Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion

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Understanding the transmission dynamics and persistence of avian influenza viruses (AIVs) in the wild is an important scientific and public health challenge because this system represents both a reservoir for recombination and a source of novel, potentially human-pathogenic strains. The current paradigm locates all important transmission events on the nearly direct fecal/oral bird-to-bird pathway. In this article, on the basis of overlooked evidence, we propose that an environmental virus reservoir gives rise to indirect transmission. This transmission mode could play an important epidemiological role. Using a stochastic model, we demonstrate how neglecting environmentally generated transmission chains could underestimate the explosiveness and duration of AIV epidemics. We show the important pathogen invasion implications of this phenomenon: the nonnegligible probability of outbreak even when direct transmission is absent, the long-term infectivity of locations of prior outbreaks, and the role of environmental heterogeneity in risk.

stochastic model | mathematical model | demographic stochasticity | waterfowl | epidemic

Avian influenza viruses (AIVs) are an important class of infectious agents, both as a model for the influenza viruses that infect millions of people each year and as a generator of the genetic variation that might give rise to a future pandemic strain (1, 2). In contrast to the dominant human strains (3–5), the dynamics, control, and management of transmission remain poorly understood even in historically prevalent low pathogenic avian influenza viruses (LPAIVs) (2, 6). Given the recent emergence of H5N1 highly pathogenic avian influenza virus (HPAIV) and its continued introduction into new territories with attendant impacts on domestic waterfowl, poultry, and human populations, a thorough understanding of influenza evolution and epidemiology takes on a new urgency (6, 7).

For many infectious diseases, transmission theory assumes that the majority of infections is caused by direct interactions between infectious and susceptible individuals (8, 9). The presence of additional transmission modes, particularly environmental transmission, gives rise to mechanisms that alter the conditions for pathogen invasion and persistence (10). Based on a number of lines of reasoning, we believe environmental transmission of LPAIVs occurs in natural populations:

1. *Environmental persistence of LPAIVs.* LPAIV persistence in incubations intended to mimic aquatic environments may last many months, depending on environmental conditions (Fig. 1; ref. 11). Importantly, infectivity of persistent viruses has been unambiguously demonstrated (12, 13). These experiments might explain the routine isolation of many AIV subtypes, including H5, from unconcentrated surface water (14), mud and soil swabs (15), and from aquatic environments where previous outbreaks have been documented (16).
2. *Studies in poultry farms.* Contaminated pond water and drinking water have been repeatedly implicated in farm outbreaks (17–19). Most tellingly, in one experiment, ducks reared under infection-free conditions became infected when placed in pens positioned in contaminated Minnesota marshes (20, 21).
3. *Natural history observations.* The high incidence of infection among juvenile birds—even very early in the breeding season—is inconsistent with direct contact transmission, which would require high frequency of early interactions between ducklings and nonsiblings (1).
4. *Epidemiological evidence.* Roche et al. (22) have argued that neither density- nor frequency-dependent direct transmission captured the observed pattern of infections during an outbreak of LPAIV in the Rhône delta, France. By contrast, they considered the predictions of a model including water-borne transmission and the data in strong agreement.

For these reasons, environmental transmission could be important in AIV epidemiology (23). It is known that indirect transmission chains, which the standard susceptibles–infectives–removals (*SIR*) theory does not account for, alter the characteristic timescale of the transmission cycle and patterns of long-term persistence (24). To understand how, we introduce the following simplified model for the within-season dynamics of a migratory waterfowl population and show that environmental transmission qualitatively changes the structure of an epidemic, with implications for invasion. In what follows, we study this model at parameterizations based on data from LPAIVs.

A Mixed Transmission Model

To simultaneously account for demographic stochasticity—crucial for understanding the probability of epidemic takeoff and extinction—and the large size of the environmental virus population, we adopt a hybrid dynamical system composed of a stochastic birth–death process for susceptible and infected birds, and an ordinary differential equation for virus kinetics. Denoting the number of susceptibles by S and infectives by I, we then define $p_{(m,n)}$ as the probability of m susceptibles and n infectives at time t to arrive at the Kolmogorov forward equation:

$$\dot{p}_{(m,\,n)} = p_{(m+1,\,n-1)}\left[\beta(m+1)(n-1)+\frac{\rho}{L}(m+1)\frac{V}{V+\kappa}\right] - p_{(m,\,n)}\left[\beta mn+\frac{\rho}{L}m\frac{V}{V+\kappa}+\gamma n\right]+p_{(m,\,n+1)}[\gamma(n+1)], \quad [1]$$

