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AVIAN INFLUENZA SHEDDING PATTERNS IN WATERFOWL: IMPLICATIONS FOR SURVEILLANCE, ENVIRONMENTAL TRANSMISSION, AND DISEASE SPREAD

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ABSTRACT: Despite the recognized importance of fecal/oral transmission of low pathogenic avian influenza (LPAI) via contaminated wetlands, little is known about the length, quantity, or route of AI virus shed by wild waterfowl. We used published laboratory challenge studies to evaluate the length and quantity of low pathogenic (LP) and highly pathogenic (HP) virus shed via oral and cloacal routes by AI-infected ducks and geese, and how these factors might influence AI epidemiology and virus detection. We used survival analysis to estimate the duration of infection (from virus inoculation to the last day virus was shed) and nonlinear models to evaluate temporal patterns in virus shedding. We found higher mean virus titer and longer median infectious period for LPAI-infected ducks (10–11.5 days in oral and cloacal swabs) than HPAI-infected ducks (5 days) and geese (7.5 days). Based on the median bird infectious dose, we found that environmental contamination is two times higher for LPAI- than HPAI-infectious ducks, which implies that susceptible birds may have a higher probability of infection during LPAI than HPAI outbreaks. Less environmental contamination during the course of infection and previously documented shorter environmental persistence for HPAI than LPAI suggest that the environment is a less favorable reservoir for HPAI. The longer infectious period, higher virus titers, and subclinical infections with LPAI viruses favor the spread of these viruses by migratory birds in comparison to HPAI. Given the lack of detection of HPAI viruses through worldwide surveillance, we suggest monitoring for AI should aim at improving our understanding of AI dynamics (in particular, the role of the environment and immunity) using long-term comprehensive live bird, serologic, and environmental sampling at targeted areas. Our findings on LPAI and HPAI shedding patterns over time provide essential information to parameterize environmental transmission and virus spread in predictive epizootiologic models of disease risks.

Key words: Avian influenza, ducks, environment, geese, highly pathogenic avian influenza virus, low pathogenic avian influenza virus, surveillance.

INTRODUCTION

Wild aquatic birds play an important role in the maintenance and spread of all 16 hemagglutinin and 9 neuraminidase subtypes of low pathogenic avian influenza (LPAI) viruses (Olsen et al., 2006), but their role in the transmission and spread of highly pathogenic (HP) AI has been widely debated. Replication of AI viruses in ducks occurs primarily in the intestinal tract, with high concentrations of infectious LP virus shed in feces (Webster et al., 1978). As a result, the local transmission of LPAI in wild birds likely depends on fecal/oral transmission via contaminated wetlands (Hinshaw et al., 1979, 1980), whereas geographic spread of AI viruses depends mostly on the patterns of viral shedding and the migratory behavior of infected birds (Lebarbenchon et al., 2009). Some species of wild birds may shed large amounts of HPAI H5N1 virus without exhibiting clinical disease (Tumpey et al., 2002; Keawcharoen et al., 2008), suggesting they could serve as long-distance carriers. However, HPAI viruses have been identified in migrating live birds only rarely (Chen et al., 2006; Gaidet et al., 2008). Despite the recognized importance of fecal/oral transmission, little is known about the length or quantity of AI virus shed by wild waterfowl into the environment and the importance of these factors in AI epizootiology.

Monitoring studies for AI in wild

waterfowl species have reported marked variations in the distribution of LPAI prevalence and subtypes among species, years, and geographic locations (Olsen et al., 2006; Munster et al., 2007; Wallensten et al., 2007; Parmley et al., 2008). Heterogeneity in LPAI virus detection from wild birds or the environment may be related to species- and age-dependent differences in shedding routes (cloacal vs. oral), shedding duration, virus titers, and other virus characteristics. Virus detection may also be affected by surveillance and monitoring protocols. Prevalence surveys have primarily used virus isolation and real-time reverse transcriptase polymerase chain reaction (rRT-PCR) of cloacal swab samples but have not usually considered the duration of virus shedding or differential detection among AI virus strains. Recently oral swabs have also been collected to enhance detection of HPAI H5N1 (Parmley et al., 2009; Pasick et al., 2010), which is shed primarily in the respiratory tract of wild birds (Sturm-Ramirez et al., 2004, 2005; Hulse-Post et al., 2005; Pantin-Jackwood et al., 2007).

