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Article

Keywords: avian influenza virus, poultry, live bird markets, epidemiology, mathematical modelling

Posted Date: November 23rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3616508/v1

Additional Declarations:
There is **NO** Competing Interest.

This study made use of secondary data collected in a previous field study. Ethical approval was obtained from both Chattogram Veterinary and Animal Sciences University and City University of Hong Kong.

Modelling the transmission dynamics of H9N2 avian influenza viruses in a live bird market

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Abstract

H9N2 avian influenza viruses (AIVs) are a major concern for the poultry sector and human health in countries where this subtype is endemic. By fitting a model simulating H9N2 AIV transmission to data from a field experiment, we characterise the epidemiology of the virus in a live bird market in Bangladesh. Many supplied birds arrive already exposed to H9N2 AIVs, resulting in many broiler chickens entering the market as infected, and many indigenous backyard chickens entering with pre-existing immunity. Most susceptible chickens become infected within one day spent at the market, owing to high levels of viral transmission within market and short latent periods, as brief as 5.3 hours.

Although H9N2 AIV transmission can be substantially reduced under moderate levels of cleaning and disinfection, effective risk mitigation also requires a range of additional interventions targeting markets and other nodes along the poultry production and distribution network.

Introduction

H9N2 Avian influenza virus (AIV) is considered to be the most prevalent AIV in poultry globally[1]. Despite being classified as a low pathogenic virus, H9N2 AIV is responsible for substantial economic loss for the poultry industry[2,3]. Infection is typically associated with moderate to severe respiratory symptoms, delayed growth, reduced egg production and increased mortality, specially when co-infection with other pathogens is involved[4]. Some H9N2 AIV lineages are known to be zoonotic, with resulting symptoms being typically mild. Co-circulation with other AIV subtypes may lead to the emergence of reassortant viruses with increased pathogenicity and/or zoonotic potential[5–7]. H9N2 appears to be involved in the origin of several novel zoonotic AIVs, whose number has been rapidly increasing since 2013[8]. AIVs with H9N2-derived genes include H7N9[9], H5N1, H10N8[10–12] and, more recently, H3N8[13].

In many Asian countries, prevalence of H9N2 AIVs is particularly high in live bird markets (LBMs), with estimates in Bangladeshi markets as high as 80%[14,15]. LBMs play a central role in marketing of poultry in developing countries, being the place of choice for many people to purchase meat for consumption. At the same time, high prevalence of AIV infection among traded poultry is concerning due to the risk of zoonotic spillover to humans[5,16,17]. In LBMs, the latter may be exposed to AIV through contaminated dust particles, water, surfaces and the slaughtering of infected birds. LBMs are also known to promote the mixing and evolution of AIVs, in that they enable the intermingling of multiple poultry species from many distant locations and diverse farming systems[18–20]. Over the last 25 years, public health concerns around LBMs have prompted health authorities in several Asian countries to take steps to control AIV transmission in these settings; adopted measures included enhanced hygiene protocols, bans on overnight poultry storage, as well as periodic rest days[21–26]. Temporary and permanent market shutdowns have also been employed in response to outbreaks of emerging zoonotic AIVs[27].

The central role played by LBMs in disseminating AIVs, including H9N2 viruses, calls for a better understanding of AIV transmission dynamics in these settings, which is paramount to design and implement effective and appropriate interventions. Previous field research focused on specific epidemiological aspects of AIV transmission, e.g. contamination in the environment[28–31], or involved cross-sectional investigations of AIV circulation in LBMs[15]. Unfortunately, linking results from these studies to viral dynamics is not straightforward. Challenge and transmission experiments in which live virus is inoculated artificially into chickens, and eventually transmitted onwards[15,32], allow to estimate important properties of AIV epidemiology. However, because these experiments are con-

ducted within a controlled environment, it remains difficult to draw general conclusions about AIV transmission in LBMs.

Here we aimed to fill these gaps by modelling H9N2 AIV transmission in an LBM. Mathematical modelling has proven useful to study AIV transmission dynamics in LBMs, but such investigations have been mostly theoretical so far[22]. Our work is instead grounded on a longitudinal dataset of H9N2 AIV acquisition in exotic and indigenous chickens in an LBM in Chattogram, Bangladesh[33]. Using Bayesian methods, we estimated quantities of epidemiological relevance, including H9N2 AIV transmission rate, host-specific latent periods, and quantified within-market prevalence as well as the likelihood of prior chicken exposure to H9N2 before entering the LBM. Finally, we leveraged these results to assess the impact of a range of hypothetical veterinary public health interventions on H9N2 AIV transmission.

Results

Parameter inference

Our model simulated transmission of avian influenza viruses (AIVs) among chickens in an LBM in Chattogram, Bangladesh. There, a fast turnover of poultry (Fig. S1A) drew together a steady supply of susceptible animals and unsold chickens offered for sale in previous days, thus creating opportunities for amplification of AIVs.

