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Sema Nickbakhsh [a,1], Matthew D. Hall [b,c,2], Ilaria Dorigatti [d], Samantha J. Lycett [b,c,3], Paolo Mulatti [e], Isabella Monne [e], Alice Fusaro [e], Mark E.J. Woolhouse [b,c], Andrew Rambaut [b,c,f], Rowland R. Kao [a,*]

[a] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, UK
[b] Institute of Evolutionary Biology, University of Edinburgh, EH9 3FL, UK
[c] Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh EH9 3FL, UK
[d] MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, London W2 1PG, UK
[e] Istituto Zooprofilattico Sperimentale delle Venezie, Italy
[f] Fogarty International Center, National Institutes of Health, Bethesda, MD 20892-2220, USA

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ABSTRACT

It is well known that highly pathogenic avian influenza (HPAI) viruses emerge through mutation of precursor low pathogenic avian influenza (LPAI) viruses in domestic poultry populations. The potential for immunological cross-protection between these pathogenic variants is recognised but the epidemiological impact during co-circulation is not well understood. Here we use mathematical models to investigate whether altered flock infection parameters consequent to primary LPAI infections can impact on the spread of HPAI at the population level. First we used mechanistic models reflecting the co-circulatory dynamics of LPAI and HPAI within a single commercial poultry flock. We found that primary infections with LPAI led to HPAI prevalence being maximised under a scenario of high but partial cross-protection. We then tested the population impact in spatially-explicit simulations motivated by a major avian influenza A(H7N1) epidemic that afflicted the Italian poultry industry in 1999–2001. We found that partial cross-protection can lead to a prolongation of HPAI epidemic duration. Our findings have implications for the control of HPAI in poultry particularly for settings in which LPAI and HPAI frequently co-circulate.

1. Introduction

Avian influenza is a viral disease of poultry which, as well as having severe socio-economic consequences, continues to pose a significant threat to public health and global food security (McLeod et al.; Sonaiya, 2007). The highly pathogenic (HPAI) form, associated with up to 100% flock mortality, has been a particular concern for governments worldwide (Alexander, 2000). The prevention and control of emergent HPAI in the poultry source is critical to ensure the economic and public health threat is not extended to new settings or to novel virus subtypes in the future.

A necessary though insufficient determinant of avian influenza pathogenicity in poultry is a mutation to the virus's genome causing enhanced cleavability of the viruses' haemagglutinin (HA) glycoprotein. This mutation is believed to evolve during circulation of LPAI viruses, often of wild bird origin (de Jong et al., 2009), among domestic poultry (Banks et al., 2001; Garcia et al., 1996; Horimoto and Kawaoka, 1994; Pasick et al., 2005; Suarez et al., 2004). Therefore, although wild bird reservoirs can be a direct source of both LPAI and HPAI incursions in domestic poultry, in many cases, the emergence of novel HPAI mutants is consequent to pre-existing LPAI outbreaks in poultry. When these evolutionary transitions arise, the emergent HPAI will co-circulate with the antigenically similar but pathogenically distinct LPAI viruses (Monne et al., 2014).

This particular scenario of HPAI arising through the prolonged circulation of LPAI therefore provides an opportunity to study the ecological relationship between different pathogenic variants of avian influenza.

In the context of human disease, it is known that the local co-circulation of different viruses produces the potential for epidemiological interference, whereby exposure to one virus alters the infection trends of another, most likely as a consequence of temporary immunity (Bang, 1975; Dietz, 1979). Recent animal studies have contributed to the growing evidence that temporary non-specific innate immune responses may lead to interference among influenza viruses (Laurie et al., 2015). In the case of antigenically similar variants of a pathogen such as LPAI and HPAI, interference may similarly manifest at the host scale through immunological cross-protection (CP); the prevention or partial inhibition of an individual host to future infections may lead to altered transmission dynamics during pathogen co-circulating. In the case of influenza, cross-protection as a consequence of adaptive immunity is expected since neutralizing antibodies and cross-reactive cell-mediated immunity are known to act across homo- and heterosubtypically diverse viruses (Ekiert et al., 2011; Seo and Webster, 2001; Wang et al., 2010). Hereafter we collectively refer to the cross-virus effects of adaptive and innate immune responses as immunological cross-protection.

Greater consideration to the effect of immunological cross-protection on the co-circulatory dynamics of LPAI and HPAI could prove valuable for predicting the long-term epidemiological success of emergent HPAI mutants. Based on theories of ecological competition (Ojosnegros et al., 2012), it is anticipated that immune-mediated competition for susceptible hosts will favour infection with LPAI viruses over HPAI viruses because of their comparatively low virulence, longer associated infectious periods, and consequently greater population-level transmissibility (all other factors being equal). However, it has been hypothesised that HPAI can invade a poultry population despite high mortality rates as a consequence of partial immunological cross-protection (de Leo and Bolzoni, 2012). This is readily explained by the outcome of prior exposures to homo-and heterosubtypic avian influenza viruses which may result in attenuated disease, thereby prolonging the infectious periods of HPAI-infected birds without full protection against viral shedding (Fereidouni et al., 2009; Seo and Webster, 2001; van der Goot et al., 2003, 2005).