Our goal was to evaluate shedding patterns of LPAI and HPAI in infected waterfowl (ducks and geese). This information is crucial to understanding the potential contribution of infected birds to environmental contamination, evaluating the potential spread of LPAI and HPAI viruses by migratory waterfowl, understanding the dynamics of AI epizootiology, and guiding future AI surveillance programs.

MATERIALS AND METHODS

Data sources

We based our evaluation on data from laboratory experiments that involved AI exposure studies in waterfowl species (wild and domestic ducks or geese) via nasal or oral inoculations (Table 1). We considered laboratory challenges where 1 to 15 individuals were inoculated with a virus isolated from wild birds (species in the orders Anseriformes and Pelecaniformes). Viruses were considered as HP or LP, based on their ability to cause disease in chickens (World Organization for Animal Health, 2010). Analyses on HPAI viruses included H5 and H7 subtypes, when not otherwise stated. All but one laboratory challenge used doses of $\geq 10^5$ 50% egg infective dose per ml of inoculum (EID_{50}/ml). We included only studies in which virus isolation was carried out in eggs. We used each experiment as one unit for the analysis (instead of an individual bird) because only a few publications provided bird-specific information on the course of the infection until death or recovery. For studies with individual-specific data, we pooled daily results from birds within each experiment. All statistical and modeling analyses (described below) were carried out in R 2.11.0 (R Development Core Team, 2010).

Shedding duration

In most experiments, virus titer or presence/absence of virus in swabs were determined at varying time intervals postinoculation (p.i.). This resulted in an infectious period that was right censored (birds were still shedding at the last sampling time) or interval censored (birds stopped shedding virus between two nonconsecutive sampling days). In addition, because the onset of shedding was not specifically known, we used the inoculation time as the initiation of shedding. As a consequence, shedding duration also includes the latent (noninfectious) period, which is negligible in wild birds (median duration: 1.2 days with LPAI and 0.3 day with HPAI; Hénaux et al., 2010). Because published challenge studies did not report individual shedding duration, we used the last day virus was isolated from any inoculated bird as the event; therefore the data consist of the maximum shedding duration per experiment. We determined the median duration of shedding using Kaplan-Meier survival analysis (Fay and Shaw, 2010). We used nonparametric bootstrapping with 1000 resamplings to evaluate 95% confidence interval. We used weighted logrank tests (Fay and Shaw, 2010) to evaluate the effect of virus pathogenicity (LP vs. HP), shedding route, and species (ducks vs. geese) on shedding duration.

Virus titer and viral shedding

We extracted, from published laboratory challenges, mean virus titer in cloacal swab, oral swab, and feces from infectious individuals at various times p.i. We focused on laboratory challenges conducted on ducks only; virus titer data from goose species were too sparse for separate estimates. To compare virus titers among studies, we used EID_{50}/ml as the standard unit of virus titer. HPAI titers reported in 50% tissue culture infective doses ($TCID_{50}$) per ml were converted

TABLE 1. Low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) virus subtypes, origin of virus isolates, laboratory species, and data sources included in our analyses.