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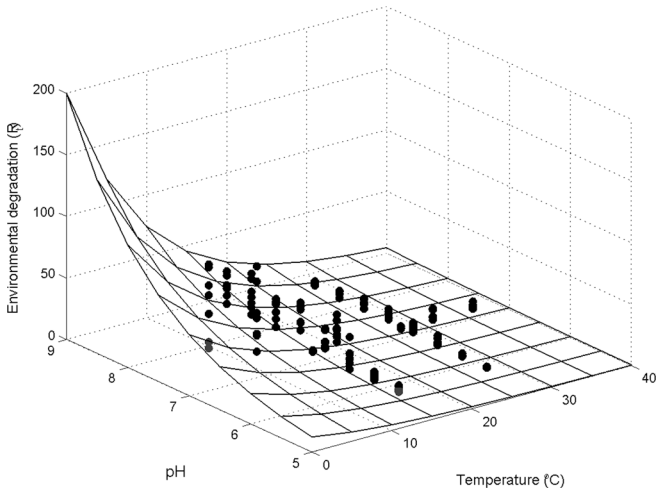

Fig. 1. Effects of pH and temperature on the environmental persistence time of H2N2 isolates, as measured by R_t (11). The quantity R_t denotes the number of days required for viral abundance to decline by one $\log_{10}$ unit (12) and is directly related to our model parameter η in Eq. **1**: $\eta = \log_e 10/R_t$. The surface represents a regression model fit to the data.

where β gives the rate of any direct (fecal/oral) transmission, ρ is the per capita consumption rate and is scaled by the lake volume, L, and γ is the recovery rate of infectives. The constant host population is given by $S + I + R = N$, where R denotes the number of recovered birds. The environmental transmission term is the product of the consumption rate of lake water by susceptible birds ($\frac{\rho}{L}S$) and the probability that the concentration of virus consumed results in infection ($\frac{V}{V+\kappa}$), where κ is a shape parameter that determines infectious dose (25). This formulation highlights the two timescales presumed to be involved in transmission. On one timescale, transmission may be considered as essentially direct because the force of infection scales with both the duration of shedding and the abundance of infectious birds (26). On a longer timescale, susceptible birds chronically encounter infectious particles that persist in the environment. These encounters occur at a low rate that scales with the abundance of the environmental reservoir (24).

The stochastic dynamics of susceptibles and infectives are coupled to virus concentration (V) by using the following differential equation:

$$\dot{V} = \omega I - \eta V, \qquad [2]$$

where ω is the rate at which infecteds shed virus and η is the decay rate of virus in water. Empirical estimates of all parameters are presented in Table 1. We obtain from the mean field equations for this model an expression for the basic reproductive ratio, R_0 (see supporting information *(SI) Appendix*):

$$\begin{aligned} R_0 &= \frac{\beta S_0}{\gamma} + \frac{\omega\rho S_0}{L\kappa\eta\gamma}, \\ &= R_0^{\text{direct}} + R_0^{\text{env}}. \end{aligned} \qquad [3]$$

This expression separates the contributions of direct transmission (βSI) and environmental transmission ($\frac{\rho}{L}S\frac{V}{V+\kappa}$) and shows that the critical value ($R_0 > 1$) can be achieved even when R_0^{direct} is less than unity. The implication is that the pathogen may persist even when active infections are no longer present.

Results

Epidemic Curve. By decomposing the infected class into indirectly and directly infected fractions, we show in Fig. 2 how the relative contributions of direct (dark-blue line) and environmental (green dash-dot line) transmission change over the course of an outbreak in the mean field model. We see the effects of environmental transmission are most pronounced during epidemic takeoff and the epidemic tail (Fig. 2 *Insets*). Specifically, when there are few infectious birds at the start of an epidemic ($I_0 < \frac{1}{\beta}\frac{\rho}{L}\frac{V_0}{V_0+\kappa}$), transmission due to the consumption of contaminated water dominates epidemic takeoff. Nonetheless, the timing and the size of the epidemic peak are determined by direct transmission. Finally, the decline in the epidemic is initially proportional to the decline in direct transmission, but the final decline scales with the long tail of environmental transmission.

