Risk assessment for recrudescence of avian influenza in caged layer houses following depopulation: The effect of cleansing, disinfection and dismantling of equipment.

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Short title: Cleansing and disinfection risk assessment

Abstract

Following an outbreak of **highly pathogenic avian influenza virus (HPAIV)** in a poultry house, control measures are put in place to prevent further spread. An essential part of the control measures based on the European Commission Avian Influenza Directive 2005/94/EC is the **cleansing and disinfection (C&D)** of infected premises. C&D includes both preliminary and secondary C&D and the dismantling of complex equipment during secondary C&D is also required, which is both costly to the owner and also delays the secondary cleansing process hence increasing the risk for onward spread. In this study a quantitative risk assessment is presented to assess the risk of re-infection (recrudescence) occurring in an enriched colony caged layer poultry house on restocking with chickens after different C&D scenarios. The risk is expressed as the number of restocked poultry houses expected before recrudescence occurs. Three C&D scenarios were considered namely (i) preliminary C&D alone, (ii) preliminary C&D plus secondary C&D without dismantling and (iii) preliminary C&D plus secondary C&D with dismantling. The source-pathway-receptor framework was used to construct the model and parameterisation was based on the three C&D scenarios. Two key operational variables in the model are (i) the **time between depopulation of infected birds and restocking with new birds (TbDR)** and (ii) the proportion of infected material that by-passes C&D, enabling virus to survive the process. Probability distributions were used to describe these two parameters for which there was recognised variability between premises in TbDR or uncertainty due to lack of information in the fraction of by-pass. The risk assessment estimates that the median (95% credible intervals) number of repopulated poultry houses before recrudescence are $1.2\ 10^4$ (50 to $2.8\ 10^6$), $1.9\ 10^5$ (780 to $5.7\ 10^7$) and $1.1\ 10^6$ ($4.2\ 10^3$ to $2.9\ 10^8$) under C&D scenarios (i), (ii) and (iii) respectively. Thus for HPAIV in caged layers undertaking secondary C&D without dismantling reduces the risk by 16-fold compared to preliminary C&D alone. Dismantling has an additional, although smaller, impact, reducing

the risk by a further six-fold and thus around 90 fold compared to preliminary C&D alone. On the basis of the 95% credible intervals, the model demonstrates the importance of secondary C&D (with or without dismantling) over preliminary C&D alone. However, the extra protection afforded by dismantling may not be cost beneficial in the context of reduced risk of onward spread.

Key words: Notifiable avian disease; outbreak; control; policy; poultry house.

Implications

Disease caused by highly pathogenic avian influenza virus (HPAIV) severely impacts on the profitability of poultry farming. It is important to ensure that levels of residual HPAIV infectivity in the poultry house are sufficiently reduced to ensure recrudescence does not occur. The outputs of the work presented here have important benefits through supporting reductions in both labour costs to the farmer and in the time to complete secondary cleansing and disinfection by not having to dismantle and rebuild complex equipment. The results of the risk assessment will help inform policy-makers and industry in their decision-making and the risk assessment model could be applied to other avian pathogens such as Newcastle disease virus using appropriate data.

Introduction

Avian influenza is an infectious viral disease in birds, including both domestic poultry and wild birds. Infections caused by avian influenza viruses in poultry cause two forms of the disease that are distinguished by their pathogenicity. The low pathogenicity phenotype generally only causes mild clinical signs, while the **highly pathogenic avian influenza (HPAI)** phenotype results in very high mortality rates in most poultry species. Disease caused by **highly pathogenic avian influenza virus (HPAIV)** may have a severe impact on the profitability of poultry farming and infected poultry flocks are typically culled (in developed countries) with potential contacts to other poultry establishments being traced so as to contain the spread of disease. Several HPAIV subtypes are currently circulating and are considered endemic in parts of the world such as south-east Asia. During the period January 2013 to August 2018 (OIE, 2018), 12 different HPAIV subtypes were reported worldwide with Europe reporting the highest virus diversity (7 subtypes).

New virus strains with altered transmission and infection properties may emerge through genetic reassortment and mutation. During the winters of 2016/17 and 2017/18 multiple incursions of HPAIV into Europe including the **United Kingdom (UK)** (Hansen et al., 2018) occurred. The outbreak of HPAI H5N8 virus induced disease across Europe in the winter of 2016/17 was particularly severe affecting both wild birds and poultry and was the largest ever recorded in Europe in terms of number of poultry outbreaks, geographical extent and number of dead wild birds (Alarcon et al., 2018). The HPAI H5N6 virus which emerged in the Netherlands in late 2017 caused many events in wild birds in the UK and Republic of Ireland in that winter (Roberts et al., 2018) but did not affect poultry in the UK and resulted in only limited wild bird mortality in continental Europe with very few poultry outbreaks. HPAI is a notifiable disease internationally and following an outbreak in poultry, control measures are put in place to prevent further spread. Effective and rapid control of HPAIV in poultry is important to prevent its spreading from an infected poultry house to

other poultry flocks through infection of wild birds or through fomite transmission. An essential part of the control measures based on the European Commission Avian Influenza Directive 2005/94/EC (EU, 2005) is the **cleansing and disinfection (C&D)** of infected premises. Cleansing and disinfection includes preliminary and secondary C&D and the dismantling of complex equipment during secondary C&D is also required. Preliminary C&D is Government funded and involves spraying all parts of the premises and any contaminated material remaining with disinfectant to 'damp down' any virus in the environment. Secondary C&D is at the owner's expense and requires cleansing the premises, including equipment and installations, to remove organic debris, degreasing and disinfecting and then repeating the process.

In the absence of epidemiological evidence and data on how effective dismantling is in preventing further outbreaks of HPAIV in a poultry house after C&D, a quantitative risk assessment model is developed here to assess the probability that newly introduced immunologically-naive chickens used to restock a poultry house become infected (recrudescence) with HPAIV after C&D has taken place. Three C&D scenarios in a caged layer house are assessed, namely preliminary alone, preliminary plus secondary without dismantling and preliminary plus secondary with dismantling with data drawn primarily from HPAIV H5N1 scenarios.

Materials and methods

Risk analysis and risk assessment

The terms risk analysis and risk assessment have different meanings. Risk analysis is the complete process for handling a threat. Risk assessment is a defined stage of the risk analysis process. Thus the risk analysis process is hazard identification followed by the risk assessment itself and finally risk management with risk communication important for all three stages (OIE, 2019). The risk assessment estimates the risks associated with the

hazard and may be qualitative or quantitative. It should be noted that hazard and risk are different. The hazard is the pathogen, HPAIV in this case, while the risk is the probability of an adverse event from the hazard occurring, namely recrudescence of HPAI in the restocked poultry. Risk assessment is one of a number of tools to help manage and prevent poultry diseases like HPAIV through predicting the risks of outbreaks and assessing by how much various control processes reduce those risks. Other tools include epidemiological case studies based on previous outbreaks to identify and rank those factors which contribute to incursion, transmission and spread of such diseases. The advantage of risk assessment is that it can used to predict the probability of outbreaks occurring so that preventative actions may be implemented through risk management and policy (Goddard et al., 2012), hopefully before an outbreak occurs.