Following our experimental design, explained in detail in[33], we focused on exotic broiler (BR) and local, backyard-raised (BY) chicken types, which represent a large share of chickens traded daily in the LBM (Fig. S1B). We further distinguished between control (c) and intervention (i) chickens, according to whether they were recruited at the market or from farmers, respectively. We assumed these chickens could differ in terms of prior exposure to AIVs, possibly due to our intervention, which consisted in applying strict biosecurity measures during the collection and transport of farm-acquired chickens before introducing them to the LBM. Control chickens, instead, were recruited from market vendors among those recently supplied by mobile traders.

We fitted our model to H9N2 Polymerase chain reaction (PCR) positivity data[33]. We considered samples with a cycle threshold (Ct) < 40 as positive, in accordance with the laboratory protocols of the Australian Animal Health Laboratory (Geelong, Australia, http://www.csiro.au/places/AAHL). A more conservative criterion for positivity ($Ct < 33$) was also considered throughout the analysis. We obtained posterior estimates and credible intervals (C.I.) for thirteen parameters listed in Table 1; these include H9N2 AIV transmissibility β, latent periods $T_{E,b}$ for types b =BR and BY (panels Fig. 1A-C, respectively) and probabilities of prior exposure $\rho_{g,b}$ for different combinations of chicken type and recruitment group $g = c, i$. A description of prior distributions for each fitted parameter can be found in Table S1, while posterior marginal distributions and pairwise plots are shown in Fig. S2.

Table 1: **Fitted parameters.** Description of fitted parameters.

Name	Description
β	Transmissibility
σ_{BR}	Latent to infectiousness rate (broiler)
σ_{BY}	Latent to infectiousness rate (backyard)
μ	Recovery rate
η	Positivity waning rate
λ_{BR}	inverse scale past exposure time (broiler)
λ_{BY}	inverse scale past exposure time (backyard)
κ_{BR}	shape past exposure time (broiler)
κ_{BY}	shape past exposure time (backyard)
$\rho_{c,BR}$	Prior exposure prob. (control, broiler)
$\rho_{i,BY}$	Prior exposure prob. (intervention, broiler)
$\rho_{c,BR}$	Prior exposure prob. (control, backyard)
$\rho_{i,BY}$	Prior exposure prob. (intervention, backyard)

From our model's output, we found a shorter latent period in exotic broiler compared to backyard chickens (Fig. 1B,C), lasting an average of 5.3 hours for exotic broiler, and 1 days for backyard chickens. With a more conservative criterion for positivity ($Ct < 33$ instead of $Ct < 40$), these estimates increased to 6.1 hours and 1.3 days. In these exercises we assume that infected chickens would test positive only from the point where they start shedding, i.e. since the onset of infectiousness. We also found remarkably high levels of transmission in the LBM, which translated into more than 80% of chickens entering the market as susceptible, becoming infected within 20 hours, regardless of whether we set the threshold for positivity to $Ct = 40$ or $Ct = 33$ (Fig. 1D). However, we estimated higher transmission under $Ct = 40$, where more than 80% of poultry became infected within 10 hours, in contrast to nearly 55% for $Ct = 33$. This was likely due to the fact that the latter threshold corresponds to less positive samples in the data with respect to $Ct = 40$.

We also obtained posterior estimates for the proportions of chickens that were already infected (i.e. latent or infectious, E+I) or immune to H9N2 (R) at recruitment, for any combination of chicken type and recruitement group (Fig. 1E,F, show exotic broilers and backyard chickens, respectively). Interestingly, we found different patterns across chicken types: in the case of exotic broilers, most chickens with prior exposure to H9N2 were either infectious or latent, with only a minor proportion of them being immune (Fig. 1E). In contrast, most previously-exposed backyard chickens were immune to H9N2 (Fig. 1F). Our results thus tentatively suggest that prior infection occurs close to marketing age for broilers, whereas in backyard chickens it may occur further in the past, which is consistent with the latter being raised for a longer time compared to broilers (more than 6 months and up to 1 month for backyard and broiler chickens, respectively). See also Fig. S3 for distributions of time since exposure.

We also found differences between control and intervention chickens already

at recruitment. In the broiler case, intervention chickens were less likely to be already exposed at recruitment compared to their control counterparts (odds ratio 0.44-0.58, depending on Ct, see Fig. 1E). However, the reverse was the case in backyard chickens, with a larger proportion of intervention chickens being already exposed to H9N2 compared to controls (odds ratio 2.37-2.13, depending on Ct).