Thus, we conclude that AIV invasion success is substantially altered by the inclusion of environmental transmission.

Implications for Pathogen Invasion. To assess the invasion consequences of environmental transmission, we study the probabilities of outbreak under a range of conditions. We solve the full hybrid model by using Gillespie's direct method (27) for Eq. **1**, updating V after each event according to Eq. **2**. In Fig. 3*A*, we show that when $R_0^{\text{direct}} < 1$, environmental transmission can boost R_0, resulting in successful AIV invasion. Within the deterministic direct transmission framework, $\beta < \gamma$ would guarantee the failure of the pathogen to invade. Similarly, within a stochastic direct transmission setting, the likelihood of invasion in this region would be very small. Hence, the region of positive probability in Fig. 3*A* to the left of the black line is solely attributable to the effects of environmental transmission. We explore the mean length of environmental transmission chains in Fig. 3*B*, which demonstrates that when $R_0^{\text{direct}} < 1$, environmental transmission consistently gives rise to small outbreaks, typically with <10 infected birds in our population. However, these sparks may spasmodically lead to much larger epidemics, amplified by direct transmission events (Fig. 3*C*).

Next, we ask how the persisting environmental pool of virus, which results in ongoing exposure of susceptibles to pathogens, affects outbreak probability after an epidemic. For instance, we envision an epidemic among arriving migrants sparked by the residual environmental reservoir. Accordingly, we study how the probability of a new outbreak changes in the ensuing months. This is equivalent to studying the early phases of the dynamics when the initial conditions are given by $S(0) = N, I(0) = 0$ and $V(0) = V_0 > 0$.

Three findings emerge (Fig. 4*A*). (*i*) In small lakes, there is a noticeable chance of a large secondary outbreak, even in the absence of infected birds initially. (*ii*) Environmental transmission represents a lesser problem in large lakes because of dilution. (*iii*) Finally, distinguishing between likely (mean) and extreme (99th percentile) scenarios reveals a disparity of two orders of magnitude between the volume of lake at which secondary outbreaks are unexpected (the median) and where they occur with a small but meaningful (1%) probability.

To characterise the role of initial virus density (V_0) in this scenario, we plot probability contours of outbreaks that infect 10% or more of the population (Fig. 4*B*). These contours are flat with respect to V_0, implying that the environmental reservoir of virus may represent a long-term source of infections irrespective of the interval before migrants arrive. Despite the indifference of contours to V_0 over many orders of magnitude, initial virus concentration determines the predicted distribution of epidemic sizes (see Fig. 4*B Insets*).

Discussion

Our work has shown that the mixture of direct and environmental transmission characteristic of avian influenza viruses gives rise to transmission chains that are unaccounted for in standard *SIR* theory. Although a subtle component of a primary outbreak, these

Table 1. A list of the model parameters, along with their biological description, the values or ranges explored, and the references for these choices

Parameter	Biological description	Value (range)	References
N	Host population size	10^4	
β	Direct transmission rate	0.0078 (0.002–0.01) year^{-1}	22
$1/\gamma$	Infectious period	7 days	1
ρ	Consumption rate	10^4 (10^3–1.5×10^4) liters/year	36
$1/\eta$	Persistence	30 (4–90) days	11
L	Lake volume	10^8 (10^7–10^{12}) liters	
ω	Shedding rate	10^{12} EID_{50}/year	1
κ	Infection shape parameter	10^2 (1–10^3) EID_{50}	37

environmental transmission chains could play a significant role in generating secondary outbreaks and in the persistence of LPAIV. A similar role for environmental transmission has been proposed for other systems. For example, outbreaks of the Nuclear Polyhedrosis Virus in forest defoliators such as the Gypsy Moth have been shown to be sparked by environmental contamination of egg masses (28). In cholera, the environmental reservoir is considered to affect mainly long-term persistence, with a limited transmission contribution during violent epidemics (29).

