Supplementary Information for Silent spread of H5N1 in vaccinated poultry

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The Model

The model is individual based. Each bird has parameter values randomly chosen from appropriate distributions, and we track the infection status of each and every bird. Each bird is assigned a latent period, an asymptomatic period, a symptomatic period, a susceptibility, infectiousness rates for virus excreted in faeces and exhaled and an antibody titre quantified as a haemagglutination inhibition test. All model parameters and their distributions are given in Supplmentary Table 1.

In terms of the model, floor reared birds are a special case of caged birds with just one cage. The status of the birds and the infectiousness of airborne virus and virus in faeces are updated every hour. At hour 0 we assume that a small amount of infective faeces (equal to the amount of infective faeces one bird excretes in an hour) contaminates a single cage in a caged system or is deposited on the floor in a floor–reared system. The rate of infection r_i, of susceptible bird i in cage j is given by the susceptibility of bird i (which can be reduced by vaccination) multipled by the infectiousness of infective faeces and airborne virus and divided by the number of birds per cage (thus assuming frequency–dependent transmission), i.e.,

$$r_i = \frac{s_i}{1+P_i} \cdot \frac{T_{\mathrm{f},j} + T_{\mathrm{a},j}}{n}, \tag{1}$$

where s_i is the unvaccinated susceptibility of bird i, n the number of birds per cage, $T_{\mathrm{f},j}$ the infectiousness of infective faeces in cage j, $T_{\mathrm{a},j}$ the infectiousness of airborne virus in cage j and P_i the reduction in susceptibility caused by vaccination.

We assume that susceptibility drops exponentially with vaccine–induced antibody titre which is conventionally quantified by the haemagglutination inhibition (HI) test[1]. We use HI in the model because there is good data on its distribution within flocks[2]. We assume that birds with HI titres below a certain threshold H_t, are not protected even though they may have been vaccinated. Thus P_i is given by

$$P_i = \begin{cases} 0 & \text{if } H_i < H_t, \\ 10^{a_P(H_i - H_t)} - 1 & \text{if } H_i \geq H_t, \end{cases} \tag{2}$$

where H_i is bird i's antibody titre quantified by the HI test and a_P the rate at which susceptibility decreases with antibody titre. In the main text we assume $a_P \to \infty$, which means that the vaccine is 100% effective and that vaccinated birds with $H_i \geq H_t$ cannot be infected. We also consider here $a_P = 0.05$ and 0.1, which means that the vaccine is imperfect and that vaccinated birds can become subclinically infected. In reality, many factors determine whether a vaccinated bird becomes infected, including age, species, challenge dose, health, antibody titre, infections of immuno–suppressive diseases and cross–reactivity of other AI serotypes[3,4,5,6,7].

Once birds become infected they are latent (asymptomatic and non–infectious) for L hours. They then become asymptomatic and infectious for A hours. We assume that the infectiousness of virus excreted by birds is constant throughout their infectious periods. At the end of the asymptomatic period birds have a probability p_S, of becoming symptomatic otherwise they die. Symptomatic birds are infectious for S hours and then die.

At the beginning of a simulated hour we reduce the infectiousness of infective faeces T_{f}, in all cages by a proportion d. The default value of d models decay of infectiousness in wet faeces at 25°C. We next calculate the infectiousness of any new faeces (reduced if birds have been vaccinated) that has been excreted by all infectious birds in each cage, which, for cage j, is given by

$$T_{\mathrm{f},j}^{\mathrm{new}} = \sum_{i \in \text{infectious bird in cage } j} \frac{E_{\mathrm{f},i}}{1+V_i} \tag{3}$$

where $E_{\mathrm{f},i}$ the infectiousness of faeces excreted by infectious bird i (if unvaccinated) in one hour and V_i the reduction in the faeces' infectiousness caused by vaccination of bird i. Similar to the derivation of P_i in Equation 2, V_i is given by

$$V_i = \begin{cases} 0 & \text{if } H_i < H_t, \\ 10^{a_V(H_i - H_t)} - 1 & \text{if } H_i \geq H_t. \end{cases} \tag{4}$$

where a_V is the rate of reduction of faeces' infectiousness with HI titre.