Our results, in particular posterior estimates of latent periods and probability of prior exposure, are robust to prior assumptions on transmissibility β and time to viral clearance–i.e. the sum of infectious and latent periods–(Fig. S4 and S5). Furthermore, all scenarios yielded large levels of transmission. These translated, under default priors, into sustained transmission of AIV in the absence of repeated external introductions (Fig. S6). Finally, our inferential procedure was able to recover model parameters in the context of synthetic data simulated from the same generative process used for inference (Fig. S7). In particular, we show that inference succeeds in a range of scenarios where model parameters differ across chicken types and recruitment groups and in the presence of moderately biased prior assumptions about shedding time.

Modelling interventions

In the last 20 years, LBMs have often been the target of veterinary public health interventions aiming to mitigate AIV transmission. Yet, effectiveness of individual measures is difficult to assess and are likely to vary between different social, economic and political contexts. Here, we leveraged our inferential results to evaluate the impact of various potential control measures to reduce H9N2 transmission in an LBM. In doing so, we considered different modes of transmission, namely direct and mediated by environmental contamination, and assessed sensitivity of our results to each assumption. With environmentally-driven transmission, the force of infection was assumed to be proportional to environmental contamination $I_{env}(t)$; $I_{env}(t)$ accumulates due to shedding from infectious chickens and decays progressively at rate Θ. We did not attempt to fit this model to data; rather, we mapped each value of "direct" transmissibility β from previous posterior samples into an appropriate value of environmental transmissibility (β_{env}) yielding similar prevalence levels. The exact mapping, suggested by[22] and derived in the Materials and Methods section, is:

$$\beta \longrightarrow \beta_{env} = \beta \cdot (1 - e^{-\Theta}) \,. \tag{1}$$

Note that this relation depends on the decay rate Θ and that a slower decay corresponds to a smaller β_{env}, which compensates for the longer persistence in the environment. Here we consider three values of Θ, namely $\Theta^{-1} = 10, 3, 1$days, corresponding to slow, intermediate and fast decay, respectively. These values are based on actual estimates from the scientific literature and capture a broad range of environmental conditions (see Supplementary Text S1.3). Fig. S8 shows a numerical validation of our mapping.

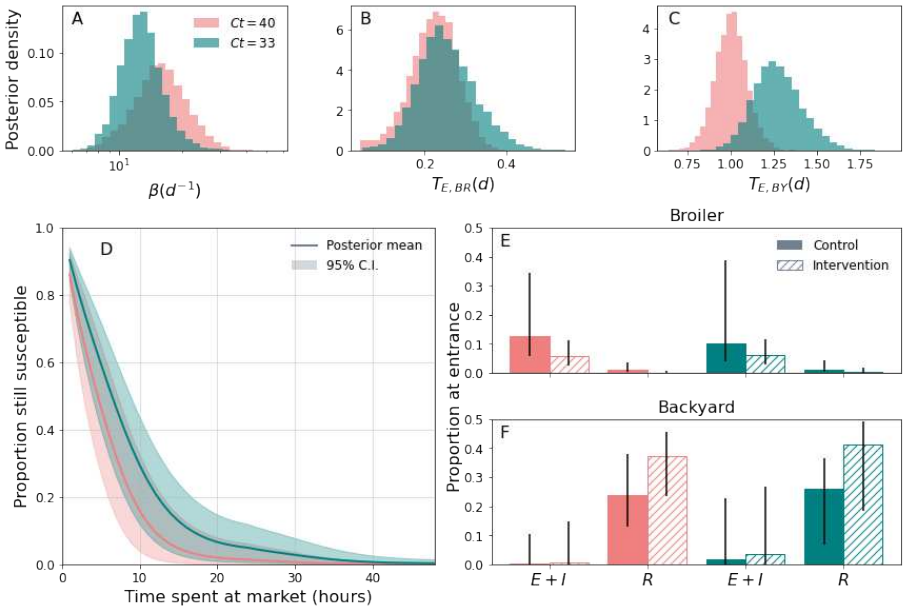

Figure 1: **Model fit results.** Posterior distributions for β (A), $T_{E,BR}$ (B) and $T_{E,BY}$ (C) obtained from fits to $Ct = 40$ (coral) and $Ct = 33$ (teal) data. (D) Average posterior probability of a chicken remaining susceptible after a given amount of time spent at the market and 95% C.I. (shaded area). (E-F) Average proportions of exotic broiler and backyard chickens in either control (solid) or intervention (dashed) groups entering the market as latent or infectious ($E + I$) or recovered (R). For both fits we set prior hyper-parameters $l_\beta = 0.005$ and $\bar{T}_{EI} = 5$ days (see S1.2). Results in (D) are based on 30000 simulations based on 3000 samples from the posterior, each simulation tracking 10^6 experimental chickens; all other panels are based on 8360 posterior samples, obtained after discarding the first 10000 MCMC iterations and keeping one sample every 1000th iteration.