Subtype[a]	Virus isolate[b]	Laboratory species	Data source[c]
LP H?N?	A/Duck/Alberta/19/1976	*Anas platyrhynchos*	1
LP H4N6	A/Duck/Alberta/24/1976		
LP H7N3	A/Duck/Alberta/49/1976		
LP H5N2	A/Duck/Alberta/57/1976		
LP H11N9	A/Duck/Memphis/546/1974		
LP H7N7	A/Duck/Victoria/1976	*Anas platyrhynchos*	2
LP H3N2	A/Duck/Hokkaido/5/1977	*Anas platyrhynchos*	3
LP H3N2	A/Duck/Hong Kong/24/1976	*Anas platyrhynchos*	4
	A/Duck/Hong Kong/315/1978		
	A/Duck/Hong Kong/7/1975		
HP H7N7	A/Goose/Leipzig/137-8/1979	*Anser anser*	5
	A/Goose/Leipzig/187-7/1979		
	A/Goose/Leipzig/192-7/1979		
HP H5N1	A/Duck/Hong Kong/y283/1997	*Anser anser*	6
	A/Goose/Hong Kong/w355/1997		
HP H5N1	A/Duck/Anyang/AVL-1/2001	*Anas platyrhynchos*	7
HP H5N1	A/Goose/Hong Kong/437-4/1999	*Anas platyrhynchos*	8
	A/Goose/Hong Kong/437-6/1999		
	A/Goose/Hong Kong/437-4/1999	*Anser anser*	
	A/Goose/Hong Kong/437-6/1999		
	A/Goose/Hong Kong/437-8/1999		
	A/Goose/Hong Kong/437-10/1999		
	A/Goose/Hong Kong/485-3/2000		
	A/Goose/Hong Kong/485-5/2000		
HP H5N1	A/Duck/Anyang/AVL-1/2001	*Anas platyrhynchos*	9
HP H5N1	A/Duck/Fujian/01/2002	*Tadorna tadorna*	10
	A/Duck/Fujian/17/2001		
	A/Duck/Guangxi/35/2001		
	A/Duck/Shanghai/35/2002		
	A/Duck/Zhejiang/11/2000		
HP H5N1	A/Goose/Hong Kong/739.2/2002	*Anas platyrhynchos*	11
	A/Rosybilled Pochard/Hong Kong/821/2002		
	A/Teal/Hong Kong/2978.1/2002		
HP H5N1	A/Mallard/Vietnam/16D/2003	*Anas platyrhynchos*	12
HP H5N1	A/Duck/Thailand/71.1/2004	*Anas platyrhynchos*	13
	A/Duck/Vietnam/40D/2004		
	A/Mallard/Vietnam/16D/2003		
HP H5N1	A/Duck/Shanghai/16/2004	*Tadorna tadorna*	14
	A/Duck/Shanghai/16/2004	*Anser anser*	
HP H5N1	A/Duck/Jiangxi/1653/2005	*Anas platyrhynchos*	15
	A/Duck/Jiangxi/1657/2005		
	A/Duck/Jiangxi/1657/2005	*Anser anser*	
	Bar-Headed Goose/QH/65/2005		
HP H5N1	A/Muscovy/Vietnam/453/2004	*Anas platyrhynchos*	16
HP H5N1	A/Egret/Hong Kong/757.2/2002	*Anas platyrhynchos*	17
HP H5N1	A/Wild Duck/Hunan/021/2005	*Anas platyrhynchos*	18
	A/Wild Duck/Hunan/211/2005		
HP H5N1	A/Whooper Swan/Mongolia/244/2005	*Anas crecca*	19
	A/Whooper Swan/Mongolia/244/2005	*Aythya americana*	
	A/Whooper Swan/Mongolia/244/2005	*Aix sponsa*	
	A/Whooper Swan/Mongolia/244/2005	*Anas platyrhynchos*	
	A/Whooper Swan/Mongolia/244/2005	*Anas acuta*	
	A/Duck meat/Anyang/2001	*Anas crecca*	
	A/Duck meat /Anyang/2001	*Aythya americana*	
	A/Duck meat /Anyang/2001	*Aix sponsa*	

Table 1. Continued.

Subtype[a]	Virus isolate[b]	Laboratory species	Data source[c]
	A/Duck meat /Anyang/2001	*Anas platyrhynchos*	
	A/Duck meat /Anyang/2001	*Anas acuta*	

[a] Virus pathogenicity (LP vs. HP) is based on their ability to cause disease in chickens (World Organization for Animal Health, 2010).

[b] Virus nomenclature follows Swayne (2008) and includes antigenic type, host animal from which the virus was isolated (omitted for human isolates), geographic origin of the isolate, unique laboratory reference identification number, and year of isolation.

[c] Data sources: 1=Webster et al., 1978; 2=Westbuty et al., 1979; 3=Kida et al., 1980; 4=Higgins et al., 1987; 5=Röhm et al., 1996; 6=Shortridge et al., 1998; 7=Tumpey et al., 2002; 8=Webster et al., 2002; 9=Tumpey et al., 2003; 10=Chen et al., 2004; 11=Sturm-Ramirez et al., 2004; 12=Hulse-Post et al., 2005; 13=Sturm-Ramirez et al., 2005; 14=Tian et al., 2005; 15=Chen et al., 2006; 16=Middleton et al., 2007; 17=Pantin-Jackwood and Swayne, 2007; 18=Li et al., 2008; 19=Brown et al., 2006.

to EID_{50}/ml using the conversion formula: $\log_{10} EID_{50}/ml = 0.5666 \times \log_{10} TCID_{50}/ml + 4.4206$ ($R^2=0.57$; based on data from Trani et al., 2006; Yen et al., 2007; Saito et al., 2009). Studies using other units to measure virus titer were not included in this analysis. We assigned a titer of $10^{0.9}$ EID_{50}/ml to titers reported as $<10^1$ EID_{50}/ml.