An interesting question raised by results shown in Fig. 3 is why the probability of outbreak observed in simulations is negligible in a large neighbourhood around the $R_0 = 1$ isocline. We conjecture that this phenomeon is a manifestation of the J to U transition in the distribution of the final outbreak size of stochastic epidemics in finite populations, as has been previously demonstrated for the stochastic *SIR* epidemic by Ball and Nåsell (30). Consider a stochastic model with two processes, infection $\{S \to S-1, I \to I+1\}$ at rate βSI and removal $\{I \to I-1\}$ at rate γI, and initial conditions S_0 and $I_0 = 1$. Until an infection occurs, the expected number of infecteds is

$$\langle I \rangle = I_0 e^{-\gamma t}.$$

Thus, the expected number of locally derived (second generation) infections is approximately

$$\int_0^\infty \langle \beta SI \rangle dt = \beta S_0 I_0 \int_0^\infty e^{-\gamma t} dt = \frac{\beta S_0 I_0}{\gamma} = R_0^{\text{direct}} I_0. \quad [4]$$

Now consider an infection process $\{S \to S-1, I \to I+1\}$ at rate $(\rho/L)SV/(V+\kappa)$, where V satisfies Eq. **2**, with initial conditions $S_0, I_0 = 0$ and V_0 (i.e.,the environmental component of our mixed transmission model). At all times before the first infection, the virus concentration is approximated by

$$V = V_0 e^{-\eta t}.$$

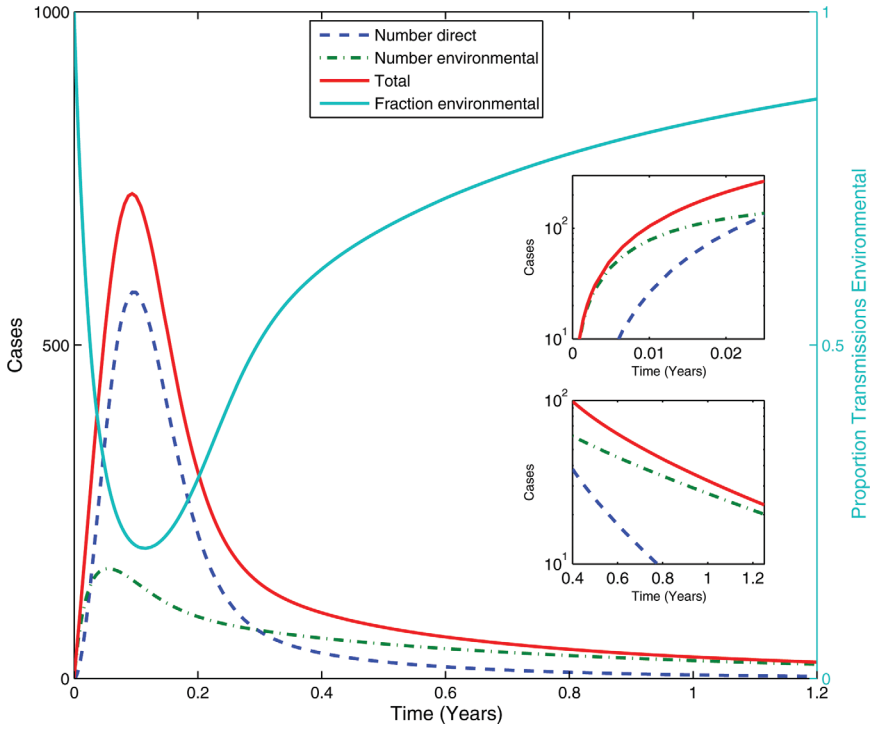

Fig. 2. Illustration of an epidemic, assuming an entirely susceptible population ($S_0 = 10^4$), with a single infected bird and 10^2 virus in the environment (measured in EID_{50} per liter). *Inset* plots show details of the initial (*Upper*) and final (*Lower*) phases of the epidemic (note logarithmic scale on *y* axis). Epidemic takeoff and tail are shown to be determined by environmental transmission (green dash-dot line). Model parameters were $\kappa = 10^2$, $\omega = 10^{12}$ per year, $1/\eta = 30$ days, $\rho/L = 1$ per year, $\beta = 0.006$ per year per individual, $1/\gamma = 7$ days. These parameters result in $R_0^{\text{direct}} = 1.15$ and $R_0^{\text{env}} = 1.6 \times 10^7$. Detailed descriptions of model parameters and sources for their numerical values are presented in Table 1. The figure was generated by integrating the mean field equations, described in *SI Appendix*.

