopulation of the poultry. We detected viral shedding and exposure to AIV in waterfowl and estimated approximately 15% viral shedding and 60% antibody prevalence. In waterfowl, we did not detect shedding or exposure to the HPAIV that affected the poultry facility. We also conducted camera trapping around poultry carcass depopulation composting barns and found regular visitation by four species of medium-sized mammals. We provide preliminary data suggesting that peridomestic wildlife were not an important factor in the transmission of AIV during the poultry outbreak, nor did small birds and mammals in natural wetland settings show wide evidence of AIV shedding or exposure, despite the opportunity for exposure.

Key words: Highly pathogenic avian influenza, low pathogenic avian influenza, peridomestic wildlife, RT-PCR, serology.

INTRODUCTION

Since the detection of highly pathogenic avian influenza viruses (HPAIV) on the west coast of North America in 2014, HPAIV has spread into the Central and Midwestern US with severe economic repercussions to the poultry industry during spring and summer, 2015. The primary virus involved in these outbreaks was HPAIV H5N2 (hereafter EA/NA H5N2). The EA/NA HPAIV was a reassortment between the Eurasian HPAIV H5N8 and North American low pathogenic avian influenza viruses (LPAIV) that commonly circulate in wild birds. The original H5N8 virus has also been found in a Midwestern poultry flock; however, EA/NA H5N2 predominated as the virus infecting poultry in the Central and Midwestern US (Lee et al. 2015; US Department of Agriculture, Animal and Plant Health Inspection Service [USDA APHIS] 2015).

Wild migratory birds have been implicated in the long-distance movement and reassortment of the EA/NA H5N2 over multiyear scales (Lee et al. 2015), yet there is no evidence to support a hypothesis that wild migratory birds are responsible for transmission to poultry (Clark and Hall 2006; Bui et al. 2016). Additionally, there are alternative explanations for the regional transmission and emergence of EA/NA H5N2 as an epidemic poultry disease. For example, peridomestic species such as European Starlings (*Sturnus vulgaris*), House Sparrows (*Passer domesticus*), rodents (e.g., *Peromyscus* spp., *Mus musculus*), raccoons (*Procyon lotor*), and others may be exposed at affected poultry facilities. These species are capable of epidemiologically linking wildlife with poultry, can be abundant at agricultural operations, and their role in the ecology of avian influenza is unknown.

We assessed the potential role of peridomestic wildlife species in the ecology of avian

influenza in a poultry epizootic. We sampled peridomestic wildlife for AIV and antibody to AIV at 1) a poultry farm that was affected with EA/NA HPAIV in April 2015, 2) a nearby poultry facility that was unaffected by any HPAIV, and 3) a nearby natural area. We also monitored wildlife visitation to poultry carcasses and manure composting barns using camera traps.

MATERIALS AND METHODS

Field sampling

We conducted capture and sampling of wildlife from 10–29 September 2015 and camera trapping from 8–14 October 2015. Sites were in Jefferson and Dane counties, Wisconsin, US and included a previously EA/NA H5N2-affected poultry facility, a poultry facility unaffected by HPAIV, and a natural area comprised of a complex of connected wetlands and interspersed upland habitats managed by the Wisconsin, US Department of Natural Resources. All sites were within 7.5 km of the previously HPAIV-affected poultry facility (geographic coordinates are not provided for confidentiality).