We assume that only a proportion l, of the faeces excreted are retained in cages due to loss through openings in the cages. The remaining faeces in a cage are spread equally among neighbouring cages up to a distance z_{f} cages away. We have no estimate for how far faeces can be spread in caged flocks; we assume in the main text spread to only contiguous cages, although faeces could be moved farther distances on feed conveyor belts, for example. However, it is known that spread of infection is slower in caged systems than in floor–reared systems[8,9], so spread is localised in caged systems.

Also at the beginning of each simulated hour we calculate the infectiousness of airborne virus exhaled in each cage. We assume that viral shedding rates from the cloacal and trachael are reduced by the same proportion in a vaccinated bird. Virus exhaled in cage j is given by

$$T_{\mathrm{a},j}^{\mathrm{new}} = \sum_{i \in \text{infectious bird in cage } j} \frac{E_{\mathrm{a},i}}{1+V_i} \tag{5}$$

where $E_{\mathrm{a},i}$ is the infectiousness of airborne virus excreted by infectious bird i (if unvaccinated) in one hour. Airborne virus is assumed to spread equally among neighbouring cages up to a distance z_{a} cages away. We assume that airborne virus is less infectious than in faeces[9]. There is no quantitative estimate for the relative infectiousness of these transmission routes, so we assume that transmission in faeces is ten times more likely than through inhalation (see Sensitivity Analysis for different assumptions).

At the end of a vaccinated bird's asymptomatic period, a check is made to determine if it will become symptomatic. If it becomes symptomatic another check is made at the end of its symptomatic period to determine if it dies. The probability p_i, of bird i becoming symptomatic or dying is given by

$$p_i = \frac{1}{1+S_i}, \tag{6}$$

where

$$S_i = \begin{cases} 0 & \text{if } H_i < H_t, \\ 10^{a_S(H_i - H_t)} - 1 & \text{if } H_i \geq H_t, \end{cases} \tag{7}$$

is the reduction in the probability of becoming symptomatic or dying caused by vaccination and a_S is the rate of reduction in the probability of becoming symptomatic or dying with HI titre. In addition, the infectious period of vaccinated birds with $H_i \geq H_t$ that do not become symptomatic or become symptomatic but do not die is assumed to decay linearly with the log of their HI titre. For bird i, this is given by

$$\text{infectious period} = \begin{cases} I_{\mathrm{v},i} + \log_2 H_t - \log_2 H_i & \text{if } H_t \leq H_i \leq I_{\mathrm{v},i} + H_t, \\ 0 & \text{otherwise} \end{cases} \tag{8}$$

where we have assumed that a drop of 1–log in HI titre translates into a 1–day reduction in infectious period. For unvaccinated birds and vaccinated birds with $H_i < H_t$ infectious period is determined by the distributions of asymptomatic and symptomatic periods.

The values of a_P, a_V and a_S are not known. However, current vaccines reduce viral shedding by approximately 10,000–fold at a HI titre of about 1:40, this translates into a value of 0.1 for a_V. We also perform simulations of a poorer vaccine with a 100–fold reduction in virus shedding rate. This translates into a value of 0.05 for a_V. Parameters a_P and a_S are assumed to have the same value as a_V.

The flock is checked D_e times per day, in practice, usually once in the morning and once in the evening. A tally of dead birds is kept. Detection can occur in a variety of ways:

- Mortality greater than D_d% in two consecutive 24 hour periods.
- Mortality greater than D_w% over the last week.
- Reduction in feed or water intake or egg production of D_S%. Our proxy for this is the number of symptomatic birds divided by the number of birds alive.
- Fraction of cages with the number of dead birds greater than $D_p n$ is greater or equal to D_c.
- Death of a single infected sentinel bird.

As well as death due to infection, birds also stochastically die of other causes at a constant background rate of b per day.

In a caged system we model two methods of placing sentinels in cages: at most one sentinel per cage in randomly chosen cages (main text), and randomly chosen cages containing just sentinels (sensitivity analysis).