The risk assessment here is based on quantifying the amount of infectivity that restocked poultry (the receptor) are exposed to from infectious HPAIV remaining in the poultry house (the source) through all the conceivable exposure pathways within the poultry house (the pathway). Conceptually these risk assessments, known as “source-pathway-receptor” models, are relatively simple mathematically although the pathways may be complex depending on the system being studied. The structure of the risk assessment has to be appropriate for the system and the hazard. Thus the source-pathway-receptor model is well suited to environmental/process risk assessments involving a series of protective barriers. Another risk assessment approach is the entry-exposure-consequence assessment used for import risk assessment for exotic livestock diseases (OIE, 2019) and is often qualitative as for example for importation of lumpy skin disease virus into the UK through cattle hides (Gale et al., 2015). Qualitative assessment does not require mathematical modelling skills to carry out and so is often the type of assessment used for rapid, reactive, evidence-based decision making (Kelly et al., 2018).

The choice of qualitative or quantitative in risk assessment depends on the nature of the available data and the complexity of the model and also the scope of the risk question as set by the risk manager. Qualitative risk assessment can be applied in the absence of sufficient numerical data but where there is at least some basic knowledge, expert opinion or other understanding of the magnitude of the risks for each of the risk assessment steps. The model here allows for by-pass of the C&D process and is too complex for qualitative risk assessment. Also being a multiple barriers model (i.e. including removal of manure at the poultry house, destruction of virus by C&D and decay with time) it is not necessarily suited to combining multiple low qualitative conditional probabilities using a risk matrix approach (Kelly et al., 2018). Furthermore adding qualitative probabilities from several parallel streams as required here is not straight forward. The risk assessment approach here is therefore quantitative and complements a previous qualitative assessment (Horigan et al., 2019).

Once the basic mathematical model as defined by the equations relating levels of HPAIV in the poultry house at point of culling to the risk of infection in the restocked poultry have been set out, there are several different approaches for quantitative risk assessment including deterministic and probabilistic. The deterministic approach calculates the arithmetic mean for each step in the source-pathway-receptor model and tends to deal with uncertainty by using worst case assumptions particularly where data are lacking (Gale, 2004 and 2005). The probabilistic approach produces a distribution of risks to accommodate the uncertainty and/or variation and thus naturally provides 95% credible intervals in addition to the median probability. This is important because it allows the risk manager to be 97.5% confident that the risk is not higher.

Model overview

The quantitative model is based on observations made during a site visit to a laying house which housed 129 000 chickens in enriched colony cages. The model is based on three parts of the feed stream (namely the metal trough, the moving hopper and the moving chain) and on three parts of the waste stream (namely the manure belt, the cross-conveyor, and the manure air drying equipment). In addition the floor is included. Colony cages are not specifically considered, but are included as part of the manure belt which runs directly underneath the cages. Moving parts were included because of their capacity to generate dusts, although poultry are unlikely to have direct contact with moving chains, for example.

The approach uses the “source-pathway-receptor” model developed previously for assessing the infection risks from pathogens through environmental routes involving treatment processes such as composting and sewage sludge processing followed by pathogen decay in the environment (Gale, 2004 and 2005). The source term is the amount of infectivity in the poultry house at the point of culling and removal of the infected birds. The receptor in this model is the whole chicken flock used for restocking the poultry house after the given C&D scenario. By assuming that the dose-response is linear such that just a single HPAI virion is able initiate infection in a poultry host, albeit with low probability, it does not matter whether one chicken in the restocked flock ingests the whole dose (and all the other chickens are not exposed) or whether each and every chicken has an equal portion of the dose. This approach is equivalent to calculating an arithmetic mean individual bird exposure as has been used previously for environmental source-pathway-receptor risk assessments (Gale, 2004 and 2005) and avoids the need to estimate the exact dose ingested by each and every one of the individual restocked birds. Furthermore by assuming that a certain fraction of the residual infectivity is inhaled or ingested by the incoming flock, the total number of restocked birds is not required in the exposure calculation.The whole flock exposure is then used to calculate the **probability of at least**

one chicken becoming infected in the poultry house ($p_{outbreak}$), since it would only need one bird to be infected for the entire restocked population to succumb. The probability $p_{outbreak}$ is thus in effect the probability of recrudescence in that poultry house, and its inverse represents the average number of similar poultry houses deploying the C&D scenario before one had a recrudescence.

The source term: Virus loadings in the poultry house

The quantitative model is based on a large layer poultry house with 129 000 chickens (reflecting a site visit made in December 2016). It is assumed that 50% (i.e. 64 500) of the birds are infected and shedding HPAIV at the point of culling. The unit of infectivity in the exposure assessment is the **egg infectious dose 50% (EID_{50})** which is the dose required to infect 50% of inoculated embryonated fowls eggs (when given to each and every egg in the group) in laboratory assay. The viral titre contributions to the source term expressed as EID_{50} units are estimated as described in Supplementary Material S1 for:-

1. The **total HPAIV infectivity from the three bird matrices namely feathers, faeces and oropharyngeal secretions which goes into the "manure" (EID_{50_manure})** calculated from data in Yamamoto *et al.* (2008) and Scottish Government (2016) together with unpublished data from the **Animal and Plant Health Agency (APHA)**; and
2. The **airborne particulate HPAIV infectivity which settles as dust ($EID_{50\_airborne}$)** calculated from data of Spekreijse et al. (2011).

Distribution of mass fractions of infected material to different feed and waste streams. The source term considers three waste and three feed streams within the poultry house, together with the floor, as shown in Figure 1 for enriched colony caged layer houses. It is assumed that 99.9% of the manure produced is removed daily during normal operation of the poultry house and that this would have been removed in the 24 hours prior to culling.

Therefore 0.1% of the manure is still present in the poultry house after culling and removal of the infected poultry. It is assumed that all of the airborne fraction settles as dust after removal of the poultry. The **fractions of manure (f_{stream_manure})** and the **fractions of airborne particulate ($f_{stream\_airborne}$)** assumed to be entering each of the three feed streams and the three waste streams, together with the floor are set out in Table 1. These are estimated on the basis of the site visit. The **source term infectivity in a given stream immediately after depopulation of the infected poultry ($EID_{50\_source\_stream}$)** is given by:-

Equation 1 $$EID_{50\_source\_stream} = EID_{50\_manure} \times 0.001 \times f_{stream\_manure} + EID_{50\_airborne} \times f_{stream\_airborne}$$

The pathway term: Assessing the barriers and total exposures to re-stocked poultry

The pathway from residual infectivity in the poultry house at the point of depopulation of the infected flocks to the restocking with the new poultry flock is set out in Figure 2. The pathway is used to calculate the total exposure to the receptor in terms of EID_{50} units and sets out the barriers which act to decrease the exposure to the receptor. These include natural decay in addition to destruction of virus by the C&D process.

Modelling virus decay during the period between depopulation and restocking. Viruses cannot multiply outside the host and undergo natural decay once outside the host.