Unveiling the potential importance of immunological cross-protection on the competitive success of HPAI viruses is however compounded by other infection life history traits. For example, owing to their enhanced cleavability, it is reasonable to presume HPAI viruses have a lower infectious dose, shorter latency, and greater per capita transmission rate than LPAI. Furthermore, virus survivability as well as the environmental component of avian influenza transmission (via infectious faeces thus removing the dependency on host survival), further obscure understanding into the likely influence of LPAI infections on the spread of HPAI through immunological CP. At the population scale, non-immune related or 'ecological' mechanisms of interference are also possible through interventions that alter population demography (Rohani et al., 2003). In the poultry setting, such an effect is plausible through national veterinary disease interventions that enforce the culling of infected flocks thereby temporarily altering the demography of susceptible poultry.

Here we aimed to use mathematical models to quantify the impact of immunological cross-protection on the spread of emergent HPAI within a commercial poultry population at both within-flock and between-flock scales. This study was motivated by a major epidemic of avian influenza A(H7N1) that afflicted the Italian poultry industry during 1999 to 2001. This epidemic is an important example of HPAI emergence through the mutation of a precursor LPAI virus before the statutory control of LPAI was introduced (Busani et al., 2009; Capua and Marangon, 2000; Monne et al., 2014).

2. Two-pathogen within-flock infection transmission model

We first investigated the impact of immunological CP on HPAI transmission dynamics within a single flock using a two-pathogen deterministic S-E-I-R model under frequency-dependent transmission. This model was adapted to reflect the spread of avian influenza within a densely populated commercial flock which is known to occur through aerosol and environmental transmission pathways. Fig. 1 provides a schematic overview of the model; S, E, I, R denotes the proportion of birds in the infection classes Susceptible, Exposed (infected but not yet infectious), Infectious and Removed, and an additional F class represents contamination of the poultry house environment with infectious faeces. Subscripts 1, 2, and 3 denote birds infected with LPAI, HPAI, and birds co-infected with both variants respectively.

Infectious birds excrete a single gram of infectious faeces at rate ε (days^{-1}) which decays in the poultry house environment at rate σ (days^{-1}). The per capita rate at which new infections are generated via direct bird-to-bird contact through the aerosol route, and indirectly via infectious faeces, is given by β_a (days^{-1}) and β_f (days^{-1}) respectively. Newly infected birds undergo a latent period given by $1/\delta$ followed by an infectious period given by $1/\gamma$. In the case of LPAI infected birds, complete protection against both strains is acquired at a rate of γ_1 (days^{-1}), whilst birds with a primary HPAI infection die at a rate γ_2 (days^{-1}). A background level of non-avian influenza-induced mortality is applied to birds in classes S, E_1, I_1, E_2, I_2, E_3, I_3 at rate μ (days^{-1}). Parameter values and ranges are provided in Table 1.

We assume that immunological cross-protection reduces (i) the susceptibility of LPAI infected birds to secondary HPAI infection, and (ii) the mortality rate (or lengthening of the infectious period) of birds co-infected with both variants (Seo and Webster, 2001; van der Goot et al., 2003, 2005). Secondary HPAI infections are acquired at a rate of $\beta_{2a}(1-\varphi) + \beta_{2f}(1-\varphi)$ (days^{-1}), and the mortality rate of co-infected birds is given by $\gamma_2(1-\varphi)$ (days^{-1}), where φ denotes the proportional reduction in both transmission and mortality rates as a consequence of immunological cross-protection.

The full model ordinary differential equations are as follows:

$$\frac{dS}{dt} = -S\left(\beta_{1a}I'_1 + \beta_{1f}F_1\right) - S\left(\beta_{2a}I'_2 + \beta_{2f}F_2\right) - \mu S$$

$$\frac{dE_1}{dt} = S\left(\beta_{1a}I'_1 + \beta_{1f}F_1\right) - \delta E_1 - \mu E_1$$
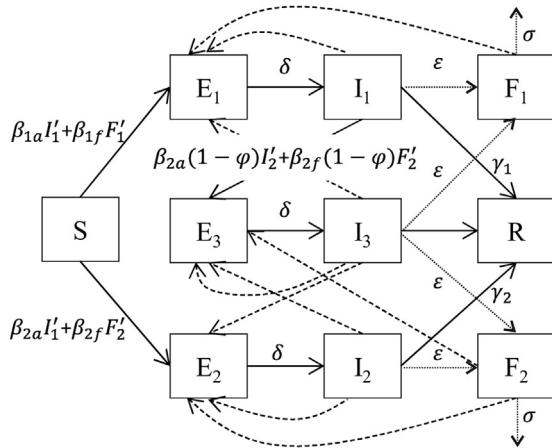
$$\frac{dI_1}{dt} = \delta E_1 - \left(\beta_{2a}(1-\phi)I'_2 + \beta_{2f}(1-\phi)F_2\right)I_1 - \gamma_1 I_1 - \mu I_1$$