To start with, we implemented three measures based on either (i) early removal/culling of unsold chickens, (ii) control of chickens entering the market or (iii) preemptive immunisation through vaccination. Fig. 2 displays effectiveness of various interventions, computed as the reduction in cumulative daily prevalence relative to a baseline scenario with no intervention (See Fig. S9 and S10 for prevalence dynamics over a single day). Green and yellow bars correspond to direct and environmental transmission, respectively. In the latter case we present a single value of Θ, but our results are independent of this choice.

In (i), unsold chickens are automatically removed from the market if still unsold after a time T_m. Fig. 2 shows that (i) is not effective at reducing prevalence (A,D), unless chickens are removed after 1 day or less. Indeed, high levels of transmission, combined with a short latent period in broilers (Fig. 1B), lead to a rapid build-up of infectious chickens well before T_m. This result holds, both qualitatively and quantitatively, regardless of whether we consider direct (green) or environmental (yellow) transmission.

Intervention (ii) aims at reducing the proportion of exposed chickens entering the market, either as the result of control measures acting upstream, e.g. by enhancing farmers' and traders' compliance with bio-security practices. In practice, we implement (ii) by reducing the proportion of previously exposed chickens from $\rho_{c,b}$ to $(1-r)\rho_{c,b}$, where r represents the intervention's strength. Panels B,E in Fig. 2 reveal that a reduction in $\rho_{c,b}$ by a factor $r = 0.9$ alone (filled bars) is not sufficient to lower transmission significantly. Indeed, latent & infectious chickens arriving at the LBM, albeit fewer compared to baseline, are still able to sustain high levels of transmission. Effectiveness of (ii) is even smaller in the presence of environmental transmission due to AIV persistence in the environment, which is not directly affected by the intervention. However, a combined control strategy involving both (i) and (ii) proves superior to individual measures (hatched bars).

With intervention (iii) a proportion p of chickens are immunised through vaccination, and are assumed to be completely protected from AIV infection. This measure not only reduces the number of chickens entering the market while infectious or latent, but also reduces overall susceptibility to AIV in the flock. Fig. 2C,F show that preemptive vaccination is particularly effective at reducing transmission; in particular, the reduction arising from vaccinating just 20% of all chickens is comparable to that of the most stringent implementations of interventions (i) or (ii).

The inclusion of environmental transmission in our model allowed us to explore the impact of sanitation, which is often adopted in the context of LBMs. Here, sanitation is assumed to reduce environmental contamination by a factor δ. First, we note that while direct and environmental transmission were shown to yield similar stationary dynamics (Fig. S8) and sensitivity to interventions (i) to (iii) (Fig. 2), significant dynamical differences arose in presence of sanitation. Specifically, Fig. 3A shows that after depopulating and disinfecting the LBM, baseline prevalence levels were recovered rapidly under direct transmission, but not under environmental transmission. The mechanistic reason lies in the "inertia" inherent to the environmental reservoir, relative to an equivalent model

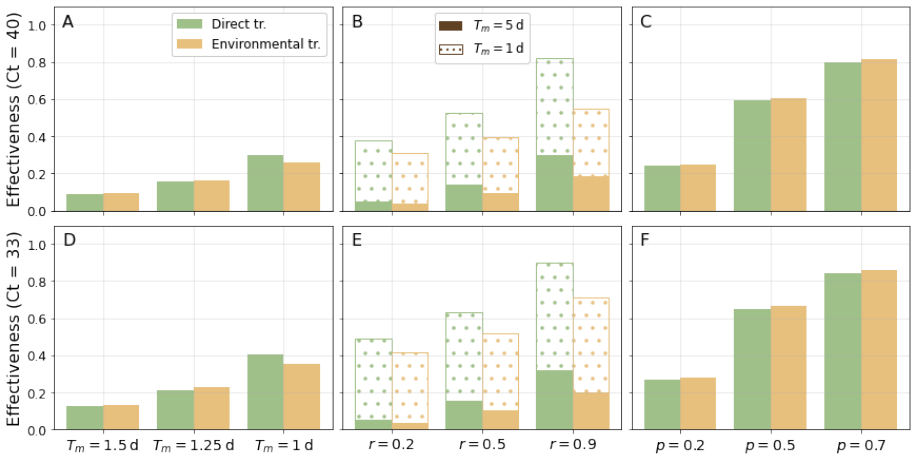

Figure 2: **Effectiveness of intervention measures.** Results for early removal/culling of unsold chickens (A,D), control of chickens entering the market (B,E) and preemptive immunisation through vaccination (C,F). Bars represent mean reduction in average, cumulative daily prevalence with respect to a baseline scenario with no intervention, based on 5000 simulations from 500 posterior samples. Green and yellow bars correspond to direct and environmental transmission, respectively. In the latter case we set $\Theta^{-1} = 3$ days for the sake of visualization. In (B,D), solid and hatched bars correspond to a maximum length of stay of 5 (baseline) and 1 days, respectively. First and second rows are based on posterior distributions obtained from fits to $Ct = 40$ and $Ct = 33$ data, respectively.