The **decimal reduction time (D_t)** is the time for a 10-fold decrease (i.e. 1 $\log_{10}$) or 90% decrease in the virus loading. D_t times for HPAIV H7N1 A/ostrich/Italy/984/2000 and H5N1 A/turkey/Turkey/1/2005 in chicken faeces were 3.33 days and 12.05 days at 4°C and 0.83 days and 4.41 days at 20°C, respectively (C. Warren, personal communication). As is well known from other studies of virus inactivation, decay is more rapid at the higher temperature of 20°C compared to 4°C. The D_t time used for decay of HPAIV in this risk assessment is 10 days. Although the D_t for H5N1 at 4°C is >10 days at 12.05 days, the

value of 10 days takes into account that temperatures may exceed 4°C even during the winter period (particularly in 2016). Furthermore the temperature in the shed with birds present is higher, although the temperature will fall after depopulation. Using D_t times for chicken faeces as in the models here is a worst case scenario because the D_t times measured in poultry litter were much shorter at <5 min and <10 min for H7N1 and H5N1 respectively at both 4°C and 20°C (C. Warren, personal communication). For the purpose of risk assessment, decay is assumed to occur over the **time period between depopulation of the infected poultry and restocking with the new birds (*TbDR*)**. Minimum and maximum values of 40 and 90 days respectively were used to define a uniform distribution for *TbDR* (see Supplementary Material S2).

Modelling virus inactivation by cleansing and disinfection. In this risk assessment, the overall inactivation of HPAIV by C&D is modelled by summing the titres surviving in two separate 'portions':-

1. The bulk phase, which undergoes efficient cleansing and disinfection; and
2. The by-pass phase, which misses efficient cleansing and disinfection altogether so that no pathogen inactivation takes place.

This is based on the method developed by Gale (2004) for removal of pathogens by composting of catering waste and simplifies the risk assessment methodology into estimating:-

1. The **fraction of pathogen surviving in the properly cleansed and disinfectant-treated bulk phase portion (γ)**; and
2. The **fraction of debris and organic material (and hence associated viruses) in those parts within each stream where C&D cannot reach and which therefore by-passes the bulk phase and effective C&D (f_{bypass})**.

The overall **fraction of input pathogen surviving C&D for each stream ($f_{survive\_stream}$)** is thus calculated as:-

Equation 2 $$f_{survive\_stream} = (1 - f_{bypass}) \times \gamma + f_{bypass}$$

Values of γ are allocated in Table 1 for each of the six streams and the floor on the basis of the measured decrease in total aerobic bacteria counts in the most closely related equipment during C&D of an operational poultry house as reported by Lucyckx et al. (2015). This is described in Supplementary Material S2. Minimum and maximum values of f_{bypass} to represent the proportions of organic material (and hence associated viruses) which by-pass C&D within each stream for preliminary and secondary C&D with and without dismantling are set out in Table 2. These were used to define a uniform distribution for f_{bypass} and were based on what is thought to be operationally achievable as set out in Supplementary Material S2.

Calculation of exposures to restocked poultry through inhalation of dust and ingestion. The **fractions inhaled** (f_{inhale}) of the remaining infective material (after conversion to dust through moving parts in the equipment or other disturbance in the restocked poultry house) by the restocked poultry are set out in Table 1 for each of the streams together with the **fractions ingested** (f_{ingest}) through feeding and pecking. In the absence of data, these are based on expert opinion and assumptions as set out in Supplementary Material S2. Exposures (in EID_{50} units) to the restocked poultry through ingestion and inhalation were calculated for each of the seven streams from $EID_{50\_source\_stream}$ (Equation 1) allowing for decay of HPAIV according to D_t over the *TbDR* period in the fraction, $f_{survive\_stream}$, (from Equation 2) of HPAIV surviving C&D in each stream. The equations are set out in Supplementary Material S2. The **total poultry exposure** ($Exposure\_EID_{50}$) was calculated as the sums of the exposures through the ingestion and inhalation routes for each of the seven streams using equations set out in Supplementary Material S2. This

represents the total exposure to the poultry in a given poultry house. The units are "EID_{50} in total poultry population per poultry house". Because the model assumes that a fixed proportion of the remaining infectivity is ingested or inhaled (according to f_{ingest} and f_{inhale} in Table 1) by the poultry flock as a whole, the risk assessment is not dependent on the number of restocked poultry. This is realistic for poultry houses with large numbers of birds where a steady state is likely to be reached over a few days, but would be less appropriate for houses with only a few birds. This avoids a more complex calculation involving the estimation of how much debris each of 129 000 chickens ingests each day and the number of days over which this could occur.

Receptor term: Calculating the risk of infection of the poultry house

The **number of chicken ID_{50}s ingested by the chicken flock as a whole within the poultry shed ($N_{Chicken\_ID50}$)** is calculated from the total poultry exposure ($Exposure\_EID_{50}$) using the poultry infectivity data for HPAIV H5N1 of Aldous et al. (2010) as described in Supplementary Material S2. The probability of at least one infected chicken in the poultry house, and hence the probability of an outbreak in the poultry house, $p_{outbreak}$, is then given by:-

Equation 3 $$p_{outbreak} = 1 - (1 - p_{50})^{N_{Chicken\_ID50}}$$

where p_{50} is the risk of infection from a single chicken ID_{50} when given to a chicken (i.e. 0.5). The inverse of $p_{outbreak}$, is the number of infected poultry houses cleansed according to the given C&D procedure before recrudescence in the restocked poultry is expected to occur in one. The number of infected chickens in the poultry house could be calculated from $N_{Chicken\_ID50}$ as done for livestock grazing on land to which composted catering waste had been applied (Gale, 2004) and would be greater than one for high values of $N_{Chicken\_ID50}$. However, the number of infected chickens in the poultry house is of little interest here as we are not modelling severity of consequence or the probability of

311 detection of the infected flock (which would increase with higher numbers of infected
312 birds). If a chicken ingests more than one ID_{50} (due to spatial heterogeneity) it is
313 preventing other chickens in that house from being infected. With high values of
314 $N_{Chicken\_ID50}$, then $p_{outbreak}$ (i.e. the probability of one or more infected chickens) in Equation
315 3 tends to 1 and with just one chicken infected, recrudescence has occurred.

316 *Running the model*

317 The model was run in R Studio with 1 000 iterations using the equations and parameters
318 as set out in this paper and in the Supplementary Material S1 and S2. This number of
319 iterations gave convergence of the probability distributions and outputs. The R code is set
320 out in Supplementary Material S3. For each of the 1 000 iterations a single value is used
321 for each of the input parameters in the equations of the model giving a single estimate of
322 the output, $p_{outbreak}$. Values for most of the parameters in the model are constant and are
323 the same for each iteration, for example D_t is always 10 days. However, for each iteration,
324 the programme draws a random value for f_{bypass} for each of the feed and waste streams
325 and for the floor and also draws a random value for *TbDR* from their respective uniform
326 distributions with minimum and maximum values specified in Table 2 for f_{bypass} and
327 between 40 and 90 days for *TbDR*. Thus the model output, $p_{outbreak}$, is different for each
328 iteration giving 1 000 different versions of $p_{outbreak}$ which are represented by the frequency
329 distribution in Figure 3.