Daily change in virus titer was estimated using nonlinear least-squares regression (Pinheiro and Bates, 2000). We assumed that virus titer v_t on day t follows a loglogistic distribution f as $v_t=c \times f(t)$, where c scales the probability density function to the $\log_{10}$-transformed shedding data. Preliminary analyses using Gamma, Lévy, or Weibull distributions provided similar or worse fit to the data. Model fit was quantified by calculating the coefficient of determination R^2 (Motulsky and Christopoulos, 2004, pp. 34–35). We evaluated differences in shedding patterns between LP and HP viruses and route of shedding (oral vs. cloacal vs. feces) by comparing the scale, shape, and c parameters of the loglogistic models with a z test using the significance level $\alpha=0.05$. We considered that a significant difference for at least one of the distribution parameters (scale, shape, or c) indicated a significant difference in shedding patterns.

The product of the cumulative area under the estimated loglogistic cloacal titer model (v_t) and the mean daily fecal excretion rate (27 g in mallards; Andrikovics et al., 2006) predicts the total amount of virus shed in feces from infection until day t. We used cloacal titers because virus titers in feces were collected for only a few LPAI studies. To compare routes of virus shedding, we estimated the amount of virus shed orally by assuming 0.1 ml of oral secretions daily; we are not aware of specific data on the daily volume of oral secretions. We graphically estimated the period of detection (from each swab) as the maximum duration of virus shedding above the minimum detectable titer ($10^{0.8}$–$10^{1.0}$ EID_{50}/ml; Tumpey et al., 2002; Hulse-Post et al., 2005; Pantin-Jackwood et al., 2007).

Virus detection in oral and cloacal swabs

We estimated the relative ability of isolating LPAI and HPAI virus (from at least one bird in each experiment) from a cloacal swab [P(C+)] compared to an oral swab [P(O+)] using Bayes' Theorem:

$$\frac{P(C+)}{P(O+)} = \frac{P(C+|O+)}{P(O+|C+)}.$$

The conditional probability of isolating the virus from the cloacal swab *given that* it had been isolated from the oral swab P(C+|O+) was the number of times AI virus was isolated from both swabs divided by the number of times AI virus was isolated from the oral swab. Similarly, P(O+|C+) was the conditional probability of isolating the virus from the oral swab *given that* it had been isolated from the cloacal swab. The 95% confidence interval (CI) for the relative rate of virus isolation was calculated following Motulsky (1995, p. 285). LPAI data included several subtypes, but HPAI data included H5N1 subtype only.

RESULTS

Shedding duration

We found a median shedding duration of 10–11.5 days, with no difference (log-rank test: $P=0.28$) between oral and cloacal swabs from LPAI-inoculated ducks (Table 2). However, the upper limit of the

TABLE 2. Median estimated duration of shedding (with 95% confidence intervals) of low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) viruses. Estimated duration (in days) was derived from Kaplan-Meier survival curves (see text for details). Here 95% confidence intervals were estimated by nonparametric bootstrap (with 1,000 randomized samples).

AI virus	Species	Swab	n_{risk}, n_{events}[a]	Median shedding duration (95% CI)[b]	Data source[c]
LPAI	*Anas platyrhynchos*	Cloacal	11, 6	11.5 (9.0–18.5)	1–4
		Oral	11, 7	10 (9.0–12.5)	1–4
HPAI	*Anas platyrhynchos, Anas acuta, Anas crecca, Aythya Americana, Aix sponsa*	Cloacal	33, 22	5.0 (4.0–7.5)	5–15
		Oral	37, 25	5.0 (4.5–7.0)	5–16
	Anser anser	Cloacal	14, 9	7.5 (7.0–14.0)	7, 12, 17–18
		Oral	14, 10	7.5 (7.0–8.0)	7, 12, 17–18

[a] n_{risk} corresponds to the number of experiments, and n_{events} denotes the number of experiments within which all inoculated birds stopped shedding before the end of the experiment.

[b] Median shedding duration denotes the median time from virus inoculation to the day the last infectious bird in the challenge experiment stopped shedding.