with direct transmission. This inertia is expressed by the apparent trade-off between environmental transmissibility β_{env} and persistence in the environment, as quantified by Θ. We stress that while this effect follows from Eq. 1, it is not an artefact: β_{env} and Θ should be expected to behave in this way, with, e.g., longer persistence in the environment (smaller Θ) corresponding to slower relaxation. This is indeed confirmed by Fig. 3B,C, where we compare three values of Θ and use $Ct = 40$ and $Ct = 33$ posterior samples, respectively. At low Θ, the typical relaxation time is at least 15 days and increases rapidly with disinfection δ. As Θ increases, the relaxation time becomes shorter and less dependent on the disinfection rate.

Consistently with Fig. 3A-C, we found increasing returns from routinely (daily) disinfecting the market when Θ is small, even if disinfection is not perfect (Fig. 3D-G). A multi-pronged approach featuring interventions (i) and (ii) and small levels of disinfection, say $\delta = 0.3$, is able to curb cumulative daily prevalence by more than 80% for any explored value of Θ and in both parameter configurations (Fig. 3G). Preventing 90% of prior infections (Fig. 3E) proved more effective than just limiting maximum length of stay to 1 day (Fig. 3F)

when coupled with routine disinfection, but not in absence of it (i.e. $\delta = 0$).

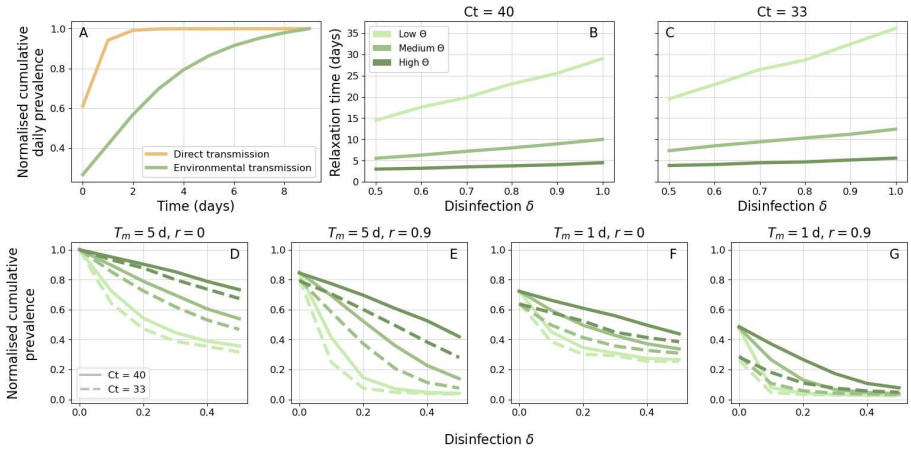

Figure 3: **Effectiveness of market depopulation and disinfection under direct vs environmental transmission** (A) Cumulative daily prevalence, expressed as a fraction of its stationary value, after depopulating and fully disinfecting ($\delta = 1$) the LBM, under direct (yellow) and environmental (green) transmission. In the latter case we set $\Theta^{-1} = 3$ days. (B,C) Average relaxation time as a function of disinfection δ, based on $Ct = 40$ and $Ct = 33$ posterior distributions. Light to dark lines correspond to $\Theta^{-1} = 10, 3, 1$ days, respectively. Relaxation time is defined as the time at which cumulative daily prevalence crosses a given threshold value for the first time since LBM depopulation. Here, this threshold is set to a fraction (0.95) of expected cumulative daily prevalence in the pre-intervention period. We compute 500 relaxation times from as many posterior samples, using 10 independent simulations to estimate mean cumulative daily prevalence. (D,G) Cumulative daily prevalence under various combinations of reduced length of stay (from left to right), reduced probability of prior exposure (from left to right) and disinfection, on the x-axis, for varying rates of environmental decay. Prevalence is calculated relative to a scenario with no interventions and the same Θ. Results corresponding to solid and dashed lines are based on samples from $Ct = 40$ and $Ct = 33$ posterior distributions, respectively.

Discussion

In this work we characterised H9N2 transmission patterns in a single LBM in Bangladesh by fitting a mechanistic transmission model to a longitudinal dataset collected in the context of a field experiment.

Our results confirm the important role of LBMs as hotspots of AIV transmission. We found high prevalence of H9N2 AIV, in agreement with previous studies and LBM surveillance in Bangladesh[15,16]. Our simulations further sug-

gest that H9N2 AIV prevalence varies considerably during a single day due to high transmission rates. Such an effect has been illustrated in previous modelling work[22], and should be accounted for by AIV surveillance initiatives and in the design of chicken sampling strategies in general.