330 *Validation of the model*

331 Sargent (2011) discussed validation techniques for simulation models. Event validity
332 where the output of the model is compared with epidemiological data is difficult due to the
333 lack of case-control studies on recrudescence of HPAI after C&D. Extreme condition tests
334 and sensitivity analyses where parameter values are altered gave expected outputs. For
335 example setting f_{bypass} to 0 or 1 in Equation 2 gives $f_{survive\_stream}$ equal to γ and 1.0

respectively as expected and reducing the percentage of infected birds in the source term from 50% to 5% increased the predicted average number of houses before a recrudescence by 10-fold as expected. As part of face validity (Sargent, 2011), representatives of poultry industry agreed the conceptual model represented in Figure 1 and Figure 2 was correct and that the model's input-output relationships are reasonable (Gale et al., 2018).

Results

Highly pathogenic avian influenza virus loadings in a poultry house at point of culling and removal (depopulation) of infected poultry

The total HPAIV infectivity in the poultry house at the end of depopulation and after removal of 99.9% of the manure is 3.89 10^7 EID_{50}s (Supplementary Table S1). This is mainly from cloacal/oropharyngeal secretions and feathers in the remaining manure, with settling of airborne particulate making only a small contribution. By apportioning the infectivity according to the fractions, f_{stream_manure} and $f_{stream\_airborne}$ from Table 1 in Equation 1, the amounts of infectivity in each of the feed and waste streams and on the floor at the point of depopulation are calculated (Table 3).

Predicted exposures and risks of recrudescence to restocked poultry

The estimated median HPAIV exposures to the restocked poultry in terms of EID_{50}s per poultry house are presented in Table 4. Secondary C&D (without dismantling) decreases the median exposure by 15-fold compared to just preliminary C&D alone. When dismantling is applied, the median exposures are decreased by a further 6-fold, and the overall decrease in exposure compared to preliminary C&D alone is over 88-fold. These decreases in exposure directly reduce the risks of recrudescence reflecting the linear nature of Equation 3 at low doses as shown in Table 4 by the number of poultry houses

360 treated by a given C&D scenario before recrudescence occurs in one. Thus applying
361 secondary C&D without dismantling decreases the median number of poultry houses
362 which can be restocked by 16-fold compared to preliminary C&D alone, and dismantling
363 during secondary C&D has an additional 6-fold preventative effect.

364 The uncertainty in C&D efficacy is assessed by putting in lower and upper limits for the
365 degree of by-pass (Table 2). The frequency distributions for the values of $p_{outbreak}$ predicted
366 by the model are presented in Figure 3. There is considerable uncertainty/variation in the
367 predicted risks with estimates of the number of poultry houses treated with secondary
368 C&D without dismantling ranging between 781 and 5.6 10^7 before a recrudescence
369 occurs, i.e. almost five orders of magnitude (Table 4).

370 **Discussion**

371 This study provides a risk-based approach for the control of HPAIV following an outbreak
372 in an enriched colony caged poultry house with specific reference to cleansing and
373 disinfection (C&D). It can be used as an evidence base for proportionate but effective
374 approaches to the application of C&D after an outbreak. It will inform policy-makers and
375 industry in their decision-making and could be applied to other avian pathogens such as
376 Newcastle disease virus using appropriate data. It could also be applied to other poultry
377 production systems. A source-pathway-receptor framework model is developed with data
378 for HPAIV H5N1 and the output is the expected number of infected poultry houses treated
379 with the particular C&D scenario before recrudescence occurs when restocked with
380 susceptible birds. Uncertainty and variation in the degree of by-pass and variation in the
381 total time (days) between depopulation and restocking (*TbDR*) are modelled using Monte
382 Carlo simulations based on uniform distributions such that each value has an equal
383 probability of being drawn.

384 Central to the model is the estimation of the overall inactivation of virus by C&D using
385 Equation 2 and the degree of by-pass, i.e. the proportion of the residual infective material
386 that does not come into contact with disinfectant during the C&D process. Although there
387 are no data on the degree of by-pass during C&D with and without dismantling, the level of
388 by-pass is chosen to reflect what is thought to be operationally achievable. Such an
389 approach has been used previously for composting of catering waste (Gale, 2004) and for
390 treatment of sewage sludge (Gale, 2005). Obtaining experimental measurements of by-
391 pass data would be logistically difficult in practice during C&D at an operational poultry
392 house not least from the point of view of experimental design. In particular it would be
393 difficult to quantify with reliability the infective material present in the poultry house and its
394 equipment without actually dismantling before commencing preliminary C&D and then
395 again before secondary C&D and finally after secondary C&D. In effect, dismantling would
396 be needed before and after both preliminary and secondary C&D to measure the amount
397 of infected material remaining which would be disturbed in the process.

398 The *TbDR* is variable and has a significant effect on the amount of decay and hence the
399 predicted risk. Thus with a D_t of 10 days as used here, if the *TbDR* is 40 days, then there
400 is 4-$\log_{10}$ decay. In the model, the median *TbDR* is 65 days and the maximum *TbDR* is 90
401 days over which 6.5 $\log_{10}$ and 9 $\log_{10}$ decays respectively are predicted according to the
402 model. However, this is based on the assumption that decay of the virus occurs linearly up
403 to 9 $\log_{10}$ units over 90 days. Typically experimental data for virus decay demonstrate up
404 to ~4-$\log_{10}$ decay. Thus there are uncertainties in extrapolation to greater than 4-$\log_{10}$
405 decay (i.e. over the 40 to 90 day *TbDR* period) particularly as virus decay is typically non-
406 linear with a long tail perhaps representing a more resistant subpopulation of virus/matrix
407 complex. However, since D_t values for poultry litter are in the range of <5 to <10 minutes
408 and may be more appropriate than the D_t value of 10 days used here based on HPAIV
409 decay in chicken faeces (C. Warren, personal communication), it is not considered that the

410 risk estimates presented here are over-optimistic. A further source of uncertainty in the D_t
411 time for HPAIV decay arises from the temperature and humidity conditions. Thus Guan et
412 al. (2017) show that the absolute humidity is an important parameter in the inactivation of
413 H9N2 and H6N2 virus on both non-porous and wood surfaces. This is potentially important
414 for a poultry house being cleansed and disinfected because a lot of water is used, and the
415 relative humidity could be high due to the dampness.