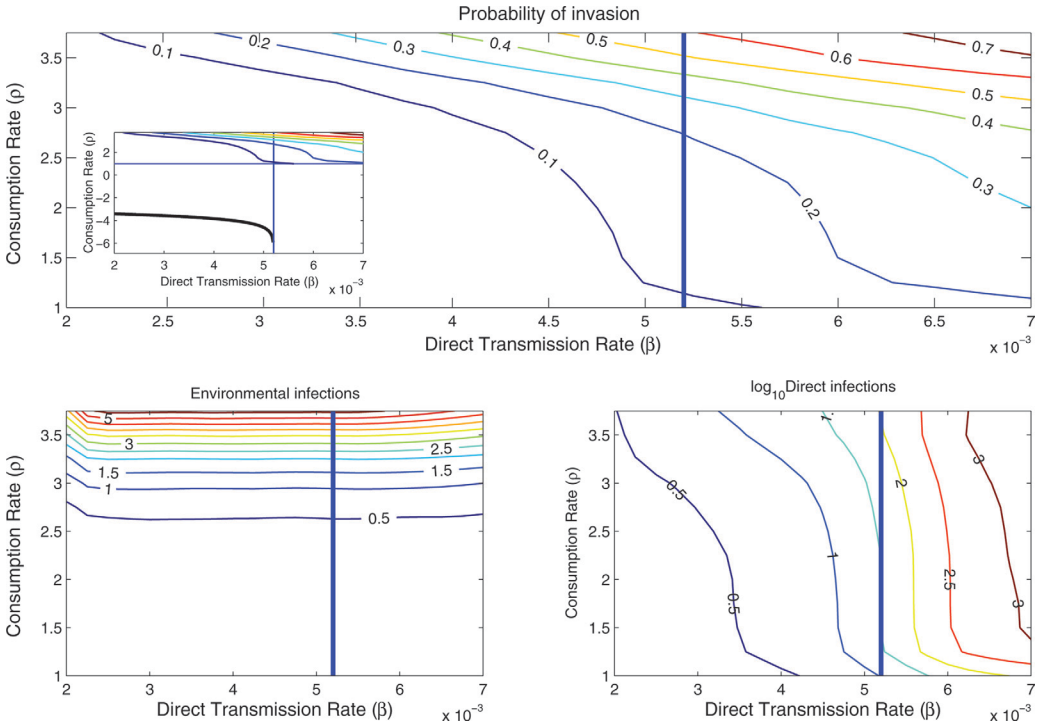

Fig. 3. AIV invasion success as a function of the direct transmission rate (β) and the rate of water consumption (ρ). (*Top*) Contours represent the probability of observing 20 cumulative infections, starting from $S(0) = 10^4$, $I(0) = 1$, and $V(0) = 10^4$. The very high frequencies in the contours were smoothed by using a convolution kernel. The black line demarcates the region $R_0^{\text{direct}} = 1$. Outbreaks to the left of this line, therefore, are mostly sparked by environmental transmission. (*Bottom*) Quantification of the mean number of environmental and direct transmission events per year. To illustrate direct transmission frequency when $R_0^{\text{direct}} < 1$, contours in the *Bottom Right* frame are plotted on a $\log_{10}$ scale. For each combination of parameter values, 1,000 stochastic realizations were generated. Other model parameters as in Fig. 2, with $L = 10^7$ and $V_0 = 10^2$. The sensitivity of these findings to changes in model parameter values are discussed in detail in the *SI Appendix*.

Hence, the expected number of infections is given by

$$\int_0^\infty \left\langle \frac{\rho}{L} S \frac{V}{V+\kappa} \right\rangle dt = \frac{\rho}{L} S_0 \int_0^\infty \frac{V_0 e^{-\eta t}}{V_0 e^{-\eta t} + \kappa} dt = \frac{\rho S_0}{L\eta} \ln\left(\frac{V_0}{\kappa} + 1\right) = R_0^{\text{env}} \frac{\gamma\kappa}{\omega} \ln\left(\frac{V_0}{\kappa} + 1\right). \quad [5]$$

Whereas the expected number of locally derived infections in the direct transmission process is linearly dependent on I_0 (Eq. **4**), there is only a logarithmic dependence on V_0 in the environmental transmission model (see Eq. **5**). The condition under which, on average, one susceptible is infected by the environmental transmission process can be obtained by setting