We conducted live trapping of small mammals using 7.6×7.6×22.9-cm Sherman traps (HB Sherman Traps, Inc., Tallahassee, Florida, USA). We set traps between 1500 and 1700 hours and checked them between 0600 and 0800 hours the following morning. We baited traps with approximately 10 g of mixed bird seed and cotton bedding and placed them along fencerows, poultry barns, feed silos, marsh edges, and in woodlots. We set 50–100 traps per trapping session and identified captured mammals to the finest taxonomic level possible using morphology. We anesthetized mammals via isoflurane inhalation in a static chamber at a maximum concentration of 8% isoflurane. Once under anesthesia as determined by nonresponse to a toe pinch, we collected a volume of blood up to 1% of body mass via retro-orbital collection (via capillary tubes that were immediately emptied into a serum separator tube; Becton Dickinson, Franklin Lakes, New Jersey, USA), maxillary/facial puncture (collected directly into a serum separator tube), or saphenous vein puncture (syringe contents immediately emptied into a serum separator tube). We also collected oral swabs for virus detection from mammals using sterile, Dacron-tipped swabs and placed them in viral transport media (VTM: Hanks Balanced Salt Solution, 0.05% gelatin, 5% glycerin, 1,500 U/mL penicillin, 1,500 μg/mL streptomycin, 0.1 mg/mL gentamicin, 1 μg/mL fungizone). We marked mammals with nontoxic paint behind an ear or on the belly fur and released them at the point of capture. We set traps for up to three consecutive nights. We did not collect blood and swabs from recaptured individuals. We moved traps to new areas of a site for any trapping subsequent to three consecutive nights to minimize recapture of individuals with a worn marking. Traps were dedicated to a single site. We disinfected traps after every capture and disinfected all traps before moving them to different locations within a site. We disinfected traps by soaking them for 5–10 min in 1% Virkon S (DuPont Animal Health Solutions, Wilmington, Delaware, USA) or 1% Maxima 256 (Brulin & Company, Inc., Indianapolis, Indiana, USA), rinsing in clean water, and allowing to air dry before next use.

We captured avian species using ladder traps (1.5×1.5×1.8 m) or mist-nets (9×2 m, 30-mm mesh; Bub 1995). The ladder traps were only used on poultry farms and baited with chicken feed and bread. They were set at dawn (0600–0700 hours), checked at least every hour, and closed by 1200 hours. We set mist nets among poultry barns, feed silos, other farm structures, and in apparent avian movement corridors on the edge of natural or peridomestic habitats. We set mist nets at dawn (0600–0700 hours), checked at least every 30 min, and closed them by 1200 hours. We collected oral-pharyngeal and cloacal swabs from captured birds using sterile, Dacron-tipped swabs and placed them in the same vial of VTM. We collected blood (≤1% of body mass by volume) via jugular venipuncture. We marked native birds with appropriately sized and uniquely identified bands. We marked nonnative birds (House Sparrows and European starlings) by clipping 2–3 cm from one of the outer tail coverts. All birds were released and we did not collect additional swab or blood samples from recaptured birds.

We collected oral-pharyngeal and cloacal swabs and blood from hunter-harvested Blue-winged Teal (*Anas discors*) and Green-winged Teal (*Anas crecca*) at the natural area during the Wisconsin early teal hunting season. We collected blood by cardiac puncture or from the body cavity of harvested birds. Oral-pharyngeal and cloacal swabs were placed in the same vial of VTM.

Camera trapping

We placed five trail cameras (Spartan SR1-BK, HCO Outdoor Products, Norcross, Georgia, USA) on the exterior of a compost barn on the previously affected farm from 8–14 October 2015. The cameras were motion activated and took color photos in daylight and used an infrared flash in the dark. We set the cameras to take one photo with a 60 s interval before another photo could be taken and set cameras 20–50 m apart

along the two sides of a barn adjacent to a crop field and a marsh. The compost barn was the active composting site of depopulated chickens from that site. We did not individually mark animals, thus we could not tell if animals recorded by different cameras in the same night or on different days were the same individual. If the same species was recorded within 10 min, we considered photographs from adjacent cameras to be the same individual. Otherwise, all photographs were considered separate animals.

Animal capture and sample collection procedures were approved by the National Wildlife Health Center Institutional Animal Care and Use Committee (EP 150722).