Mean susceptibility μ_s, is set to 1 with no loss of generality. Mean infectiousness of infective faeces is then found by conditioning the model output on the observation that all birds in floor reared systems die within 1 week of first detection[10]. With our default parameters, this gives $\mu_{\mathrm{f}} \approx 0.1$ and $R_0^{\mathrm{within}} \approx 66$ in unvaccinated floor–reared birds and $R_0^{\mathrm{within}} \approx 25$ in caged systems with 8 birds per cage.

Sensitivity Analysis

Using the parameters in Supplmentary Table 1 as default, we varied all of the parameters to test their effect on the probability of an outbreak, probability of detection, probability

of an outbreak at end of cycle and flock infectiousness. Parameters can be loosely classified as affecting housing, affecting R_0^{within}, affecting detection and affecting the effects of vaccination. In the following figures we show only those parameters in each class that have the largest effect on the variables. The results for the default parameters are shown in the upper row of graphs in each figure.

In Supplmentary Figure 1 we show the effects of different housing conditions. For flocks without sentinels, the probability of an outbreak is increased with greater mixing of birds (**c**, **d** and **e**). The probability of undetected outbreaks during the production cycle and at the end of the production cycle is reduced if spread within the flock is faster (**d**, **e** and **f**). Increasing the number of birds in direct contact greatly increases flock infectiousness (compare **a** with **c** and **d** with **e**). All negative effects of vaccination are mitigated or reduced if sentinels are used.

In Supplmentary Figure 2 we show the effect of parameters that change R_0^{within} and R_c^{within}. For flocks without sentinels, the probability of an outbreak is increased for slower decay of faeces' infectiousness and for imperfect vaccines (**d**, **e** and **f**). The probability of detecting outbreaks during the production cycle, the probability of undetected outbreaks at end of cycle and relative flock infectiousness all decrease as R_c^{within} decreases (compare **a** against all). In addition, the peak of the probability of undetected outbreaks and the peak of flock infectiousness move to lower protection levels as R_c^{within} declines. All negative effects of vaccination are mitigated or reduced if sentinels are used.

In Supplmentary Figure 3 we show the effect of different detection thresholds. Mortality thresholds and the threshold for disease symptoms (not shown) are robust to changes (**c**, **d** and **e**). The greatest uncertainty is in the number of cages containing dead birds that trigger detection (**b**). If detection is triggered by deaths in just one cage then probability of undetected outbreaks at end of cycle and flock infectiousness are significantly reduced due to faster detection. However, with only 100 sentinels, not all cages contain sentinels and therefore outbreak duration can still rise with protection levels. Having fewer cages containing only sentinels causes a similar problem (**f**).

Parameters that have negligible effect are the probability of showing symptoms if unvaccinated (p_S), the infectious period of vaccinated birds (I_{v}), mean latent period, background mortality rate (b), and infectiousness of airborne virus (μ_{a}). Changes in the threshold HI titre below which vaccination has no effect (H_t) is similar to changing the fraction of protected birds. Changes in the mean (μ_H) and standard deviation (σ_H) HI titre of a flock will affect both the fraction of birds with HI titre greater than H_t and the flock–protection level which changes R_c^{within}.

References

1. Alexander, D. J. Highly pathogenic avian influenza. In *OIE manual of standards for diagnostic tests and vaccines. 4th edition*, 212–220. World Organisation for Animal Health OIE, Paris, (2000).

2. Ellis, T. M., Sims, L. D., Wong, H. K., Wong, C. W., Dyrting, K. C., Chow, K. W.and Leung, C., and Peiris, J. Use of avian influenza vaccination in Hong Kong. *Dev. Biol.* **124**, 133–143 (2006).

3. Brugh, M., Beard, C. W., and Stone, H. D. Immunization of chickens and turkeys against avian influenza with monovalent and polyvalent oil emulsion vaccines. *Am. J. Vet. Res.* **40**, 165–169 (1979).