$$\frac{dF_1}{dt} = \varepsilon I_1 - \sigma I_1$$

$$\frac{dE_2}{dt} = S\left(\beta_{2a}I'_2 + \beta_{2f}F_2\right) - \delta E_2 - \mu E_2$$

$$\frac{dI_2}{dt} = \delta E_2 - \gamma_2 I_2 - \mu I_2$$

$$\frac{dF_2}{dt} = \varepsilon I_2 - \sigma I_2$$

Biological assumptions:

- The LPAI prevalence on day one corresponds with time to emergence of a "fit" HPAI mutant within a single bird
- Transmission occurs via aerosol and infectious faeces in the environment with a 10-fold greater rate via faeces
- LPAI infected birds are temporarily insusceptible to HPAI reflecting the action of innate immunity
- LPAI infected birds acquire a secondary HPAI infection at a rate dependent on the level of immunological cross-protection
- No secondary HPAI infections occur under 100% immunological cross-protection
- Secondary HPAI infections occur at the same rate as primary HPAI infections under 0% immunological cross-protection
- No interference between the two viral strains occurs during co-infection i.e. LPAI and HPAI are equally transmitted
- Birds singly infected with LPAI eventually acquire long-term immunity to both strains
- Birds singly infected with HPAI eventually die as a consequence of avian influenza disease
- Co-infected birds die as a consequence of avian influenza at a rate dependent on the level of cross-protection

Fig. 1. Two-pathogen within-flock model schematic. Mechanistic model representing the co-circulatory dynamics of LPAI and HPAI transmission within a single densely populated commercial poultry flock. The proportions of birds in each infection class are denoted as follows: S = susceptible, E = exposed (but not yet infectious), I = infectious, R = removed (immune or dead). An additional F class represents the build-up of infectious faeces in the poultry house environment. Subscripts 1, 2 and 3 denote infection with LPAI, HPAI, and co-infection with both variants respectively. Solid arrows indicate the transition of birds from one infection state to the next. Dashed arrows indicate the contribution of birds in classes I and F to the forces of infection that generate newly infected birds. Dotted arrows in relation to F classes indicate the build-up (inward arrows) and decay (outwards arrows) of infectious faeces in the poultry house environment. Immunological cross-protection was incorporated through the assumed action of (i) reduced susceptibility of LPAI-infected birds to secondary HPAI infection, and (ii) increased survival of birds with secondary HPAI infection. See Table 1 for full details of the model parameters.

Table 1
Two-strain within-flock model parameters.

Parameter	Description (units)	Baseline value	Sensitivity ranges	Reference
β_1	LPAI transmission rate (days^{-1})	2[*]	–	Saenz et al. (2012)
β_2	HPAI transmission rate (days^{-1})	2[*]	0.05–4	Saenz et al. (2012)
δ_1	LPAI latency loss rate (days^{-1})	1[*]	–	Saenz et al. (2012)
δ_2	HPAI latency loss rate (days^{-1})	1[*]	0.2–4	Saenz et al. (2012)
γ_1	LPAI-induced immunity rate (days^{-1})	0.125[*]	–	Saenz et al. (2012)
γ_2	HPAI-induced mortality rate (days^{-1})	0.5[*]	–	Saenz et al. (2012)
ε	LPAI/HPAI faecal excretion rate (days^{-1})	24	–	–
σ_1	LPAI decay rate (grams/days^{-1})	1.2	–	Shortridge et al. (1998)
σ_2	HPAI decay rate (grams/days^{-1})	1.2	0.001–3	Shortridge et al. (1998)
μ	Background mortality rate (days^{-1})	0.0005[‡]	–	McMullin (2006)
φ	Proportional reduction in susceptibility and mortality due to cross-protection	1	0–1	–

Subscripts 1 and 2 denote LPAI-specific and HPAI-specific parameters respectively. [*]Mean infectious periods of 8 days and 2 days for LPAI and HPAI infections respectively informed the bird "removal" rates; [†]viral decay rates in the poultry house environment assume an absence of infectivity one day post-infection. Highlighted rows are HPAI-specific parameters investigated in sensitivity analyses.