[c] Data sources: 1=Webster et al., 1978; 2=Westbury et al., 1979; 3=Kida et al., 1980; 4=Higgins et al., 1987; 5=Alexander et al., 1986; 6=Tumpey et al., 2002; 7=Webster et al., 2002; 8=Chen et al., 2004; 9=Hulse-Post et al., 2005; 10=Sturm-Ramirez et al., 2004; 11=Sturm-Ramirez et al., 2005; 12=Tian et al., 2005; 13=Brown et al., 2006; 14=Middleton et al., 2007; 15=Pantin-Jackwood et al., 2007; 16=Tumpey et al., 2003; 17=Shortridge et al., 1998; 18=Röhm et al., 1996.

95% confidence interval was higher for cloacal than oral LPAI shedding (Table 2). Accordingly, the maximum reported shedding duration was markedly longer in cloacal (18 days; Higgins et al., 1987) than oral swabs (11 days; Higgins et al., 1987; Fig. 1).

We found shorter shedding durations of HPAI viruses in ducks (5 days) than geese (7.5 days; logrank test: oral swabs: $z=-2.54$, $P=0.011$; cloacal swabs: $z=-1.88$, $P=0.061$; Table 2). Median duration of shedding did not vary significantly between routes in ducks (logrank test: $z=0.29$, $P=0.768$) and geese ($z=0.87$, $P=0.384$). However, maximum shedding duration reported in the literature appeared to be longer in cloacal (17 days; Hulse-Post et al., 2005) than oral swabs (10 days; Sturm-Ramirez et al., 2004; Pantin-Jackwood et al., 2007; Fig. 1).

Virus titer and shedding patterns

Virus titers were modeled independently for LPAI and HPAI and for each shedding route. The loglogistic model provided a good fit to all data sets (Table 3). We found that cloacal and oral LPAI virus titers rapidly peaked after inoculation, with a maximum titer of about $10^{4.7}$ EID_{50}/ml in both cloacal and oral swabs at days 2–3 (Fig. 2). The period of detectable shedding (i.e., $\geq 10^1$ EID_{50}/ml) was slightly longer in cloacal (9 days) than oral swabs (7 days; c: $z=2.70$, $P=0.01$, scale: $z=0.78$, $P=0.44$, shape: $z=-0.74$, $P=0.46$). Our analysis indicated higher virus titers (maximum titer of about $10^{6.0}$ EID_{50}/g) and longer period of detection of AI in feces (21 days) than in cloacal (c: $z=-10.04$, $P<0.001$, scale: $z=-2.32$, $P=0.02$, shape: $z=0.60$, $P=0.55$) or oral swabs (c: $z=-12.26$, $P<0.001$, scale: $z=-2.97$, $P=0.003$, shape: $z=1.47$, $P=0.14$; Fig. 2). These comparisons should be considered as preliminary given the low sample size (Table 2). Based on a mean excretion of 27 g of feces per day per duck and a median infectious period of 11.5 days (Fig. 1), we estimated that an infected duck shed a total of $10^{6.4}$ EID_{50} of virus in feces. In contrast, the same individual shed about $10^{4.1}$ EID_{50} of virus via oral secretions (considering a median shedding duration of 10 days). Our model showed that ≥90% of viral shedding occurs through cloacal and oral routes within 3 and 4 days, respectively.

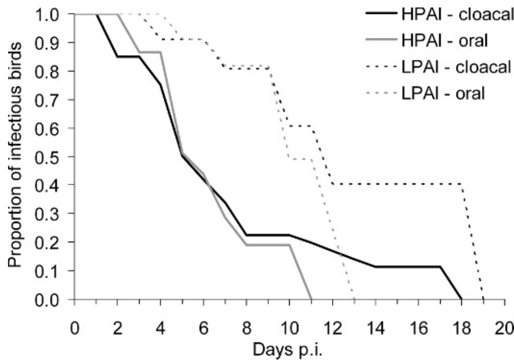

FIGURE 1. Predicted duration of shedding in ducks challenged with low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) virus. Median duration of shedding based on Kaplan-Meier survival analysis for interval-censored event time data (see text for details). Virus inoculation occurred at time $t=0$. Shedding duration by the cloacal and oral routes are represented in black and gray, respectively, in individuals challenged with LPAI (dashed lines) and HPAI (solid lines).