4. Wood, J. M., Kawaoka, Y., Newberry, L. A., Bordwell, E., and Webster, R. G. Standardization of inactivated H5N2 influenza vaccine and efficacy against lethal A/Chicken/Pennsylvania/1370/83 infection. *Avian Dis.* **29**, 867–872 (1985).

5. Stone, H. D. Efficacy of avian influenza oil-emulsion vaccines in chickens of various ages. *Avian Dis.* **31**, 483–490 (1987).

6. Swayne, D. E., Beck, J. R., Garcia, M., and Stone, H. D. Influence of virus strain and antigen mass on efficacy of H5 avian influenza inactivated vaccines. *Avian Pathol.* **28**, 245–255 (1999).

7. Seo, S. H. and Webster, R. G. Cross-reactive, cell-mediated immunity and protection of chickens from lethal H5N1 influenza virus infection in Hong Kong poultry markets. *J. Virol.* **75**, 2516–2525 (2001).

8. Sims, L. D., Ellis, T. M., Liu, K. K., Dyrting, K., Wong, H., Peiris, M., Guan, Y., and Shortridge, K. F. Avian influenza in Hong Kong 1997-2002. *Avian Dis.* **47**, 832–838 (2003).

9. Shortridge, K. F., Zhou, N. N., Guan, Y., Gao, P., Ito, T., Kawaoka, Y., Kodihalli, S., Krauss, S., Markwell, D., Murti, K. G., Norwood, M., Senne, D., Sims, L., Takada, A., and Webster, R. G. Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virology* **252**, 331–342 (1998).

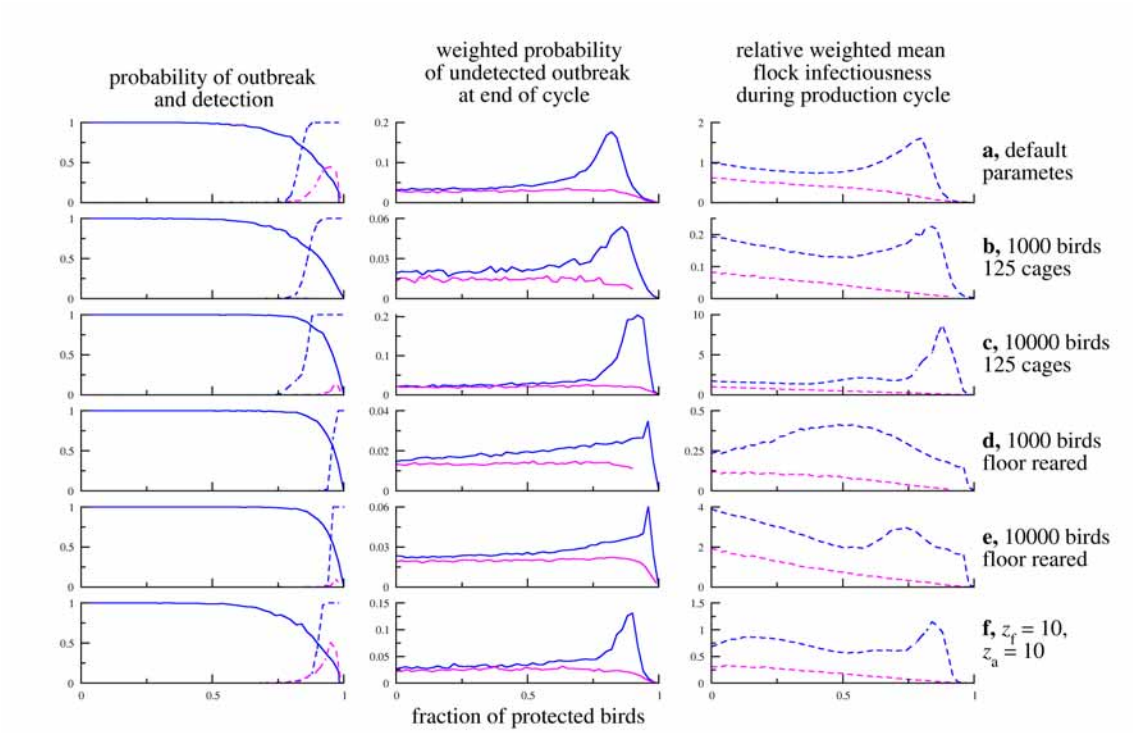
10. Elbers, A. R., Fabri, T. H., de Vries, T. S., de Wit, J. J., Pijpers, A., and Koch, G. The highly pathogenic avian influenza A (H7N7) virus epidemic in The Netherlands in 2003 - lessons learned from the first five outbreaks. *Avian Dis.* **48**, 691–705 (2004).