$$\frac{dE_3}{dt} = I_1 \left(\beta_{2a}(1-\phi)I_2' + \beta_{2f}(1-\phi)F_2 \right) - \delta E_3 - \mu E_3$$

$$\frac{dI_3}{dt} = \delta E_3 - \gamma_2 \left(1 - \phi \right) I_3 - \mu I_3$$

$$\frac{dR}{dt} = \gamma_1 I_1 + \gamma_2 I_2 + \gamma_2 \left(1 - \phi \right) I_3 + \mu \left(S + E_1 + I_1 + E_2 + I_2 + E_3 + I_3 \right)$$

$$I_1' = I_1 + I_3$$

$$I_2' = I_2 + I_3$$

The model was constructed to reflect a typical turkey flock located in northeast Italy with a median population size of 15,000 birds (although we note that our results are qualitatively insensitive to flock size). Each outbreak was initiated with a single HPAI infected bird seeded among a hypothetical background of pre-existing LPAI infected birds. All simulated HPAI outbreaks declined within 90 days; the typical duration of a production cycle of fattening female turkey farms in Italy.

In all model simulations we assumed equal latency and transmission rates for the two virus strains but a longer infectious period for LPAI. These assumptions were based on an experimental study that used viruses sampled from the Italian A(H7N1) epidemic (Saenz et al., 2012). We also conducted sensitivity analyses on the HPAI latent period, HPAI transmission rate, and HPAI virus decay rate spanning above and below that assumed for LPAI, providing a broad range of biological scenarios (Table 1). Further details of model assumptions are provided in Fig. 1.

3. Impact of immunological cross-protection at the flock level

The daily percentage of birds infected with HPAI was computed for each simulated outbreak as summarised in Fig. 2. Sensitivity analyses investigating the impact of various infection parameters demonstrated immunological cross-protection to have the great-
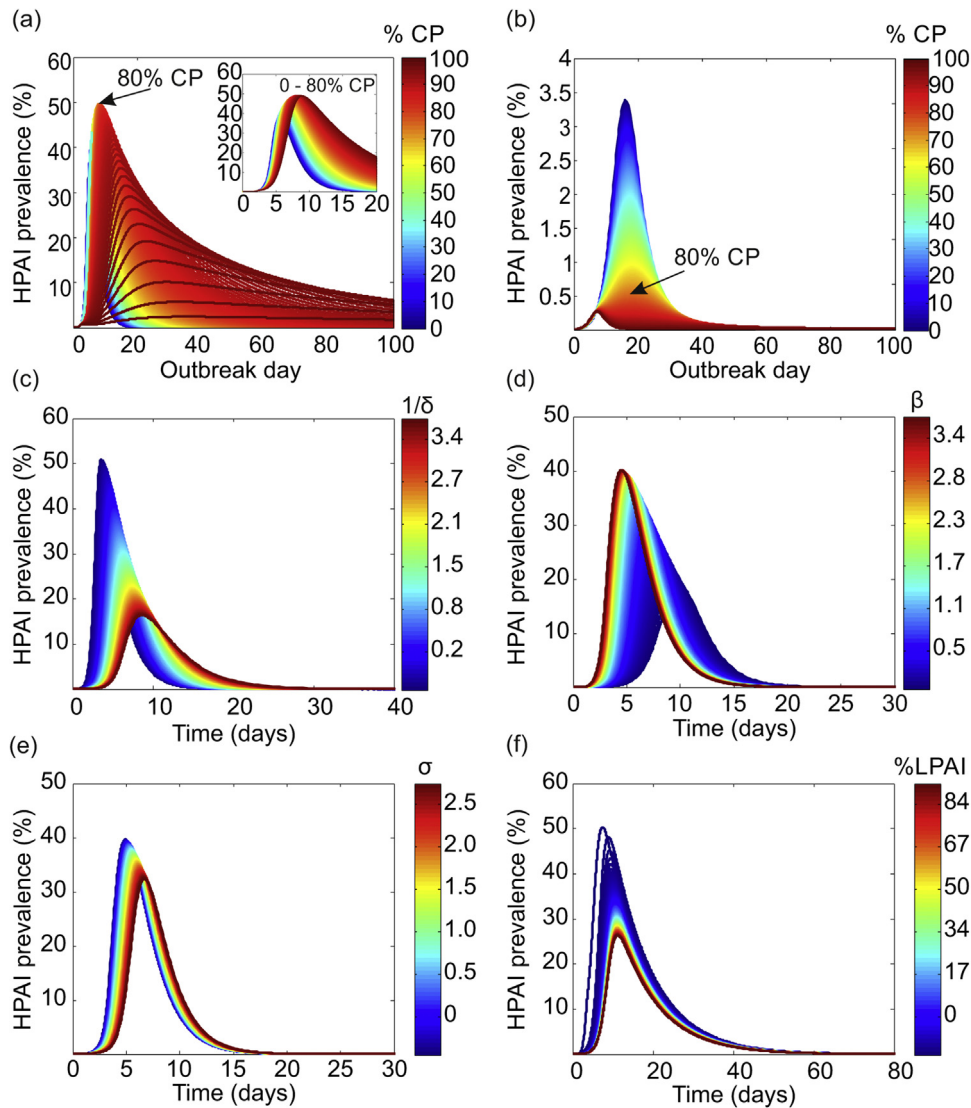
Fig. 2. Impact of immunological cross-protection on flock HPAI prevalence. Impact of immunological cross-protection (CP) on peak daily HPAI prevalence (% of birds infected with HPAI on a single day) assuming (a) aerosol and environmental transmission pathways. Inset shows results for 0%- 80% CP, and (b) aerosol transmission only. Impact of varying levels of (c) HPAI infection latent period $(1/\delta)$, (d) HPAI transmission rate (β), and (e) HPAI environmental decay rate (σ) on peak daily HPAI prevalence under 0% CP, and (f) varying the background LPAI prevalence assuming 80% CP. See Table 1 for further details of the parameter values examined.