For HPAI, our analysis included only H5N1 AI due to the lack of information on virus shedding for ducks challenged with other subtypes. Maximum HPAI titers were about $10^{4.2}$ EID_{50}/ml in oral swabs at days 2–3 and $10^{2.0}$ EID_{50}/ml in cloacal swabs at days 3–7 (Fig. 2). We found significantly different patterns between the two routes of shedding (c: $z=2.51$, $P=0.01$, scale: $z=3.00$, $P=0.003$, shape: $z=-0.07$, $P=0.94$), with higher titers in oral swabs during the week following inoculation, but longer detection of H5N1 in cloacal swabs (14 vs. 7.5 days, respectively). We estimated that an infected duck sheds $10^{4.0}$ EID_{50} of HPAI viruses through feces and $10^{3.6}$ EID_{50} through oral secretions (considering a median shedding duration of 5 days). We found that ≥90% of viral particles were shed through cloacal and oral route within 13 and 3 days, respectively.

Virus detection in swabs

The likelihood of detecting LPAI viruses was higher in cloacal than oral swabs in ducks ($\frac{P(C+)}{P(O+)}=1.15$; 95% CI=1.12–1.18, $n=6$ experiments, 39 pooled days). The likelihood of detecting H5N1 HPAI virus was slightly higher in oral than cloacal swabs in ducks ($\frac{P(C+)}{P(O+)}=0.92$; 95% CI=0.89–0.95, $n=25$ experiments, 57 pooled days) but similar in both oral and cloacal swabs in geese ($\frac{P(C+)}{P(O+)}=1.00$; 95% CI=0.97–1.03, $n=11$ experiments, 27 pooled days).

DISCUSSION

Our analysis highlighted and quantified differences between LPAI and HPAI in shedding duration, virus titers, and total viral shedding during infection in challenged ducks and geese. LPAI shedding patterns in ducks were characterized by a peak in virus titers initially following

TABLE 3. Loglogistic shedding curve parameters and model fit for low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) shedding titers in ducks. Virus titer on day t was modeled as $s_t = c \times f(t)$, where c scales the loglogistic probability density function f to the $\log_{10}$-transformed shedding data.

	LPAI			HPAI	
	Cloacal	Oral	Feces	Cloacal	Oral
R^{2}[a]	0.97	0.97	0.74	0.66	0.93
No. experiments (no. days)[b]	4 (28)	4 (28)	2 (12)	20 (42)	20 (48)
c (±SE)	32.7±5.9	25.1±2.1	87.1±23.5	39.1±18.8	27.0±4.3
Scale (±SE)	4.5±0.8	3.7±0.2	8.9±2.9	11.3±5.6	3.9±0.6
Shape (±SE)	1.9±0.3	2.5±0.2	1.5±0.2	1.6±0.4	1.8±0.3
Data sources[c]	1	1	2	3–7	3–8

[a] Goodness of fit of the model (see methods for R^2 equation).

[b] Number of experiments and pooled number of days postinfection when swabs were collected and analyzed.

[c] Data sources: 1=Webster et al., 1978; 2=Kida et al., 1980; 3=Tumpey et al., 2002; 4=Chen et al., 2004; 5=Hulse-Post et al., 2005; 6=Sturm-Ramirez et al., 2005; 7=Tian et al., 2005; 8=Sturm-Ramirez et al., 2004.

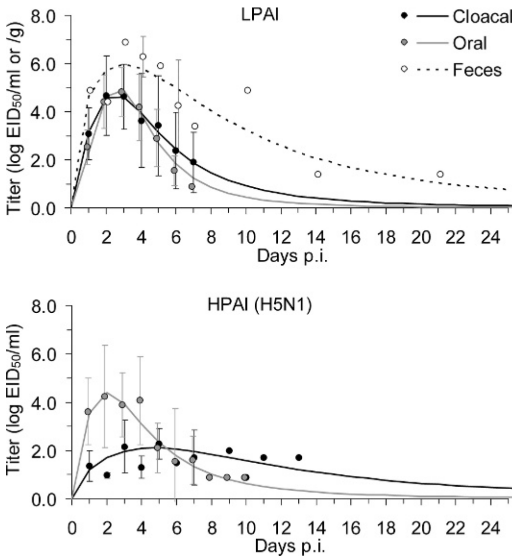

FIGURE 2. Predicted shedding curves in low pathogenic avian influenza (LPAI) or highly pathogenic avian influenza (HPAI) H5N1-challenged ducks. Dots represent mean daily titer (±SE) from published laboratory challenge data for cloacal swabs (black), feces (white), and oral swabs (gray). Lines represent shedding curves (based on a loglogistic model; Table 3) in cloacal swabs (black), feces (dashed), and oral swabs (gray). Cloacal and oral titers are in egg infective doses per ml of inoculum (EID_{50}/ml) and fecal titers in EID_{50}/g of feces.