Sample analysis for active AIV shedding

We stored swab samples cool, on blue ice, while in the field and transferred them to a –80 C freezer within 12 h of collection until lab analysis. We extracted viral RNA from swabs using the MagMAX™-96 AI/ND Viral RNA Isolation Kit (Ambion, Austin, Texas, USA) following the manufacturer's procedures. We used real-time reverse-transcriptase (RT)-PCR using the published procedures, primers, and probe designed to detect the influenza matrix gene (Spackman et al. 2002). The RT-PCR assays used reagents provided in the Qiagen OneStep® RT-PCR kit (Qiagen, Valencia, California, USA). We considered samples with RT-PCR cycle threshold (Ct) values of $\leq$38 positive for influenza virus genetic material. We used a less stringent Ct cutoff to err on the side of more virus detection as opposed to the Ct value cutoff of 35 used in typical surveillance efforts (USDA APHIS 2006). We further analyzed swab samples exceeding our matrix gene Ct threshold by RT-PCR for H5 subtypes (Spackman et al. 2002) and by EA/NA HPAIV-specific RT-PCR assays.

Serologic analysis for AIV exposure

We separated the cellular components of the blood samples from serum by centrifugation in serum separator tubes (Becton Dickinson) and stored them at –30 C. We analyzed sera using the IDEXX FlockChek* MultiS-Screen blocking enzyme-linked immunosorbent assay (ELISA; IDEXX Laboratories, Westbrook, Maine, USA) according to the manufacturer's instructions (signal-to-noise ratio [S/N] $<$0.5 was considered positive). We tested sera that were positive for AIV antibodies by ELISA with sufficient volume for hemagglutination inhibition (HI; Pedersen 2008). We obtained inactivated EA/NA HPAI H5N2 (A/Tk/AR/7791/15) virus from the USDA, Southeast Poultry Research Laboratory (Athens, Georgia, USA). All sera were adsorbed with equal volumes of 0.5% chicken red blood cells prior to HI screening at a 1:10 dilution. We subsequently titrated HI-positive sera in the screening assay using serial twofold dilutions from 1:10 to 1:1,280 and recorded titers as the highest dilution demonstrating complete inhibition.

Statistical analysis

We estimated a true prevalence, π, and the probability, D, of π exceeding a threshold prevalence of 0.01 for each site and each type of data (viral detection and antibody detection) using a binomial model that accounts for diagnostic test specificity and sensitivity fit in a Bayesian framework (Joseph et al. 1995). We did not attempt to estimate sensitivity and specificity of the viral detection PCRs or antibody assays for avian or mammalian species, but rather specified them with informative priors based on manufacturers guidelines and published literature.

Priors for prevalence

We lacked information to derive priors for avian influenza shedding prevalence and antibody prevalence of peridomestic and resident wildlife (nonwaterfowl). Therefore, we used a vague informative prior for prevalence that corresponded to an a priori hypothesis that we were attempting to detect viral shedding and antibody detection prevalence of $>$1% across all sites in small mammals and nonwaterfowl birds (prior mean prevalence of 0.014 and 95% of the probability density falling between 0.0005 and 0.072). For hunter-harvested waterfowl, we used an informative prior for viral shedding prevalence with a mean of 0.08 and 95% of the probability density falling between 0.01 and 0.24 based on prevalence rates reported from previous large-scale surveillance for LPAIV and HPAIV (Bevins et al. 2014). Lastly, we used an informative prior for serologic exposure in teal with a mean antibody prevalence of 0.48 with 95% of the probability density falling between 0.36 and 0.59 based on antibody prevalence reported from wild Blue-winged and Green-winged teals sampled in Minnesota (Brown et al. 2010). Prior distributions and parameterizations are presented in the Supplementary Material (Table S1 and Figures S2–S4).

Priors for diagnostic sensitivity and specificity

We used informative priors for the diagnostic specificity and sensitivity of the matrix gene RT-PCR with a mean sensitivity of 0.73 (95% probability density 0.63–0.81) and mean specificity of 0.99 (95% probability density 0.96–0.99) based on laboratory trials with multiple AIV

subtypes in egg inoculations and application in large-scale epidemiologic analysis of migratory waterfowl (Spackman et al. 2002; Deliberto et al. 2009).