est overall impact on flock HPAI prevalence, ranging from 38% of birds under zero CP to 50% under partial CP of 80%. Any further increases in CP beyond 80% resulted in a decrease in peak prevalence, reaching a minimum 2.56% at 100% CP (Fig. 2a). Importantly, when the environmental component of transmission was omitted, the relationship between CP and peak prevalence was reversed. This finding reveals the importance of assumed transmission pathways on the dynamics of avian influenza co-circulation within a poultry flock (Fig. 2b).

In sensitivity analyses, shortening HPAI latency to 80% less than that of LPAI (from 1 day to 5 h) corresponded with an increase in peak prevalence from 16% to 51% (Fig. 2c); increasing the rate of HPAI transmission to double that of LPAI (from 2 birds/day to 4 birds/day) resulted in an increase in peak prevalence from 18% to 40% (Fig. 2d); and decreasing the environmental decay rate of HPAI to 50% less than that of LPAI (from 1.2 g/day to 0.6 g/day) resulted in an increase in peak prevalence from 32% to 40% (Fig. 2e). The flock-level infectious period (the number of days in which at least one infectious bird was present) was also sensitive to immunological cross-protection, ranging 24 days at zero CP to 90 days at 80% CP.

Finally we investigated the sensitivity of our findings to the initial background prevalence of LPAI within a single co-infected commercial poultry flock. Under an extreme scenario where all birds were assumed LPAI-infected at day zero and assuming 80% CP (20% susceptibility to HPAI), peak HPAI prevalence was reduced from 50% to a minimum 26% of birds (Fig. 2f). However, the simulated outbreaks were largely insensitive to LPAI prevalence below 20%. In all scenarios investigated, LPAI prevalence had no notable impact on HPAI outbreak duration.

4. The avian influenza A(H7N1) epidemic in Italy, 1999–2001

The first case of LPAI of the subtype A(H7N1) was notified in northeast Italy in March 1999. By November that year the first suspected case of HPAI was reported in the poultry dense region of northeast Italy, Veneto. Phylogenetic analyses based on LPAI and HPAI viruses isolated from this epidemic indicate that the HPAI mutant emerged from the previously circulating LPAI viruses (see Supplementary material and Banks et al., 2001; Monne et al., 2014).

By the time of HPAI emergence there were an estimated 199 LPAI infected flocks and no means by which to enforce statutory control measures against the LPAI epidemic (Capua et al., 2003). Importantly for the spatial modelling analyses presented here, it is likely that LPAI viruses continued to circulate during the initial emergence of HPAI and were the likely source of a second LPAI wave post-HPAI eradication in August 2000 (see Supplementary material and Monne et al., 2014).

In April 2000 HPAI was eradicated from Italy following the implementation of statutory control measures. Of the 413 HPAI cases, 382 were confined to Veneto and its neighbouring region of Lombardia (Capua et al., 2003). Based on a phylogenetic birth-death skyline analysis conducted in BEAST2 software, median posterior effective reproduction numbers estimated for both variants were consistent with HPAI-targeted control measures successfully inhibiting HPAI epidemic growth but not LPAI. Further phylogenetic discrete-traits analysis also supported the likelihood that, in contrast to LPAI, the spread of HPAI was unconstrained by the poultry species/production type or geographical region (see Supplementary material for further details).

Here we adapted a stochastic spatial transmission model originally parameterised for the HPAI epidemic wave in northeast Italy (see Dorigatti et al., 2010 for details) by incorporating the presence of pre-existing LPAI cases at time zero of the HPAI epidemic. In this modelling framework, susceptible farms acquired infection at a rate estimated from the known timing of the 382 HPAI outbreaks, the production cycles among the total 3247 poultry farms in production during the epidemic period, and all pairwise inter-farm spatial distances. The premise of the model is that the probability of transmission from an infected to a susceptible farm decreases with increasing spatial distance in a poultry species and epidemic-phase dependent manner. The simulations also incorporate the key control measures that were enforced against HPAI during this epidemic: the culling of infected flocks followed by a ban on flock re-stocking, and pre-emptive culling of farms located within a 1 km radius of infected farms (See Dorigatti et al., 2010 for full details).