416 Other assumptions in the exposure assessment are that disinfection is highly effective at
417 inactivating the HPAIV H5N1 in the parts of the poultry house that it contacts as reflected
418 in the small values of γ for the "bulk" phase. It should be noted, however, that the actual
419 values of γ (Table 1) are not important in this risk assessment because the values of f_{bypass}
420 (Table 2) are orders of magnitude higher and therefore dominate in Equation 2. Thus when
421 f_{bypass} is much greater than γ, $f_{survive\_stream}$ tends to f_{bypass} in Equation 2. The model also
422 makes assumptions for the amounts of infectivity inhaled and ingested by the restocked
423 poultry. We consider these are worst case scenario estimates. Calculating the total virus
424 loading ($N_{Chicken\_ID50}$) on the restocked poultry population as a whole addresses potential
425 issues of the spatial and temporal heterogeneity of exposures to individuals amongst the
426 restocked birds and in effect assumes each and every chicken is exposed to the same
427 very small sub-fraction (1/129 000th) of $N_{Chicken\_ID50}$. Thus whether one bird in the flock
428 ingests the entire $N_{Chicken\_ID50}$ dose, (and all the other 128 999 birds have zero exposure),
429 or whether all 129 000 birds have an equal 1/129 000th of $N_{Chicken\_ID50}$ is not important for
430 the estimation of risk. As discussed for the model of the risks to livestock from composted
431 catering waste (Gale 2004), assuming all birds receive the same small sub-fraction as in
432 the latter scenario would predict higher risks than for the former scenario particularly for
433 high values of $N_{Chicken\_ID50}$. Equation 3 assumes that the dose-response is linear down to
434 one HPAI virion. Indeed, it is quite acceptable for $N_{Chicken\_ID50}$ in Equation 3 to be a fraction
435 of an ID_{50} as in the median exposures from Table 4 because the dose-response is linear

and Equation 3 tends to $p_{outbreak}$ = 0.69 x $N_{Chicken\_ID50}$ at low values of $N_{Chicken\_ID50}$ (Gale, 2004). A recent attempt to develop a mechanistic dose-response model for viruses (Gale, 2018) has indicated a theoretical mechanism for a threshold effect where the virus dose needs to be sufficiently high to overwhelm the host innate defences (e.g. mucins in mucus), although this has not be proven experimentally. Clearly allowing for a **minimum infectious dose (MID)** greater than one virion in the model would greatly diminish the risks predicted here to the re-stocked poultry, depending on the magnitude of that MID. This is because a single virion alone could not cause infection and those individual birds exposed to doses below the MID would not be infected in reality but according to the model here are at risk of infection. However, the exposure assessment would have to be modified to predict the actual exposure to each and every one of the 129 000 chickens taking into account all sources of variation in the source and pathway terms so as to predict how many chickens are exposed to doses above the MID. A further consideration is whether the exposure to individual poultry in the restocked birds is in one single exposure or repeated over several days or weeks. Thus, exposure to small amounts of virus distributed over a longer time might influence the virus inactivation by the immune system resulting in a higher resistance against infection than in case of exposure to the whole dose at once (Pujol et al., 2009; Marois et al., 2012). The risk assessment here takes a worst case and ignores this possibility. Indeed, it is likely that the highest exposures would occur early on for the restocked birds.

The predicted values of $p_{outbreak}$ in Figure 3 vary over some six orders of magnitude mainly reflecting the large range for *TbDR* in the uniform distribution and the assumption of log-linear decay of HPAIV over the *TbDR*. This together with more information on the degree of by-pass highlights areas of the model for which additional field data would be of use.

Overall, the probability of recrudescence of HPAI disease in caged layers following depopulation and C&D can be considered very low based on applying secondary C&D and

462 the *TbDR* of 40 to 90 days. The results presented here confirm that the dual barriers of
463 both HPAIV decay over the *TbDR* and HPAIV inactivation by the preliminary followed by
464 secondary C&D processes minimise the risks of recrudescence.. With preliminary C&D
465 alone, there is 97.5% credibility that 50 poultry houses could be restocked before a
466 recrudescence event. Applying secondary C&D without dismantling at the same level of
467 confidence this increases on average to 781 poultry houses that could be restocked before
468 recrudescence occurs. Thus on the basis of these lower 95% credible intervals, the model
469 clearly demonstrates the importance of secondary C&D without dismantling over
470 preliminary C&D alone. However, dismantling during secondary C&D only increases this
471 lower credible interval by a further five-fold to 4 200 poultry houses, and given the
472 diminishing return, it is concluded that the extra protection to the restocked chickens
473 afforded by dismantling may not justify the financial expense or the time delay in
474 completing secondary C&D with respect to minimising the risk of onward spread of HPAIV.
475 In summary, secondary C&D has substantial benefit over preliminary C&D alone by
476 decreasing the risks to restocked poultry by ~16-fold while dismantling during secondary
477 C&D only adds a further six-fold decrease in risk to the restocked poultry. The level of by-
478 pass used for these estimates is a key source of uncertainty requiring investigation with
479 experimental models.

480 **Conclusions**

481 It is concluded that dismantling complex equipment in a poultry house during secondary
482 cleansing and disinfection (C&D) may not be cost beneficial to the owner in terms of
483 protecting against further outbreaks of HPAI. However, taking into account the uncertainty
484 in the efficiency of C&D together with the variation in the time between depopulating and
485 restocking, it is concluded that preliminary C&D alone is not sufficient and that secondary
486 C&D (with or without dismantling) should be performed.

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Declaration of interest

None declared.

Ethics committee

Not relevant.

Software and data repository resources

The model was not deposited in an official repository.

References

Alarcon P, Brouwer A, Venkatesh D, Duncan D, Dovas CI, Georgiades G, Monne I, Fusaro A, Dan A, Smietanka K, Ragias V, Breed AC, Chassalevris T, Goujgoulova G, Hjulsager CK, Ryan E, Sanchez A, Niqueux E, Tammiranta N, Zohari S, Stroud D, Savic V, Lewis NS and Brown IH 2018. Comparison of 2016–17 and previous epizootics of highly pathogenic avian influenza H5 Guangdong lineage in Europe. Emerging Infectious Diseases 24, 2270-2283.

Aldous EW, Seekings JM, McNally A, Nili H, Fuller CM, Irvine RM, Alexander DJ and Brown IH 2010. Infection dynamics of highly pathogenic avian influenza and virulent avian paramyxovirus type 1 viruses in chickens, turkeys and ducks. Avian Pathology 39, 265-273.

508 EU 2005. EU Council Directive 2005/94/EC of 20 December 2005 on Community measures for the
509 control of avian influenza and repealing Directive 92/40/EEC Article 49. Retrieved on 20 January
510 2017 from http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32005L0094.

511 Gale P 2004. Risk to farm animals from pathogens in composted catering waste containing meat.
512 Veterinary Record 155, 77-82.

513 Gale P 2005. Land application of treated sewage sludge: Quantifying pathogen risks from
514 consumption of crops. Journal of Applied Microbiology 98, 380-396.

515 Gale P 2018. Using thermodynamic parameters to calibrate a mechanistic dose-response for
516 infection of a host by a virus. Microbial Risk Analysis 8, 1-13.

517 Gale P, Kelly L and Snary EL 2015. Qualitative assessment of the entry of capripoxviruses
518 into Great Britain from the European Union through importation of ruminant hides, skins
519 and wool. Microbial Risk Analysis 1, 13-18.

520 Gale P, Sechi S, Horigan V and Kelly L 2018. Quantitative assessment for the risk of
521 recrudescence of avian influenza in caged layer houses following depopulation: The effect of
522 cleansing, disinfection and dismantling of complex equipment. British Poultry Abstracts 14, 9.