We used priors for serologic sensitivity and specificity in avian species based on infection trials in multiple bird species (Brown et al. 2009a). For avian species, we used a prior sensitivity for the antibody ELISA with a mean of 0.82 (95% probability density 0.73–0.89) and a prior specificity with a mean of 0.99 (95% probability density 0.96–1.0). The sensitivity and specificity of the antibody ELISA for avian influenza antibody has not been evaluated for exposure in wild mammals, but the assay has been used successfully in swine (Tse et al. 2012; Ciacci-Zanella et al. 2016). Hence, we used sensitivity and specificity priors with similar means and greater variability as the diagnostic characteristics in avian species (mean sensitivity [95% probability density]=0.83 [0.66, 0.95]; specificity=0.98 [0.94, 0.99]). Prior and posterior distribution visualizations are presented in the Supplementary Material (Table S1 and Fig. S1).

We conducted a sensitivity analysis of our priors to examine the level of influence our choice of distributions for diagnostic sensitivity and specificity had on our posterior probability estimates. We used Uniform (0.5, 1) distributed priors for sensitivity and Uniform (0.75, 1) priors for specificity of the matrix gene RT-PCR and the antibody tests for all groups of species (Table S2). These priors created an equal probability density for all values within their respective intervals and represented a vague-informative prior for the test characteristics, relative to the priors informed by the literature (Table S1). By comparing the posterior estimates generated by these priors with those generated using the more-informative priors described above, we could assess the ability of the prior to influence the results.

Inference on prevalence

We made inference using Markov-Chain Monte-Carlo (MCMC) and Gibbs sampling implemented in OpenBUGs using 50,000 iterations with 20% burn-in. We assessed MCMC convergence visually and by using the Gelman-Rubin statistic with 3 MCMC chains initiated with different starting values (Brooks and Gelman 1998; see Supplementary Material). We report the probability, D, that prevalence is at least 0.01,

$$D = \frac{1}{M}\sum_{m=1}^{M}\hat{\pi} \geq 0.01,$$

where M is the number of iterations of values drawn from the Gibbs sampler and $\hat{\pi}$ is an indicator function equal to 1 when π_m 0.01 and 0 otherwise. We defined a significant probability of viral shedding or exposure as $D \geq 0.90$. D can also be interpreted as the compliment of traditional freedom-from-disease hypothesis test with a minimum prevalence set at 0.01. In the event that $D \geq 0.90$, we reported the mean estimated prevalence, π, and 95% credible interval (CI) and the 95% upper credible limit of the posterior of π if $D < 0.90$.

Lastly, for each estimated prevalence we overlaid posterior density plots for π on prior density plots and visually compared the two distributions. If the posterior distribution was shifted away from the prior density, this indicated the data had informed the posterior estimates of π and that results were not based solely on the prior distribution.

RESULTS

We captured and collected samples from 22 species of small birds and seven species of small mammals (Table 1). We detected no evidence of viral shedding in any of the small mammals or wild birds sampled at the poultry facilities or at the natural area (Table 2). Therefore, posterior probability (D) of viral shedding prevalence being ≥ 0.01 in any of our peridomestic samples was <0.90 (Table 2).

We detected two antibody-positive *Peromyscus* sp. using the AIV ELISA, one on each of the previously affected and unaffected farms (S/N ratio: 0.452 and 0.489, respectively). Subsequent HI screening against the inactivated EA/NA HPAI H5N2 (A/Tk/AR/7791/15) yielded no reaction in either sample. We estimated the probability of prevalence ≥ 0.01 as $D < 0.90$ for each site. We did not detect antibody in peridomestic birds at either poultry facility nor in mammals or nonwaterfowl birds in the natural area ($D < 0.90$; Table 2).

We detected avian influenza matrix gene RNA from four of 22 hunter-harvested Blue-winged Teal and Green-winged Teal from the natural area and estimated a mean shedding prevalence of 0.15 (95% CI [0.05, 0.28]). No H5 subtype RNA was detected, indicating that these were low pathogenic non-H5 AIV infections.