From a systemic perspective, high persistence and prevalence of H9N2 AIV in LBMs are concerning for the whole poultry production and distribution infrastructure in which LBMs are embedded. Although our analysis is based on data collected from a single LBM, our results are relevant to LBMs with similar features. Indeed, vendors operating in the same types of markets and locations are expected to adopt similar practices[20,25] and source chickens from overlapping catchment areas[20]. The fast turnover of susceptible chickens in LBMs is concerning since it is likely to promote amplification of AIV subtypes with short latency other than H9N2, e.g. H5N1 AIV[32]. This virus is routinely detected in Bangladeshi wholesale markets, albeit at a lower frequency compared to H9N2 AIV[14]; this likely reflects the lower abundance of traded backyard ducks, which act as the primary source of H5N1 infections in markets[15,34].

We estimated an average latent period of 5.3-6 hours and 1-1.3 days, depending on Ct threshold, for exotic broiler and backyard chickens, respectively. Short latent times in exotic broiler chickens are compatible with a fast onset of viral shedding, already after one day post-inoculation, as observed in laboratory experiments[32,35–41]. Moreover, we believe that our experimental design, which includes inter-sampling periods as short as 12 hours, is more suitable to resolve short latent periods than many laboratory experiments, which typically collect the first samples post-inoculation only after 1 day. Our estimates were robust with respect to prior assumptions about the duration of shedding, as shown in sensitivity analyses. Unfortunately, we could not reliably estimate the infectious period since our data did not include enough information about viral clearance.

Inferred proportions of chickens that were recruited directly in farms (intervention group) and that had already been exposed to H9N2 AIV prior to T_0 revealed substantial differences between broiler and backyard chickens. Specifically, we found most exposed broilers to be actively infected at recruitment, with little evidence of accrued immunity. In contrast, the majority of backyard chickens were estimated to be already immune to H9N2 AIV at recruitment. A recent study found 1% and 15.7% H9N2 AIV antibody prevalence and low viral prevalence, 0.2% and 0.5%, in broiler and backyard farms around Chattogram, respectively[42]. These prevalence values are slightly lower than estimates reported from active surveillance, which found 2.2% and 9.6% of AIV RT-PCR positivity in backyards and farms, respectively, with around a fourth of positive samples attributable to H9N2 AIV[14]. At the flock-level, H9N2 AIV prevalence around Chattogram has been estimated around 0.7% and 1.9% for backyard and broiler chickens, respectively. Another cross-sectional study of household chickens performed in the same area found a household-level prevalence of H9N2 AIV of 3.2%[43].

In absolute terms, our estimates of H9N2 AIV circulation in broilers sampled at T_0 are larger than previous estimates of viral circulation in farms. In fact, crude numbers of broiler chickens recruited in farms that tested positive for

H9N2 AIV at T_0 (5 out of 110), suggest higher viral prevalence than found by other cross-sectional studies. Analogously, we estimated a higher proportion of past infections in backyard chickens at T_0 than suggested by serological evidence. While the reasons for these discrepancies remain unknown, we note that chickens included in this study were collected towards the end of a production cycle, when they might be exposed to an increased risk of AIV infection. Nonetheless, our results remain in broad qualitative agreement with available evidence as both suggest a higher prevalence of antibodies against H9N2 AIV in backyards compared to broiler farms, in the face of larger viral circulation in broilers.

Exotic broilers recruited at farm gates were found to be less likely to be already exposed to H9N2 AIV compared to chickens recruited at LBM gates (control group), suggesting some degree of viral amplification happening along channels connecting farms to markets[15,20]. However, we found the opposite relation in the case of backyard chickens. One possible explanation is that backyard farmers included in this study saw an opportunity to sell chickens that were already sick, potentially due to AIV infection. Selling sick birds is not an uncommon practice among backyard farmers near Chattogram, who often operate in a world of compromises[44].

High levels of H9N2 AIV circulation in LBMs are concerning from a veterinary public health standpoint, and may require considerable efforts and resources to be controlled effectively. Indeed, some of our simulated interventions, like reduced length of stay and reduced probability of prior exposure, proved to be only modestly effective. Combining both interventions proved considerably more effective at reducing transmission compared to individual measures. Bans on overnight stay in Hong Kong were estimated to reduce H9N2 AIV isolation rates by more than 80%[23]. It is possible that the combination of high introduction levels and baseline within-market transmission is larger in our study, thus requiring increased efforts to reduce transmission by an amount similar to what had been observed in Hong Kong.