11. Stegeman, A., Bouma, A., Elbers, A. R. W., de Jong, M. C. M., Nodelijk, G., de Klerk, F., Koch, G., and van Boven, M. J. Avian influenza A virus (H7N7) epidemic in The Netherlands in 2003: course of the epidemic and effectiveness of control measures. *J. Infect. Dis.* **190**, 2088–2095 (2004).

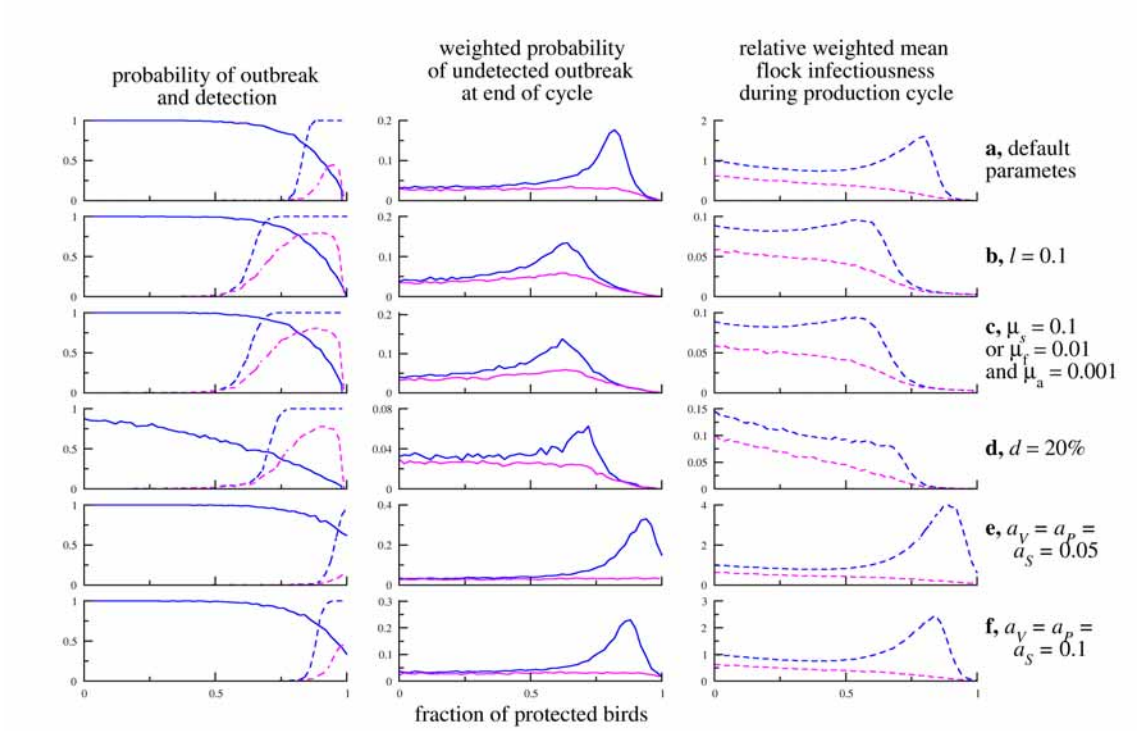
12. Elbers, A. R., Koch, G., and Bouma, A. Performance of clinical signs in poultry for the detection of outbreaks during the avian influenza A (H7N7) epidemic in The Netherlands in 2003. *Avian Pathol.* **34**, 181–187 (2005).