infection, then lower virus titers for a median duration of 10 and 11.5 days through oral and cloacal routes, respectively. HPAI-infected ducks shed viruses for a median time of 5 days via both routes; however, we found a peak in oral virus titers initially followed by lower titers, compared with constant, relatively low cloacal titers. Median cloacal and oral shedding duration of HPAI viruses were longer in geese than ducks. Based on cloacal swabs from recaptured wild mallards, Latorre-Margalef et al. (2009) reported a maximum duration of LPAI infection of 8.3 days on average, which is close to our estimates from laboratory challenge data (which included the latent—noninfectious—period). We used our results from laboratory challenges to compare LP and HPAI viral shedding and assess potential environmental contamination in wild birds.

Following initial infection, LPAI-infected ducks may shed 150 times more virus through feces than ducks infected with a HPAI virus ($10^{6.4}$ vs. $10^{4.0}$ EID_{50}, respectively); the amount of HPAI virus shed into the environment may be further reduced if individuals die rapidly from HPAI. Our estimates assumed that infected ducks shed virus every day (i.e., not intermittently). However, shedding duration may vary among individuals within the same experiment, and intermittent shedding has been observed toward the end of the infectious period in laboratory challenges (Costa et al., 2010; Jourdain et al., 2010). We doubt this individual heterogeneity substantially alters our estimate of environmental contamination for LPAI given that ≥90% of shedding occurs within 4 days p.i. In contrast, because HPAI cloacal titers are constant over time (i.e., no initial peak; Fig. 2), the duration and frequency of HPAI shedding by any individual duck may influence environmental contamination. We stress that HPAI estimates, based on cloacal titers, provide only an initial evaluation of environmental contamination via fecal material. Although no experimental studies have specifically compared matched fecal and cloacal titers, reported LPAI virus titers were usually higher in feces than in cloacal swabs (Fig. 2; e.g., Webster et al., 1978; Kida et al., 1980). Considering a median bird infectious dose BID_{50} (i.e., concentration of viral particles that yields 50% probability of infection) of $10^{3.2}$ EID_{50} for LPAI viruses (ranging from $10^{1.7}$ to $10^{5.6}$ EID_{50} in domestic ducks and geese; Swayne and Slemons, 2008) and $10^{1.3}$ EID_{50} for HPAI viruses (ranging from $10^{0.95}$ to $10^{1.5}$ in ducks; Brown et al., 2007; Middleton et al., 2007), we found that the total amount of virus shed in feces represents about 1,500 BID_{50} for LPAI, and about 800 BID_{50} for HPAI. This suggests that environmental contamination, and therefore the probability of

exposing susceptible birds to AI viruses, is about two times higher per LPAI- than HPAI-infectious duck. In addition, shedding of HPAI viruses can be dramatically reduced in waterfowl with homo- or heterosubtypic immunity to HPAI viruses due to previous infections (Fereidouni et al., 2009, Berhane et al., 2010). Therefore, birds that have been previously exposed to a LPAI virus may shed less viral particles into the environment, reducing the rate of disease exposure for susceptible birds.

Dilution of viral particles in the environment may reduce the rate of direct virus transmission. Still, wetlands are likely critical to transmission because LPAI viruses may persist in water for extended periods of time depending on environmental conditions (Stallknecht et al., 1990a, b; Zarkov, 2006; Brown et al., 2007; Stallknecht and Brown, 2009; Nazir et al., 2010), and thus susceptible birds may become exposed to AI viruses even after the departure of infectious individuals. These results concur with models developed by Breban et al. (2009) and Rohani et al. (2009), which suggested that AI cannot persist in many wild bird populations without environmental transmission. The amount of virus shed by an infected individual is thus a key component of the dynamics of AI in wild bird populations, and our results showed a large difference in environmental contamination (adjusted to mean BID_{50}) between LPAI and HPAI. The lower environmental contamination and the shorter persistence of HPAI viruses in water compared to LPAI (Brown et al., 2007; Domanska-Blicharz et al., 2010) suggest that wetlands could be a less favorable reservoir for HPAI than for LPAI. Environmental contamination and local epizootic dynamics may be further influenced by host age (Costa et al., 2010) and species composition (Brown et al., 2006), population density, and ecologic factors that influence habitat use by waterfowl species.