Pre-emptive vaccination alone proved to be particularly effective in simulations, under the assumption of complete sterilising immunity. A vaccine against H9N2 AIV is already available in Bangladesh, but its use has been limited to breeders and layers[45]. Widespread H9N2 AIV vaccination has been implemented in China and Korea. In Korea, genetic diversity of H9N2 AIV decreased suddenly after implementing vaccination in 2007[46]. Large-scale AIV vaccination stamped out H7N9 in Chinese LBMs[47] but not H9N2, likely due to vaccine failure[48]. Indeed, continued AIV evolution can jeopardise vaccination efforts, requiring effective viral surveillance to inform vaccine composition and timely roll-out of updated vaccines.

We considered two alternative modes of transmission, direct and mediated by the environment. Both scenarios were able to explain observed dynamic patterns and yielded similar results in the context of interventions targeting chickens only. Including environmental transmission allowed us to model the impact of LBM disinfection. In this scenario, moderate levels of cleaning could curb transmission significantly in simulations, specially if decay rates are small, as that corresponds to a slower accumulation of contaminated material. Peri-

odic disinfection, usually performed during rest days, has been shown to reduce H5N1 burden in Chinese LBMs[22,49]. It should be noted, however, that both transmission modes are likely to be at play at the same time; unfortunately, it was not possible to assess the relative contribution of each mode to overall transmission in this study. Overall, our analysis supports a multi-pronged approach to reduce the burden of H9N2 AIV in LBMs and makes the case for the vaccination of poultry intended to be sold in LBMs in Bangladesh.

Our study has several limitations. It focused on exotic broiler and backyard chickens, i.e. the same chicken types sampled in the field experiment. We did not include other chicken types, quails or ducks that are traded at the same market, as it would have been difficult to estimate additional parameters in the absence of appropriate data. While this could potentially bias our estimate of AIV transmissibility, which appears to be sensitive to other prior assumptions as well, we believe that our main results, e.g. estimated prevalence, are not affected by these simplifying study conditions. We did not consider seasonal variation in AIV transmission over the study period[50]. Nonetheless, explored contamination decay rate values can be sensibly mapped to environmental conditions at different times of the year.

We assumed that PCR tests could not detect infections during the latent phase, i.e. in absence of viral shedding, but were otherwise perfectly sensitive in the case of infectious and recently recovered chickens. High rates of positivity to H9N2 AIV suggest however that test sensitivity should not be a problem in our analysis. We also believe that positive outcomes were unlikely to arise from cross-reactivity induced by other AIVs, but we can not exclude cross-contamination of some samples in the laboratory. We note that immune cross-reactions between distinct AIVs may still affect susceptibility to H9N2 AIV. In addition, it has been proposed that backyard chickens are intrinsically more resistant to AIV infection compared to exotic broilers[51–54], which could partially explain differences in attack rates between them. Future analyses may consider further heterogeneities among chicken types. It should be noted, however, that increased resistance of domestic types hypothesized by previous studies could in fact be the result of earlier exposure to AIVs, as hinted by our results.

In conclusion, we found that H9N2 AIV is transmitted rapidly among chickens in LBMs with similar conditions to those in Chattogram, Bangladesh. A short latent period, specially in broilers, high transmission rates and a continuous daily supply of susceptible chickens provide fertile grounds for H9N2 AIV amplification despite short length of stay. Virus persistence in LBMs is further promoted by poor cleaning, which enables viral accumulation in the environment, and frequent introductions of infectious chickens from trade. Consequently, sustained efforts involving a diverse range of veterinary public health interventions will be required to curb circulation of this virus.

Materials and Methods

Model description

We use a SEEIRR model to simulate disease dynamics. Under the assumptions of density-dependent transmission and homogeneous mixing, susceptible (S) chickens become infected at rate $\Lambda(t) = \beta I(t)/N$, where β is the transmission rate, $I(t)$ counts the number of infectious (I) chickens at time t and N is the number of new chickens entering the market daily. Exposed (E) chickens turn infectious after an average latent period $T_E = \sigma^{-1}$ and recover after an average infectious period $T_I = \mu^{-1}$. The exposed state consists of two consecutive stages ($E_{1,2}$) with the same exit rate 2σ, yielding a gamma-distributed latent period. Recovered chickens initially enter the R_+ state and then advance to R_- at rate η. In this work we assume that only biological samples retrieved from I or R_+ chickens can result positive to PCR. We assume that the two chicken types considered here, exotic broiler and backyard chickens, share the same biological parameters, except the latent period.

We model an open population of chickens that mimics the activity of an LBM: more in detail, we assume that N_b new chickens of type $b = BR, BY$ reach the market in bulk every day, always at the same time (note that $N = \sum_b N_b$). Of these, a proportion ρ_b has already been exposed to influenza prior to entering the market. Chickens are then sold progressively over time, their length of stay being distributed as in Fig. S1A. We assume for simplicity that the distribution of length of stay of backyard chickens is the same as that of broilers. Fig. S11 shows that this assumption does not affect epidemic dynamics significantly.