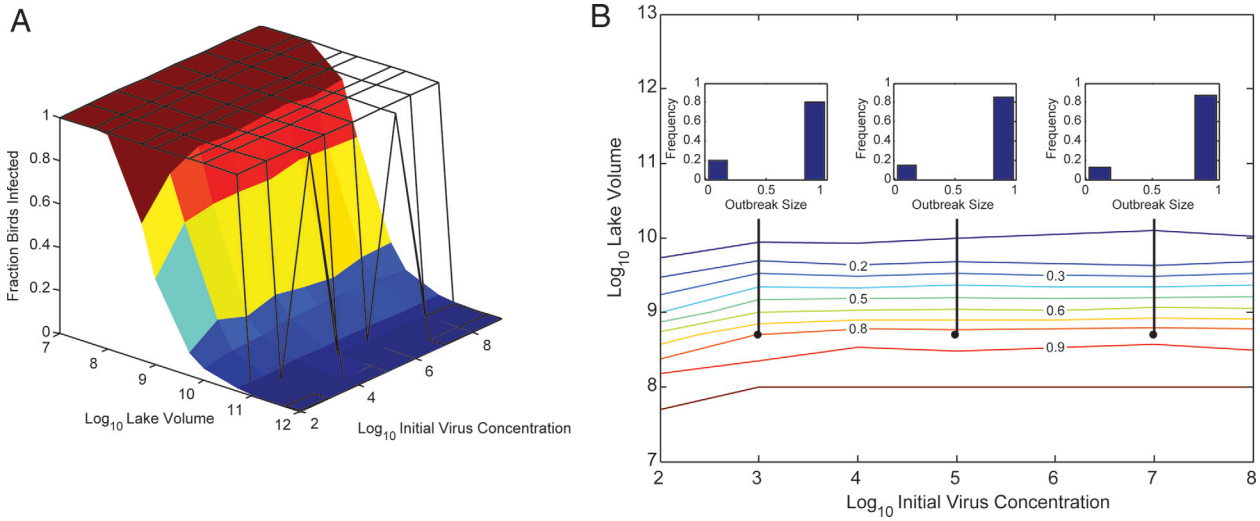

Fig. 4. Invasion implications of environmental transmission. In *A*, we assume 10^4 susceptible migrants arriving at a lake of volume L (liters), containing V_0 virus (measured in units of EID_{50} per liter). The color surface shows the cumulative fraction of birds infected, averaged over 1,000 stochastic realizations. The transparent mesh presents the outcome in the worst-case scenario (top 1% simulations). In *B*, we present a contour plot of the probability of an outbreak resulting in >100 infections. As the contours demonstrate, the outcome is largely independent of initial virus concentration, suggesting virus in the environment could represent a long-term source of infections. *Insets* demonstrate, however, that changes in V_0 affect the distribution of outbreak sizes. Model parameters were $\kappa = 10$, $\omega = 10^{12}$ per year, $1/\eta = 1$ month, $\rho = 10^4$ liters per year, $\beta = 7.8 \times 10^{-3}$ per year per individual, $1/\gamma = 7$ days. These parameters result in $R_0^{\text{direct}} = 1.5$. The sensitivity of these findings to changes in model parameter values are discussed in detail in the *SI Appendix*.

Eq. **5** to 1 and solving for R_0^{env}. Using the empirically obtained parameters described in Table 1 (and $V_0 = 10^4$), we obtain:

$$R_0^{\text{env}} = \frac{\omega}{\gamma\kappa \ln(V_0/\kappa + 1)} \approx 4.2 \times 10^7.$$

Altough environmental transmission events may be infrequent, as this argument shows, failure to account for them results in a superficial understanding of AIV epidemiology and, as shown in Fig. 4, in considerably underestimating outbreak probability. Further, in nature, virus persistence in the environment will be further complicated by local environmental conditions, such as temperature and salinity that degrade the infectious particle (31, 32), which will have implications for virulence evolution due to novel opportunities for selection (33, 34). In particular, virus persistence [or durability (33)] may trade off with traditional parameters such as the direct transmission rate (β) and the infectious period (γ) giving rise to new challenges in virulence management. For these reasons, we conclude that environmental transmission warrants serious consideration in the study of avian influenza ecology and evolution.

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