Wild birds are responsible for the maintenance and spread of LPAI (Olsen et al., 2006), but their role in the spread of HPAI remains uncertain. Reperant et al. (2010) showed that the abundance of ducks was dependent on surface air temperature and that waterfowl movements associated with cold weather, and congregation of birds along the 0 C isotherm likely contributed to the spread and geographic distribution of HPAI H5N1 outbreaks in wild birds in Europe during the winter of 2005–06. Spatiotemporal dynamics of HPAI H5N1 outbreaks have also been linked to bird migration patterns (Prosser et al., 2009; Si et al., 2009). We estimated that HPAI-infected ducks and geese may shed virus for a median duration of 5 and 7.5 days, respectively. Migratory waterfowl can travel long distances within few days; however, there is currently no evidence of long-distance HPAI virus movement by individual wild birds. Accordingly, our results suggest that the longer infectious period, higher virus titers, and subclinical infections with LPAI viruses in wild birds favor the spread of these viruses in comparison to HPAI viruses.

Evaluating the potential role of wild avifauna in the transmission and spread of HPAI viruses has been difficult. Satellite telemetry has been used to evaluate temporal and spatial movement patterns for several waterfowl species and to relate these movement patterns to HPAI outbreaks in poultry or other wild avifauna (e.g., Newman et al., 2009; Gaidet et al., 2010; Gilbert et al., 2011; Prosser et al., 2011). However, these studies lack epidemiologic information on the duration of infection and shedding patterns that would help assess potential areas where wild birds become infected or their potential role in disease spread. We suggest that a combination of movement and epidemiologic studies could improve our evaluation of the spatiotemporal risk of disease spread by infected wild birds or help determine the likely areas of wild bird infection. Because the duration of AI shedding is relatively short (Fig. 2), meth-

ods such as satellite telemetry are needed to provide daily locations of marked individuals, as well as information on habitat use and potential interactions with other wildlife and domestic birds. The estimated amount of virus shed and specific habitats used during the course of HPAI infection could provide the basis for generating transmission risk maps for either contacting HPAI infections or spreading HPAI viruses to other wild and domestic birds. This approach could help identify geographic "hot spots" or key habitats with higher risk of HPAI virus transmission among migratory wild birds and domestic poultry. Such risk maps would facilitate the development of spatially explicit strategies for disease control and surveillance.

Surveillance to acquire baseline information on viruses circulating in wild birds and detect HPAI viruses has relied primarily on analysis of cloacal swab samples (Parmley et al., 2008; Deliberto et al., 2009; Dusek et al., 2009). Our analysis confirmed that cloacal swabs were slightly more efficient in detecting LPAI viruses than oral swabs. In contrast, oral swabs provided a slightly higher detection of HPAI H5N1 virus than cloacal swabs in ducks but similar detection in geese. Comparisons of virus detection from combined oral and cloacal swabs (i.e., pooled in the field or in the laboratory) versus cloacal swab have been conducted during recent surveillance of species in the order Anseriformes. It has been found that rRT-PCR analyses to detect the AI virus matrix gene demonstrated similar or improved performances in combined samples than in cloacal swabs only (USFWS/USGS, 2009; Parmley et al., 2011; H. S. Ip, R. J. Dusek, D. M. Heisey, pers. comm.). These preliminary findings suggest that surveillance activities should rely primarily on combined oral and cloacal samples to increase detection rates and reduce laboratory costs. However, further research based on virus isolation is needed to confirm these findings. Given the amount of virus shed into the environment by infected ducks and geese, we believe environmental monitoring (through sampling of feces, water, and sediments) constitutes an important complementary approach to detect viruses circulating in waterfowl populations (e.g., McLean et al., 2007). As funding for AI surveillance decreases, hypothesis-driven monitoring (Hoye et al., 2010) should be conducted to fill the existing gaps in AI dynamics in wild birds. In particular, combining live bird sampling, serologic surveys (e.g., De Marco et al., 2003), and environmental sampling at targeted wetlands would provide a comprehensive picture of AI epizootiology, by clarifying the role of the environment and prior immunity to influenza viruses. Our findings on both LPAI and HPAI shedding patterns over time provide critical information to parameterize environmental transmission and virus spread by migratory birds in epizootiologic models (e.g., Hénaux et al., 2010) and develop strategies of control of HPAI virus spread following a spillover from infected poultry.

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