Table 1. Summary of species captured during live-capture of birds and mammals at a highly pathogenic avian influenza virus-affected poultry facility, a proximate unaffected poultry facility, and a proximate natural wetland complex, 10–29 September 2015 in Dane and Jefferson counties, Wisconsin, USA.

	No. individuals captured		
	Unaffected layer facility	Previously affected layer facility	Natural area
Avian live capture			
American Goldfinch (*Spinus tristis*)	0	2	0
Black-capped Chickadee (*Poecile atricapillus*)	0	0	9
Cedar Waxwing (*Bombycilla cedrorum*)	1	0	0
Chipping Sparrow (*Spizella passerine*)	0	1	1
Common Yellowthroat (*Geothlypis trichas*)	0	0	4
Eastern Phoebe (*Sayornis phoebe*)	0	1	0
Gray Catbird (*Dumetella carolinensis*)	0	0	31
Gray-cheeked Thrush (*Catharus minimus*)	0	0	1
House Sparrow (*Passer domesticus*)	19	67	0
Magnolia Warbler (*Setophaga magnolia*)	1	0	1
Northern Cardinal (*Cardinalis cardinalis*)	0	0	1
Northern Waterthrush (*Parkesia noveboracensis*)	0	0	1
Palm Warbler (*Setophaga palmarum*)	0	1	0
Red-winged Blackbird (*Agelaius phoeniceus*)	0	0	3
Rose-breasted Grosbeak (*Pheucticus ludovicianus*)	0	0	1
Song Sparrow (*Melospiza melodia*)	0	0	1
Sora (*Porzana carolina*)	0	0	1
Swainson's Thrush (*Catharus ustulatus*)	0	0	5
Swamp Sparrow (*Melospiza georgiana*)	0	0	24
Tennessee Warbler (*Oreothlypis peregrina*)	0	1	0
Wild Turkey (*Meleagris gallopavo*)	3[a]	0	0
Yellow-rumped Warbler (*Setophaga coronata*)	0	0	1
Avian hunter-harvest samples			
Blue-winged Teal (*Anas discors*)	0	0	16
Green-winged Teal (*Anas crecca*)	0	0	6
Mammal live capture			
Eastern chipmunk (*Tamias striatus*)		1	9
Masked shrew (*Sorex cinereus*)	3	0	3
Meadow jumping mouse (*Zapus hudsonius*)	0	0	2
Meadow vole (*Microtus pennsylvanicus*)	2	0	0
House mouse (*Mus musculus*)	7	2	1
Peromyscus sp.[b]	49	45	63
Short-tailed shrew (*Blarina brevicauda*)	6	0	3

[a] Samples collected for viral isolation from feces.

[b] Species identity (*Peromyscus maniculatus* vs. *Peromyscus leucopus*) was not resolved.

Our prior sensitivity analysis indicated all our posterior estimates were insensitive to our choice of priors for sensitivity or specificity (Tables S2, S3), and our posterior distributions for all prevalence rates were shifted away from our prior distributions, indicating the data informed posterior estimates (Figs. S2–S4).

We detected antibody to AIV in 12 of 20 hunter-harvested teal with a mean estimate of 0.59 antibody prevalence (95% CI [0.48, 0.70]). Subsequent HI testing of AIV antibody-positive sera resulted in no reaction to the inactivated EA/NA HPAIV H5N2.

We recorded wildlife visitation to the poultry compost continuously for 6 d and 6

TABLE 2. Number of samples collected, diagnostic assay results, prevalence estimates, and probability (*D*) of prevalence of viral shedding or antibody detection to avian influenza virus >0.01. Table 1 contains a breakdown of species captured 10–29 September 2015 in Dane and Jefferson counties, Wisconsin, USA. na = not applicable.