523 Goddard AD, Donaldson NM, Horton DL, Kosmider RD, Kelly LA, Sayers AR, Breed AC,
524 Freuling, CM, Muller T, Shaw SE, Hallgren G, Fooks AR and Snary, EL 2012. A quantitative
525 release assessment for the noncommercial movement of companion animals: risk of rabies
526 reintroduction to the United Kingdom. Risk Analysis 32, 1769-1783.

527 Guan J, Chan M and VanderZaag A 2017. Inactivation of avian influenza viruses on porous and
528 non-porous surfaces is enhanced by elevating absolute humidity. Transboundary and Emerging
529 Diseases 64, 1254-1261.

530 Hansen R, Brown I, Brookes S, Welchman D and Cromie R 2018. Current status of avian influenza
531 in Europe and the UK. Veterinary Record 182, 54-55.

Horigan V, Gale P, Adkin A, Brown I, Clark J and Kelly L 2019. A qualitative risk assessment of cleansing and disinfection requirements after an avian influenza outbreak in commercial poultry. British Poultry Science 60, 691-699.

Kelly L, Kosmider R, Gale P and Snary E 2018. Qualitative import risk assessment: A proposed method for estimating the aggregated probability of entry of infection. Microbial Risk Analysis 9, 33-37.

Lucyckx KY, Weyenberg SV, Dewulf J, Herman L, Zoons J, Vervaet E, Heyndrickx M and De Reu K 2015. On-farm comparisons of different cleaning protocols in broiler houses. Poultry Science 94, 1986-1993.

Marois I, Cloutier A, Garneau E and Richter MV 2012. Initial infectious dose dictates the innate, adaptive, and memory responses to influenza in the respiratory tract. Journal of Leukocyte Biology 92, 1–12.

OIE 2018. OIE situation report for highly pathogenic avian influenza. Last updated 31 August 2018. Retrieved on 30 May 2019 from http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/OIE_AI_situation_report/OIE_SituationReport_AI_August2018.pdf.

OIE 2019. Terrestrial animal health code. Section 2. Risk analysis. Last updated 28 June 2019. Retrieved on 29 November 2019 from https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_import_risk_analysis.pdf.

Pujol JM, Eisenberg JE, Haas CN and Koopman JS 2009. The effect of ongoing exposure dynamics in dose response relationships. PLOS Computational Biology 5, e1000399.

Roberts H, Gale P and Brown I 2018. Findings of H5N6 HPAI in wild birds in UK/Ireland and LPAI in poultry in France. Retrieved on 30 May 2019 from

https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/682151/avian-flu-wild-birds-H5N6-180213.pdf.

Sargent RG 2011. Verification and validation of simulation models. In proceedings of the 2011 Winter Simulation Conference, pp. 183–198. Retrieved on 3 December 2019 from https://www.informs-sim.org/wsc11papers/016.pdf.

Scottish Government 2016. Calculating the amount of poultry manure produced. Retrieved on 20 January 2017 from www.gov.scot/Resource/Doc/278281/0096546.doc.

Spekreijse D, Bouma A, Koch G and Stegeman JA 2011. Airborne transmission of a highly pathogenic avian influenza virus strain H5N1 between groups of chickens quantified in an experimental setting. Veterinary Microbiology 152, 88-95.

Yamamoto Y, Nakamura K, Okamatsu M, Miyazaki A, Yamada M and Mase M 2008. Detecting avian influenza virus (H5N1) in domestic duck feathers. Emerging Infectious Diseases 14, 1671-1672.

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571 *Table 1. Fractions of infectivity entering, surviving cleansing and disinfection (C&D) and*

572 *inhaled/ingested through the different streams within the poultry house as used in the model.*

	Manure f_{stream_manure}	Airborne particulate $f_{stream\_airborne}$	γ	f_{inhale}	f_{ingest}
Feed streams					
Metal trough	0.001	0.01	$3.16\ 10^{-4}$ (Feed pan)	0.0001	0.5
Moving hopper	0.001	0.01	$7.9\ 10^{-5}$ (Feed hopper)	0.1	0.5
Moving chain	0.001	0.01	$7.9\ 10^{-5}$ (Feed hopper)	0.1	0.5
Waste streams					
Manure belt	0.897	0.20	$2.5\ 10^{-6}$ (Loose material)	0.1	0.01
Cross conveyor	0.05	0.10	$7.9\ 10^{-5}$ (Feed hopper)	0.1	0.01
Air drying equipment	0.05	0.05	$6.3\ 10^{-5}$ (Air outlet)	0.1	0.1
Floor	0	0.62	$7.9\ 10^{-5}$ (Floor)	0.1	0

f_{stream_manure} and $f_{stream\_airborne}$, respective fractions of masses of manure and settled particulate from airborne material within different streams in the poultry house.

γ, fraction of highly pathogenic avian influenza virus surviving C&D of the 'bulk' phase based on data for total aerobic bacteria counts surviving C&D of an operational poultry house (Lucyckx et al., 2015) in the most closely related equipment given in parentheses.

f_{inhale}, fraction converted to dust during operation of poultry house and inhaled by the restocked poultry.

f_{ingest}, fraction ingested by the restocked poultry during pecking and feeding.

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577 *Table 2. Minimum and maximum values used to define the uniform distributions for the fraction by-*

578 *passing the 'bulk' phase (f_{bypass}) within the poultry house during cleansing and disinfection.*

	Feed streams			Waste streams			
Infected components	Metal trough	Moving hopper	Moving chain	Manure belt	Cross conveyer	Air drying equipment	Floor
Preliminary disinfection	0.05 – 0.20	0.01 to 0.1	0.01 to 0.1	0.1 - 0.4	0.01 – 0.1	0.01 – 0.1	0.01 – 0.1
Secondary: By-pass rate without dismantling	0.005 – 0.02	0.005 – 0.02	0.01 – 0.04	0.025 – 0.1	0.005 – 0.02	0.005 – 0.02	0.005 – 0.02
Secondary: By pass rate with dismantling	0.0025 – 0.01	0.0025 – 0.01	0.005 – 0.02	0.005 – 0.02	0.0025 – 0.01	0.0025 – 0.01	0.005 – 0.02

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584 *Table 3. Source Term: Estimated amounts of highly pathogenic avian influenza virus infectivity*
585 *(units of egg infectious dose 50%) in the different streams within poultry house at the point of*
586 *depopulation of infected poultry.*

	Feed streams			Waste streams			
Infected components	Metal trough	Moving hopper	Moving chain	Manure belt	Cross conveyer	Air drying equipment	Floor
Manure	$3.88\ 10^4$	$3.88\ 10^4$	$3.88\ 10^4$	$3.48\ 10^7$	$1.94\ 10^6$	$1.94\ 10^6$	0
Airborne particulate	$1.5\ 10^3$	$1.5\ 10^3$	$1.5\ 10^3$	$3.0\ 10^4$	$1.5\ 10^4$	$7.5\ 10^3$	$9.2\ 10^4$