Because of geographical heterogeneity in the distribution of poultry species/production types and the species/production-specific incidence of infection in northeast Italy (Fig. 3a and b), we anticipate that two key features of the pre-existing LPAI cases may affect the spread of emergent HPAI among the poultry population: (i) the number of pre-existing LPAI-infected flocks present upon HPAI emergence, and (ii) the proximity of LPAI infected flocks to the 'emergent' HPAI-infected farm (i.e. the first farm in which an epidemiological fit HPAI mutant emerged). Based on the findings from our discrete-traits phylogenetic analyses (see Supplementary material), we made no demographic-related constraints on the spread of HPAI among the seeded LPAI cases.

5. Modelling the epidemiological impact of immunological cross-protection and LPAI-targeted control

We first investigated the effect of poultry demography on the opportunity for a LPAI-infected farm to acquire a secondary HPAI infection. Note that in all spatial simulations, the infection status of an individual farm was assumed to reflect the aggregated status of all associated flocks. To do so we seeded LPAI cases at random, either within 50 km of the 'emergent' HPAI-infected farm (d_{50km}) or under no distance constraint (d_{All}), whilst also varying the background LPAI prevalence.

The spatial model simulations confirmed a huge opportunity for secondary HPAI incursions to occur in a commercial poultry setting even when only a small number of LPAI cases were present upon HPAI emergence; at least one LPAI farm experienced secondary HPAI infection in 81% of simulations when the background
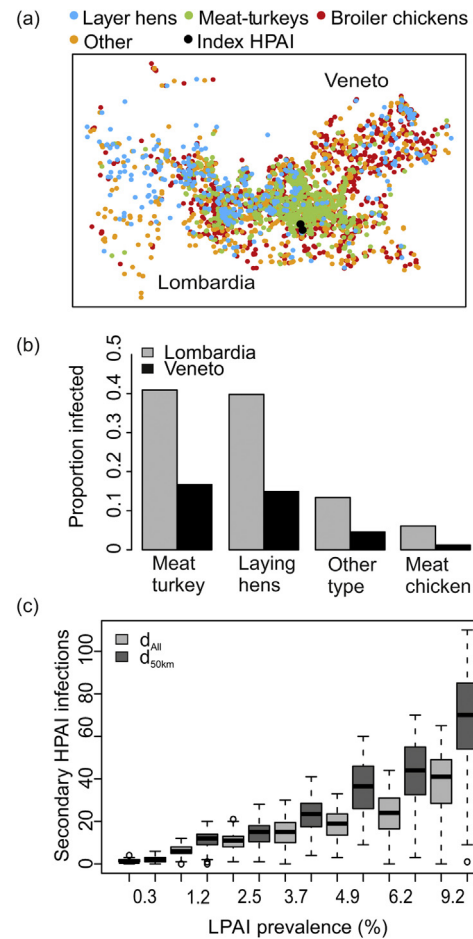

Fig. 3. Assessing the opportunity for epidemiological interference. (a) Spatial distribution of poultry farms located in Lombardia and Veneto, northeast Italy; (b) Region and production/species-specific incidence of HPAI infected farms; (c) Inter-quartile range of secondary HPAI infections across 100 model simulations for varying LPAI prevalence (corresponding with case numbers of 10, 40, 80, 120, 160, 200 and 300 infected farms) and distance to the 'emergent' HPAI-infected flock. d_{All} = no proximity constraint placed on location of LPAI cases; d_{50km} = LPAI cases constrained to within 50 km of the index HPAI farm. Note that these simulations were run in the absence of immunological cross-protection assumptions.

prevalence of LPAI was only 0.3% (n = 10 LPAI cases). The total number of secondary HPAI infections in a single epidemic simulation increased with LPAI prevalence but was less sensitive to the proximity of LPAI to the 'emergent' HPAI farm (Fig. 3c).

We then investigated the impact of immunological cross-protection (CP) on HPAI epidemic risk (the proportion of simulations resulting in >10 infected farms), epidemic size (cumulative number of new HPAI cases) and epidemic duration (number of days between first and last HPAI cases). We explored three scenarios: 80% CP, 100% CP, and LPAI-targeted control through culling. Motivated by our findings on the within-flock dynamics (see Section 3), the 80% CP scenario was based on the following assumptions: (i) LPAI-infected premises had 80% protection against (i.e. 20% susceptibility to) secondary HPAI infection for the duration of the production cycle, (ii) HPAI instantaneously displaced pre-existing LPAI outbreaks upon co-exposure, and (iii) farms in which LPAI displacement occurred had an 80% lengthening of the infectious period for the duration of the production cycle.