	Viral detection (PCR)						Antibody detection			
	Swabs collected	MA[a]	*D*	π 95% CI or UCL[b]	H5[c]	icA[d]	Sera collected	AIV ELISA[e]	*D*	π 95% CI or UCL[b]
Unaffected layer facility										
Birds	24	0	0.51	0.043	na	na	18	0	0.52	0.046
Mammals	66	0	0.37	0.030	na	na	45	1	0.57	0.045
Previously infected layer facility										
Birds	74	0	0.35	0.029	na	na	73	0	0.33	0.027
Mammals	47	0	0.43	0.035	na	na	47	1	0.56	0.044
Natural area										
Birds	85	0	0.32	0.027	na	na	82	0	0.31	0.025
Mammals	78	0	0.34	0.028	na	na	67	0	0.35	0.028
Teal[f] (hunter harvest)	22	4	0.99	0.05, 0.28	0	0	20	12	1	0.48, 0.70

[a] Reverse transcriptase PCR for avian influenza matrix (MA) gene.

[b] 95% Credible interval on prevalence (π) reported when probability of detection at $\pi \geq 0.01$ was $D \geq 0.90$, otherwise 95% upper credible limit (UCL) if $D < 0.90$.

[c] Reverse transcriptase-PCR for avian influenza H5 subtypes performed only for MA-positive samples.

[d] Reverse transcriptase-PCR for avian influenza icA highly pathogenic subtype performed only for MA-positive samples.

[e] Enzyme-linked immunosorbent assay (ELISA) for avian influenza antibodies of any subtype.

[f] Combined samples of Blue-winged (*Anas discors*) and Green-winged (*Anas crecca*) teals (Table 1).

nights. We recorded raccoon, opossum (*Didelphis virginiana*), striped skunk (*Mephitis mephitis*), domestic cat (*Felis catus*), and House Sparrow activity adjacent to and on the compost pile. All mammals were photographed between 1730 and 0645 hours and House Sparrows only during daylight hours (approximately 0700 to 1800 hours). Raccoons were the most frequent visitor and were the only mammal to be recorded visiting in groups (Table 3).

DISCUSSION

There was no evidence that peridomestic species maintained transmission or experienced widespread exposure to the highly pathogenic EA/NA H5N2, or any avian influenza virus, on poultry farms. In addition, there was no evidence of active infection or exposure to any AIV in small birds or mammals in proximity to a natural area where there was evidence of active shedding of LPAIV in waterfowl. There have been few

TABLE 3. Number of visits and distribution of group size per visit recorded on remote cameras surrounding a poultry compost facility 8–14 October 2015 in Jefferson County, Wisconsin, USA.

		Group size per visit			
	No. visits	1	2	3	4+
Raccoon (*Procyon lotor*)	16	11	3	2	0
Opossum (*Didelphis virginiana*)	4	4	0	0	0
Skunk (*Mephitis mephitis*)	4	4	0	0	0
House cat (*Felis catus*)	2	2	0	0	0
House Sparrow (*Passer domesticus*)	4	0	0	0	Small flocks of 4–16 individuals

instances of wildlife sampled during or after outbreaks of HPAI at poultry facilities, but our negative results regarding viral shedding and antibody prevalence are similar to previous wildlife examined in association with HPAI outbreaks in poultry (Nettles et al. 1985). During the 2015 EA/NA H5N2 poultry outbreak, the USDA reported detection of the highly pathogenic EA/NA H5 in lung tissue from a European Starling with no detections from oral-pharyngeal or cloacal swabs (USDA APHIS 2015). Serologic sampling of peridomestic wildlife has been limited to the same few surveys, with no evidence of antibody reported by Nettles et al. (1985), and with USDA APHIS (2015) reporting detection of antibody to the EA/NA H5 influenza virus in three peridomestic birds. The single viral detection and three antibody detections reported by the USDA were all collected at one HPAIV-affected poultry facility, with no virus or antibody reported at other affected or control facilities (USDA APHIS 2015).