Parameter	description	Default value or distribution	units	Reference
Flock and housing parameters				
c_{row}	number of cages in a row	250		
c_{col}	number of cages in a column	5		
n	number of birds per cage	8		
b	daily background mortality	0.05%		
l	fraction faeces retained in cage	1		
z_{f}	dispersal distance of infective faeces	1	cages	
z_{a}	dispersal distance of airborne virus	1	cages	
Individual bird parameters				
L	latent period	$48 + \text{Binomial}(48, 0.25)$	hours	[11]
A	asymptomatic period	$24 + \text{Binomial}(24, 0.25)$	hours	[11]
S	symptomatic period	$\text{Binomial}(96, 0.05)$	hours	[11]
s	susceptibility	$\text{Gamma}\left(\frac{\mu_s^2}{\sigma_s^2}, \frac{\sigma_s^2}{\mu_s}\right)$		
H	antibody titre	$\text{Gamma}\left(\frac{\mu_H^2}{\sigma_H^2}, \frac{\sigma_H^2}{\mu_H}\right)$		
E_{f}	infectiousness of virus excreted in faeces in one hour	$\text{Normal}(\mu_{\text{f}}, 0.25\mu_{\text{f}})$		
E_{a}	infectiousness of airborne virus excreted in one hour hour	$\text{Normal}(\mu_{\text{a}}, 0.25\mu_{\text{a}})$		
Virus parameters				
μ_s	mean susceptibility	1		
σ_s	standard deviation of susceptibility	0.1		
p_S	probability of showing symptoms if unvaccinated	0.8		
μ_{f}	mean infectiousness of virus excreted in faeces in one hour	0.1		
μ_{a}	mean infectiousness of airborne virus excreted in one hour hour	0.01		[8,9]
d	percentage reduction in faeces infectiousness per hour	5%		[9]
Vaccine and vaccination parameters				
H_{t}	antibody titre below which vaccine has no effect	1		
μ_H	average antibody titre	75		[2]
σ_H	standard deviation of antibody titre	65		[2]
a_V	rate of decrease of virus excretion with antibody titre	∞		
a_P	rate of decrease of susceptibility with antibody titre	∞		
a_S	rate of decrease of chance of symptoms or death with antibody titre	∞		
I_{v}	maximum infectious period of vaccinated birds	168	hours	
Detection threshold parameters				
D_e	times per day birds are checked	2		
D_d	daily mortality threshold	0.5%		[10]
D_w	weekly mortality threshold	3%		[12]
D_s	symptoms threshold	5%		
D_p	fraction of birds dead in a cage	50%		
D_c	fraction of cages with dead $> D_p n$	1%		