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594 *Table 4. Median values and 95% credible intervals (brackets) as predicted by the model for highly*
595 *pathogenic avian influenza virus exposures to a restocked chicken flock in a poultry house, and the*
596 *risk of infection of the poultry house.*

	Total poultry exposure (*Exposure_EID*$_{50}$) as egg infectious dose 50% units per poultry house	Probability (per poultry house) of infection of poultry house ($p_{outbreak}$)	Number of poultry houses/sheds before one outbreak ($1/p_{outbreak}$)
Preliminary C&D alone	0.30 (1.3 10^{-3} to 71.2)	8.3 10^{-5} (3.6 10^{-7} to 2.0 10^{-2})	1.2 10^{4} (50 to 2.8 10^{6})
Preliminary followed by secondary C&D without dismantling	2.0 10^{-2} (6.4 10^{-5} to 4.7)	5.1 10^{-6} (1.7 10^{-8} to 1.3 10^{-3})	1.9 10^{5} (7.8 10^{2} to 5.7 10^{7})
Preliminary followed by secondary C&D with dismantling	3.4 10^{-3} (1.2 10^{-5} to 0.87)	0.95 10^{-6} (3.4 10^{-9} to 2.4 10^{-4})	1.1 10^{6} (4.2 10^{3} to 2.9 10^{8})

C&D: Cleansing and disinfection.

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List of figure legends

Figure 1: Source term contributions of highly pathogenic avian influenza virus as egg infectious dose 50% (EID_{50}) units from infected chickens in manure (EID_{50_manure}) and as particulate matter in the air which settle as dust ($EID_{50\_airborne}$) at point of depopulation of poultry house. The fractions of manure (f_{stream_manure} shown as percentages in normal font) and the fractions of airborne particulate ($f_{stream\_airborne}$ shown as percentages in italic font) entering each stream are from Table 1.

Figure 2: Pathway detailing the fate of highly pathogenic avian influenza virus infectivity as egg infectious dose 50% (EID_{50}) units to calculate total exposure to restocked chicken poultry flock (Receptor) after cleansing and disinfection (C&D) and virus decay over the time between depopulation of infected chickens and restocking with new chickens (TbDR).

Figure 3: Frequency distribution for the values of the probability of recrudescence per chicken poultry house ($p_{outbreak}$) as predicted by 1 000 iterations of the model for a) preliminary cleansing and disinfection (C&D) alone; b) preliminary C&D followed by secondary C&D without dismantling; and c) preliminary C&D followed by secondary C&D with dismantling.

Supplementary Material S1

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Risk assessment for recrudescence of avian influenza in caged layer houses following depopulation: The effect of cleansing, disinfection and dismantling of equipment.

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The source term: Virus loadings in the poultry house

Loadings from the three bird matrices, namely feathers, faecal (cloacal), and oropharyngeal secretions.

Titres of **highly pathogenic avian influenza virus (HPAIV)** are typically reported as **egg infectious dose 50% (EID_{50})** units. The **total HPAIV infectivity from the three bird matrices namely feathers, faeces and oropharyngeal secretions which goes into the "manure" (EID_{50_manure})** is calculated from data in Yamamoto *et al.* (2008) and Scottish Government (2016) together with unpublished data from the **Animal and Plant Health Agency (APHA)**. There are few published shedding data for HPAIV in chickens and the risk assessment therefore draws on published data for other bird species. Yamamoto *et al.* (2008) presented H5N1 viral titres in feathers, oropharyngeal swabs and cloacal swabs from three domestic ducks inoculated with H5N1. The titres were not only highest in the feathers (3.8 to 6.9 $\log_{10}$ EID_{50} /ml) but also were detected for longer periods of time (8 days post infection) compared to in cloacal and oropharyngeal secretions. Although the presence of H5N1 virus in bird feathers is an important consideration, feathers are lost infrequently compared to oropharyngeal and cloacal secretions which are produced daily, and therefore feathers may only make a small contribution to the "manure" in the poultry sheds. Viral titres of H5N1 were higher in oropharyngeal secretions than in cloacal secretions and the data for oropharyngeal secretions are therefore used in this risk assessment. This represents a worst case scenario because much more cloacal secretion is produced than oropharyngeal. According to Yamamoto *et al.* (2008), the highest duck oropharyngeal EID_{50} was at 4 days post infection at $10^{3.7}$ EID_{50}/ml. It is assumed that 1 ml

equates to 1 g of manure and therefore the viral loading is $10^{3.7}$ EID_{50}/g manure produced by an infected bird. APHA unpublished data indicate that peak shedding titres for H5N1 clade 2.2 virus in chickens and turkeys are similar at $10^{3.0}$ to $10^{4.0}$ EID_{50}/ml.

Manure production data for poultry are used to estimate the amount of solid secretion produced per bird per day. Caged layers (over 17 weeks in age) have been reported to produce 0.84 tonnes of manure per 1 000 birds per week (Scottish Government, 2016). This is equivalent to 120 g per bird per day. The total viral infectivity produced in the poultry house at point of culling from cloacal, oropharyngeal and feathers is calculated as 120 g/bird/day x 64 500 infected birds x $10^{3.7}$ EID_{50}/g = 3.88 10^{10} EID_{50}/day. This is in the form of manure, which is removed from the house at a constant rate by the moving manure belt. For example, 129 000 poultry would produce 15.4 tonnes of manure per day, and the poultry house would rapidly fill up with manure if it were not removed. It is assumed that 99.9% of manure is removed from the poultry house each day and that this would have been removed in the 24 hours prior to culling, with 0.1% being left in the poultry house after culling and removal of the infected poultry. Thus 15.4 kg of manure are left each day, representing 3.88 10^{7} EID_{50} (EID_{50_manure}) in the poultry house at the point of culling and depopulation (Table S1).

Airborne infectivity which settles as dust.

The **airborne particulate HPAIV infectivity which settles as dust ($EID_{50\_airborne}$)** is calculated from data of Spekreijse et al. (2011). To estimate HPAIV H5N1 loadings from air and dust in the poultry house immediately prior to the point of culling, the number of airborne EID_{50} produced per infected chicken per day is calculated from the air sampling data of Spekreijse et al. (2011) who collected 20 air samples over 10 days, i.e., two samples per day in each of two rooms with chickens experimentally infected with HPAIV H5N1. Each sample was collected over 10 minutes at a rate of 8 m^3/minute and thus represents 1.33 m^3. In one room of volume 22 m^3, one air sample contained $10^{1.6}$ EID_{50} on day 2 and another on day 3 contained $10^{1.3}$ EID_{50} (totalling 59.8 EID_{50} in the two samples combined). The other 18 samples from that room collected over days 1 to 10 were negative. As a worst case scenario only the data from the two positive shedding days (days 2 and 3) are used here. The total volume sampled in that one room over those two days (i.e. four samples) was 4 x 1.33 m^3 = 5.33 m^3 of air. Thus 13 infected birds produced 59.8 EID_{50} in 5.33 m^3 of air. Assuming this was representative of the 22 m^3 volume of the whole room, then 13 infected birds produced 246 EID_{50} in the room as a whole. The number of airborne EID_{50} is thus 18.9 per bird in the first room. In the second identical room, however, 56 birds were infected but no airborne infectivity was detected. Combining the results from the two rooms gives 59.8 EID_{50} in 10.67 m^3 (8 air samples over two days) which is 5.6 EID_{50} per m^3. Over 44 m^3 (i.e. the two 22 m^3 volume rooms), this is 246 EID_{50} in both rooms from a total of 69 infected birds over two days. The airborne output per infected bird is therefore 3.57 EID_{50} per infected bird. Since data from two days are used, the estimated airborne infectivity per infected bird per shedding day is 1.78 EID_{50}. For the H5N1 HPAIV infected chickens, the mean infectious period (days of shedding) was 1.3 days (Spekreijse et al., 2011). Assuming 64 500 H5N1-infected birds are present at the time of culling and depopulation then the total airborne loading ($EID_{50\_airborne}$) is 64 500