Shriner et al. (2012) detected serum antibody to LPAIV in wild small mammals in association with an infection at a captive gamebird facility. More broadly, infection and exposure of wild mammals and non-waterfowl avian species have been rarely reported in North America (Slusher et al. 2014) or worldwide (Takakuwa et al. 2013; Yamaguchi et al. 2014; Yu et al. 2014), although many species have demonstrated a capacity for infection, shedding, and serologic conversion in laboratory settings (Clark and Hall 2006; Brown et al. 2009a; Reperant et al. 2009; Romero Tejeda et al. 2015; Root et al. 2016). Although we found two AIV ELISA-positive *Peromyscus* sp., follow-up HI screening against the inactivated highly pathogenic EA/NA H5N2 was negative. While we concluded that there was no evidence of exposure to the poultry outbreak strain of HPAIV, we cannot confidently characterize the ELISA-positive *Peromyscus* as true exposures to any avian influenza viruses (vs. false positives) because of the uncertainty in the specificity of the ELISA, which was developed for birds and not for mammals (Table 2). To adapt this test for use with wild mammal samples, we chose to use the manufacturer's suggested criteria for positive cut-off value and adjust our analytic methods for inference about prevalence by adding variability to our diagnostic sensitivity and specificity criteria rather than adjusting the cut-off criteria (Tse et al. 2012). In addition, statistical analysis with less-informative priors and more variation in diagnostic specificity and sensitivity produced similar results (Table S2).

There was also no evidence that non-waterfowl wildlife in a nearby natural area were widely exposed to or infected with AIV, even with evidence of active shedding in waterfowl. We estimated that the shedding prevalence of avian influenza virus in Blue-winged and Green-winged teals was 5–28%. Blue-winged and Green-winged teals were the only duck species open to hunting during our sampling, but previous analysis of large-scale monitoring of duck species for avian influenza viruses suggests that shedding prevalence was likely similar in the numerically dominant Mallard (*Anas platyrhynchos*; Farnsworth et al. 2012).

Finally, we recorded a potential exposure route to poultry-derived influenza viruses in multiple medium-sized mammal species through their utilization of composted manure and carcasses. While we were not able to collect biologic samples to test for infection or exposure, raccoons and house cats have been confirmed with either active AIV infection or antibodies to AIV (Kuiken et al. 2004; Weber et al. 2007; Hall et al. 2008). In addition, the avian and mammalian species we sampled at the poultry facilities were primarily House Sparrows and *Peromyscus* mice; both are well adapted to commensal lifestyles with agriculture facilities.

The timing of sampling was restricted to 5 mo after the active infection of poultry was contained via depopulation, thus limiting our probability of detection. We do not know the extent to which exposure to HPAIVs in peridomestic species leads to infection, the extent to which disease follows infection across the range of species we sampled (but see Brown et al. 2009b), nor the dynamics of antibody response in peridomestic species.

Three hypotheses that could lead to reduced ability to detect evidence of exposure because of the timing of our sampling are that 1) detection probability was very small because HPAIV exposure caused mortality to peridomestic species, 2) any antibody response to exposure waned before our sampling, or 3) infection negatively impacted capture probability. Owing to the lack of information about AIV dynamics in peridomestic wildlife, we cannot evaluate the extent to which the first two factors may have impacted antibody detection. Even so, there were no reports of wildlife mortality during the extensive outbreak investigations (USDA APHIS 2015). However, given our low estimated prevalences, differential detection rates between infected and noninfected individuals would only minimally impact our estimates and do not affect our overall conclusions (Jennelle et al. 2007).

More-extensive surveys with better temporal resolution during an HPAI outbreak are needed to fully evaluate the role of peridomestic wildlife in an avian influenza epizootic setting. Nonetheless, our results provide preliminary data suggesting that peridomestic wildlife were not widely exposed nor were likely to be important in epizootic transmission of viruses during the poultry outbreak at this facility by showing any evidence of infection or exposure 5 mo after the outbreak. Additionally, small birds and mammals are unlikely to play an important role in AIV transmission in natural wetland habitats, despite the opportunity for exposure.

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SUPPLEMENTARY MATERIAL

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