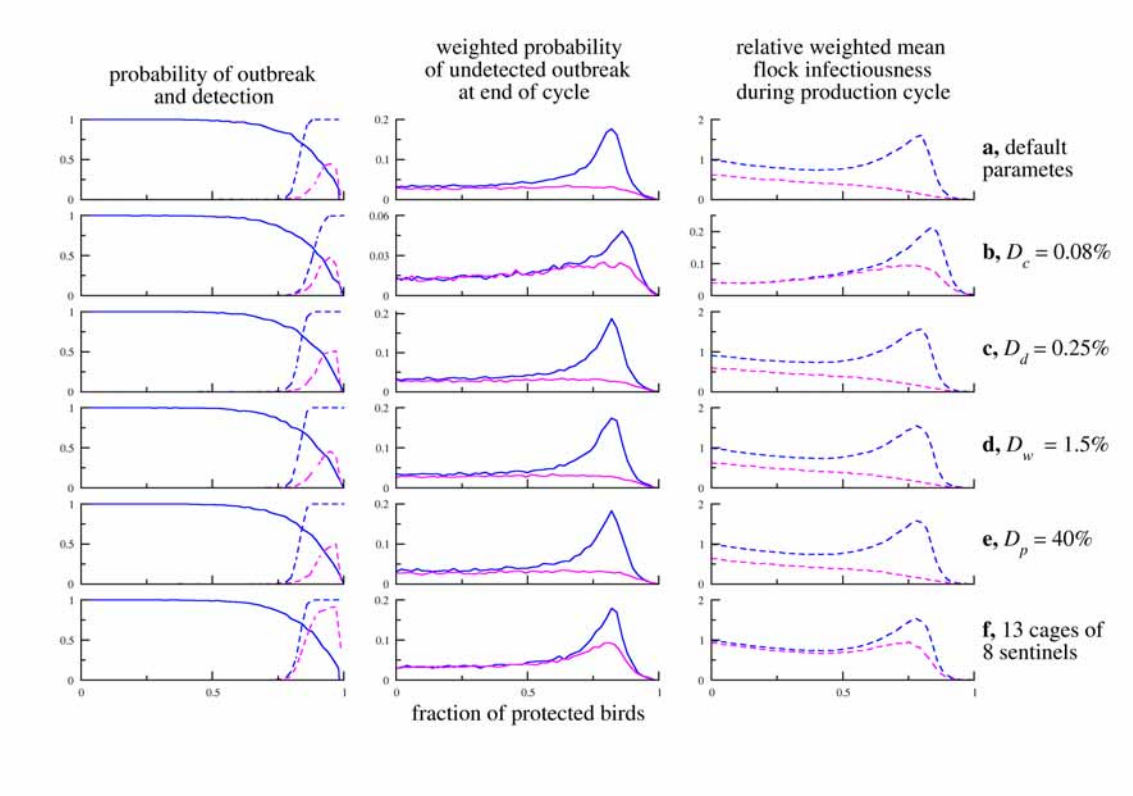
Supplementary Table 1: Model parameters and their default values



Supplementary Figure 1: **Variation in flock structure and housing conditions.** Effect on probability of an outbreak for no or 100 sentinels (solid blue line, left–hand column), probability of undetected outbreaks for no sentinels (dashed blue line, left–hand column) and 100 sentinels (dashed magenta line, left–hand column) against fraction of birds protected. Probability of undetected outbreak at end of production cycle, weighted by the probability of outbreak (middle column, blue line: no sentinels, magenta line: 100 sentinels). Mean flock infectiousness during the production cycle weighted by the probability of outbreak and relative to an unvaccinated flock with no sentinels using default parameters (right–hand column, dashed blue line: no sentinels, dashed magenta line: 100 sentinels) **a**, Default parameters as given in Supplmentary Table 1. **b**, 1,000 birds in 125 cages with 8 birds per cage. **c**, 10,000 birds in 125 cages with 80 birds per cage. **d**, 1,000 floor–reared birds. **e**, 10,000 floor reared birds. **f**, Spread of faeces and airborne virus up to 10 cages away in a 1,250 cage system with 8 birds per cage. Fraction of birds protected is varied from 0 to 1 in steps of 0.02, and variables are averaged over 1,000 simulated incursions of contaminated faeces into a single cage.



Supplementary Figure 2: **Variation in R_0^{within} and R_c^{within}.** **a**, Default parameters as given in Supplmentary Table 1. **b**, 90% of infective faeces lost through cage floor, compared to none. **c**, 10 times lower mean susceptibility or, equivalently, infectiousness of faeces and airborne virus. **d**, Faster decay of faeces' infectiousness, modelling drying of faeces within 2 days instead of a week. **e**, An imperfect vaccine that causes a 100–fold reduction in susceptibility, infectiousness, symptoms and dieing at a HI titre of 1:40. **f**, An imperfect vaccine that causes a $10,000$–fold reduction in susceptibility, infectiousness, symptoms and dieing at a HI titre of 1:40.



Supplementary Figure 3: **Variation in detection parameters**. **a**, Default parameters as given in Supplmentary Table 1. **b**, Just one cage containing 50% dead birds needed to trigger detection. **c**, Daily mortality detection threshold halved. **d**, Weekly mortality detection threshold halved. **e**, Fraction of dead birds in a cage needed to trigger detection reduced to 40%. **f**, 13 randomly chosen cages each containing 8 sentinels compared with 100 cages each containing 1 sentinel (see Fig. 1d in paper).