In contrast, in the 100% CP scenario we assumed that LPAI infected premises were fully protected against secondary HPAI infections for the duration of the production cycle. In both 80% and 100% CP scenarios, flocks resumed complete susceptibility to HPAI upon flock re-stocking at the start of the subsequent production

cycle. Similar to 100% CP, the LPAI-target control scenario assumed LPAI-infected farms were insusceptible to HPAI but in this case as a result of flock depopulation (along with HPAI-infected premises), as part of national control measures (note that at the time of the epidemic statutory control measures were only enforced against HPAI). In contrast to the 100% CP scenario, this removal of susceptible flocks was induced for a longer period of time—the duration of the HPAI epidemic.

We found that both immunological CP and LPAI-targeted control led to a reduction in the lower limit of HPAI epidemic risk to below that found in the absence of LPAI. The impact of 100% CP was similar to that produced by LPAI-targeted control; both scenarios led to a notable decrease in the lower limit of epidemic risk (Fig. 4a) and a significant decrease in median epidemic size (Fig. 4b) compared to in the absence of LPAI. However, despite these seemingly beneficial effects for the control of HPAI epidemics, 80% CP led to a significant increase in median HPAI epidemic duration whilst the 100% CP and LPAI-target control scenarios produced comparable HPAI epidemic durations as those observed in the absence of LPAI (Fig. 4c). The presented results are based on a LPAI prevalence of 6.2% (n = 200 LPAI cases). We note that the presented trends were qualitatively similar for varying assumptions of LPAI prevalence (see Supplementary material).

6. Discussion

Highly pathogenic avian influenza continues to pose a significant global public health and economic threat. It is well recognised that HPAI viruses emerge through mutation of LPAI in domestic poultry. During any emergence of HPAI that culminates in an epidemic it is evident that the precursor LPAI variant must co-circulate, at least within the single (or potentially multiple) bird(s) in which HPAI emerges as "epidemiologically fit". However, despite their co-circulation among poultry, the possibility of precursor LPAI viruses interfering with the spread of HPAI is not well understood. If we are to learn from the devastating impact caused by A(H5N1) avian influenza viruses, improved understanding of the ecological conditions that enable the invasion and spread of emergent HPAI in domestic poultry could prove valuable for the veterinary control of this disease.

Here, using mathematical models reflecting the two-pathogen transmission dynamics of avian influenza among commercial poultry, we have demonstrated the potential role of immunological cross-protection on the outcome of HPAI incursions in poultry flocks. When simulating the co-circulatory dynamics within a single flock, rather than inhibiting the spread of HPAI, our results imply primary infections with LPAI may enhance farm-to-farm transmission because of an increase in peak flock-prevalence. This finding is explained by a trade-off between the effects of immunological CP on reducing host susceptibility whilst at the same time enhancing survivability; high but partial immunological CP maximises the net effect of prolonged host survival. This finding echoes the historic notion from an evolutionary standpoint that severe disease may be sustainable when virulent pathogens are tolerated by the host (Ewald, 1983).

In the absence of comparative studies of avian influenza viral kinetics and infection life histories across homologous LPAI and HPAI viruses, we based the key infection parameters on an experimental study conducted on LPAI and HPAI viruses sampled during the Italian A(H7N1) epidemic (Saenz et al., 2012). Because HPAI-specific infection parameters may impact on the transmission of HPAI, we investigated the comparative importance of CP with these other infection parameters. For example, it is conceivable that the greater HA glycoprotein cleavability associated with HPAI mutant viruses results in a wider distribution of the virus within
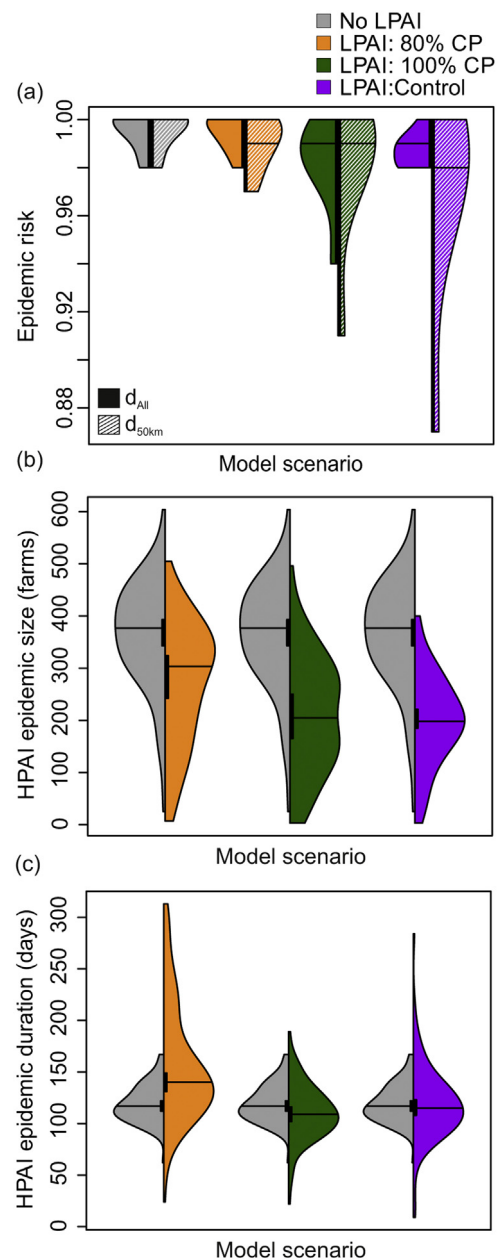