Equivalence between direct and environmental transmission

Under environmental transmission, the expression for the force of infection becomes $\Lambda_{env}(t) = \beta_{env} I_{env}(t)/N$, where $I_{env}(t)$ represents viral load in the environment at time t; its physical units are arbitrary, but chosen in a way that I_{env} increases by an amount $I(t)$ (i.e. prevalence of infectious chickens) between t and $t+1$.

A mapping between β and β_{env} that (approximately) preserves stationary viral dynamics can be obtained as follows: let $\tilde{T}$ denote the average time a single chicken spends at the market while infectious. Under direct transmission, its spreading potential is given by $\beta\tilde{T}$; under environmental transmission, the same quantity is evaluated as:

$$\beta_{env}\tilde{T}\sum_{t=0}^{\infty} e^{-\Theta t}\,, \tag{2}$$

where the last sum accounts for persistence and progressive decay of infectious faeces in the environment. Equating the two expressions yields the relation $\beta_{env} = \beta \cdot (1 - e^{-\Theta})$.

Field data collection

The field experiment consisted in caging 10 chickens together at a market stall for 84 hours, and sampling them for positivity to AIV at four time points, $T_1 = 0, T_2 = 12, T_3 = 36$ and $T_4 = 84$ hours during the duration of the experiment. Of these 10 chickens, a group of 5 were recruited directly at the market right before T_1 (control group), while the remaining 5 birds had been recruited 2.5 days in advance (T_0) from farms (intervention group) and stored in a biosecure environment before being introduced to the LBM at T_1. The experiment was repeated 30 times with exotic broilers and 34 with backyard chickens for a total of 300 and 340 chickens, respectively. In this work we removed 80 broiler chickens corresponding to 8 experimental replicates where there was a suspect of cross-contamination of samples. More details about the experimental design can be found in[33].

Fitting the model to field data

In the context of experimental data, we further distinguish between intervention (i) and control (c) chickens. This translates into four introduction parameters $\rho_{g,b}$, according to each combination of group $g \in \{c, i\}$ and type b. We assume that control and bulk (i.e. marketed chickens that were not part of the experiment) chickens are equivalent in all aspects, meaning that $\rho_b = \rho_{c,b}$. Finally, compartment-specific introduction probabilities are fully determined by specifying three hyper-parameters λ_{BR}, λ_{BY} and κ. Briefly, these set the timing of prior exposure, under the assumption that the latter is gamma-distributed with type-specific rate λ and shared shape parameter κ. Further mathematical details can be found in Supplementary Text S1.1.

We used a Bayesian MCMC approach to infer parameters θ listed in Table 1. We chose priors that penalise large values of β and set a narrow range for $T_{EI} = (\sigma_{BR}^{-1} + \sigma_{BY}^{-1})/2 + \mu^{-1}$, i.e. the average time from exposure to viral clearance; for a full account of fitted parameters' priors see Table S1. The likelihood function is multinomial (see Text S1.2), and depends on the probability of a chicken testing positive for the first time at market entrance, i.e. T_0 or T_1, or during any other time segment $[T_j, T_{j+1}]$; in addition, we also account for chickens that remain susceptible throughout the experiment or until early removal. We resort to numerical simulations to evaluate the likelihood, since an explicit representation of individual probabilities in terms of model parameters is not available. Simulations feature both bulk and recruited chickens from intervention and control groups. Importantly, we assume that recruited chickens do not contribute to transmission, but they can still be affected by exposure to infectious bulk chickens, which are way more abundant than the former. Intervention and control animals are recruited at times T_0 and T_1, respectively, and can not leave the market. From T_0 to T_1, intervention chickens are completely isolated from any source of infection, consistently with experimental conditions.

The inference routine is based on an ensemble sampler from the Python module *emcee*, version 3.1.1[55]. Briefly, this sampler runs l chains in parallel, and

makes proposals based on the collective state of all chains. We checked MCMC convergence by visual inspection, e.g. by looking at trace plots (Fig. S12), and by looking at MCMC acceptance rates.

Acknowledgments

F.P. is funded by the UKRI GCRF One Health Poultry Hub (Grant No. BB/S011269/1), one of twelve interdisciplinary research hubs funded under the UK government's Grand Challenge Research Fund Interdisciplinary Research Hub initiative. The authors would like to acknowledge the use of the University of Oxford Advanced Research Computing (ARC) facility in carrying out this work.

Author contributions

Conception and design: F.P., G.F.; **Data Collection**: L.K., D.P., G.F.; **Data Analysis**: F.P.; **Methodology**: F.P.; **Investigations**: F.P., L.K., J.L., S.G., Md.A.H., R.M., P.B., D.P., G.F.; **Visualisation**: F.P.; **Writing–original draft preparation**: F.P., G.F.

Competing interests

The authors declare no competing interests.

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