Fig. 4. Impact of immunological cross-protection and LPAI-targeted control on HPAI epidemic risk, size, and duration. Probability density function estimates based on spatial model simulations for (a) epidemic risk (probability of an HPAI epidemic i.e. >10 cases). Each scenario is based on 100 × 7 model simulations (100 simulations per LPAI prevalence investigated). Note decrease to lower limit of epidemic risk for 100% CP and LPAI-targeted control scenarios compared to in the absence of LPAI albeit with wide confidence intervals around the median estimate; (b) HPAI epidemic size; and (c) HPAI epidemic duration. Horizontal lines indicate median estimates and vertical bars indicate Binomial 95% confidence intervals around the median. For (a,c) each scenario is based on 100 model simulations and assumes a background LPAI prevalence of 6.2% (n = 200 LPAI cases) upon HPAI emergence. Density plots were produced by adapting the 'vioplot' function of the package 'violin' available in R software v. 3.1.1 (Hintze and Nelson, 1998; R Core Team, 2013). See Supplementary material for sensitivity analysis.

the host, a shorter time to infectiousness, and a greater viral load during infection, thereby resulting in a greater transmission rate and shorter latency compared with LPAI viruses. Our sensitivity analyses revealed that peak HPAI prevalence and individual flock outbreak duration were less sensitive to these alternative factors than to immunological CP.

We note that our inferences regarding the effect of immunological CP on the spread of HPAI within a poultry flock are based on assumptions built into the mathematical models and are therefore limited by the possibility of missed effects of other unaccounted for variables. In particular, further knowledge on the likely level of CP for a given bird at a given point in infection, and the potential outcome of any within-host competition dynamic between the two pathogenic variants, would help enumerate more accurate predictions for HPAI invasion success and spread at the flock level.

Using a spatial simulation model representing the farm-to-farm spread of HPAI A(H7N1) in northeast Italy (Dorigatti et al., 2010), we explored the effects of immunological cross-protection and LPAI-targeted control within a heterogeneous commercial poultry setting. We found that both cross-protection and LPAI-targeted control scenarios reduced HPAI epidemic risk as a consequence of depleting the pool of farms susceptible to HPAI incursions. However, cross-protection had the effect of prolonging HPAI epidemic duration, thereby enhancing the long-term evolutionary success of the novel and highly virulent HPAI mutants when epidemics do arise. Interestingly, this effect of prolonged epidemic duration was found under the assumption of partial (80%) but not complete (100%) immunological CP supporting the findings of a previous theoretical modelling study (de Leo and Bolzoni, 2012) that partial CP may be key to the epidemiological success of emergent HPAI.

Our findings demonstrate the measurable effect at the population level of the trade-off between reduced farm susceptibility and enhanced flock infectious periods as a consequence of immunological CP; the net effect of enhanced flock infectious periods is maximised under partial CP. Incorporating the likely heterogeneity in within-flock dynamics across different farms, and improved understanding into infection and immune kinetics of LPAI and HPAI viruses during co-infection, would improve the accuracy of the model.

7. Conclusion

Viral interference has a considerable potential to influence the epidemiology of avian influenza in a densely populated commercial poultry setting. Here, we show that, in simulation, LPAI need not inhibit the invasion of HPAI at either individual farm or population scales. On the contrary, partial immunological cross-protection may lead to the enhanced evolutionary success of HPAI. These findings may have implications for veterinary control activities to mitigate the public health and economic impacts of avian influenza. In particular, our findings regarding enhanced HPAI spread within an individual flock has importance for endemic settings such as east Asia where partial cross-protection to homo and heterosubtypic avian influenza variants is a potential mechanism for the "silent spread" of A(H5N1) within live bird markets (Imai et al., 2007). More generally, our study has implications for the effectiveness of veterinary control measures against avian influenza (Savill et al., 2006).

Conflicts of interest

None.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.epidem.2016.10.005.

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