infected birds x 1.3 days x 1.78 EID_{50} = 1.5 10^5 EID_{50}s (Table S1). It is assumed that all of this airborne infectivity has settled as dust within the house at the end of depopulation. Poultry catching normally involves unrest and wing-flapping, which potentially can redistribute the virus load in the poultry house. This together with the generation of aerosols during the cull process is assumed to be included in the estimated loading in cloacal secretions which are based on oropharyngeal titres.

Table S1. Summary of predicted levels of highly pathogenic avian influenza H5N1 virus (EID_{50}s) in a chicken poultry house at point of depopulation of poultry.

Source	Assumptions	Remaining infectivity at time of culling (EID_{50})
Cloacal, oropharyngeal and feathers (EID_{50_manure})	99.9% is removed per day as manure	$3.88\ 10^7$
Airborne particulate ($EID_{50\_airborne}$)	All settles as dust	$1.5\ 10^5$
Total	Sum of EID_{50_manure} and $EID_{50\_airborne}$	$3.89\ 10^7$
Assumes 129 000 birds in the poultry house of which 50% are infected at culling. EID_{50}: Egg infectious dose 50%		

References

Scottish Government 2016. Calculating the amount of poultry manure produced. Retrieved on 20 January 2017 from www.gov.scot/Resource/Doc/278281/0096546.doc.

Spekreijse D, Bouma A, Koch G and Stegeman JA 2011. Airborne transmission of a highly pathogenic avian influenza virus strain H5N1 between groups of chickens quantified in an experimental setting. Veterinary Microbiology 152, 88-95.

Yamamoto Y, Nakamura K, Okamatsu M, Miyazaki A, Yamada M and Mase M 2008. Detecting avian influenza virus (H5N1) in domestic duck feathers. Emerging Infectious Diseases 14, 1671-1672.

Supplementary Material S2

***animal*. The international journal of animal biosciences.**

Risk assessment for recrudescence of avian influenza in caged layer houses following depopulation: The effect of cleansing, disinfection and dismantling of equipment.

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The time period between depopulation of the infected poultry and restocking with the new birds

The minimum possible **time period between depopulation of the infected poultry and restocking with the new birds (*TbDR*)** is 42 days (EU, 2005). There are typically 2 days between depopulation and preliminary **cleansing and disinfection (C&D)**, 7 days between the first round of cleaning and the second round of cleaning in secondary C&D, and 7 days for secondary C&D. In addition, the re-population of commercial poultry holdings shall not take place for a period of 21 days following the date of completion of the final cleansing and disinfection as provided for in Article 48 the restocking information (EU, 2005). This does not take into account the extra time for decay gained by the practice of dismantling. The expert opinion estimation of *TbDR* is between 40 and 90 days (P. McMullin, personal communication), and these two values were used to define a uniform distribution.

Estimating the fraction of pathogen surviving in the properly cleansed and disinfectant-treated bulk phase portion

Virkon S is a disinfectant officially authorised for C&D in the UK. At 21°C, a 1-$\log_{10}$ inactivation of **highly pathogenic avian influenza virus (HPAIV)** H5N1 sprayed onto fomite surfaces (plastic, metal and wood) requires 3.0 - 3.5 minutes when treated immediately with 1% (w/v) Virkon S disinfectant (C. Warren, personal communication) confirming that this disinfectant rapidly inactivates HPAIV. However, there is no information on whether the inactivation is log-linear and over how many logs. Furthermore in the poultry house environment, the virus will be physically sequestered in an organic matrix (feed, debris, faeces, poultry litter and other secretions) which would not only buffer

the pH but also protect the virus through inactivating the residual disinfectant (Lucyckx et al., 2015). Thus dried faecal pats, accumulated dust, layering of faecal material and matted feathers on the muck belts are the areas to be considered not only in terms of HPAIV loading, but also in terms of the matrix for decay and inactivation (R. Davies, personal communication). To address this, total aerobic bacteria count data presented by Lucyckx et al. (2015) for C&D of an operational poultry house are used as the data source for the degree of inactivation by C&D. 1% Virkon S is effective against bacteria and viruses (Hernndez et al. 2000) and the total aerobic bacteria counts recorded by Lucyckx et al. (2015) before and after C&D are used to calculate the **fraction of pathogen surviving C&D in those parts of the poultry house that can be reached by C&D (γ)** (i.e. in the properly cleansed and disinfectant-treated bulk phase portion). These values are presented in Table 1 and represent the values of γ used in Equation 2 for the different streams.

Estimating the fraction of debris that by-passes the bulk phase

The **fractions of debris and organic material (and hence associated viruses) in those parts within each stream where C&D cannot reach with and without dismantling and which therefore by-pass the bulk phase and effective C&D (f_{bypass})** are set out in Table 2 and define the f_{bypass} parameter for each stream in Equation 2. There are no experimental data for the amount of material which does not receive effective C&D. These values are therefore determined from estimations made by visiting a chicken layer farm. The approach used was to estimate lower and upper values for a uniform distribution. For example, it was estimated that between 10% and 40% of virus in material on the manure belt could survive preliminary disinfection.

In effect, f_{bypass} reflects the efficiency of C&D at operational scale, the smaller f_{bypass}, the greater the efficiency as less of the virus-contaminated material avoids C&D. The preliminary C&D is considered not to be as efficient as secondary C&D. After preliminary C&D, a considerable amount of organic matter is still present and it is assumed for example that 25% remains on the manure belt (Table 2). During preliminary C&D, dead birds and the litter are cleared out, however there is no degreasing or scrubbing. There is only drenching with disinfectant. Thus preliminary C&D is assumed to be of relatively low efficiency (Table 2) depending on the stream. For example, it is assumed that the manure belt is least effectively cleansed, with 10 to 40% of material not being cleansed/disinfected properly. In contrast, it is assumed that only 1 to 10% of the floor is not cleansed in preliminary C&D. While the physical disturbance of preliminary C&D may produce aerosols these are negligible compared to the proportions of material assumed to be remaining overall.

In contrast to preliminary C&D, secondary C&D is much more thorough with power washes and fine brushes through greater workforce deployment to maximise removal of organic material. Degreasing and disinfection are undertaken and then repeated after 7 days. This is reflected in the smaller fractions of material that by-pass the process in secondary C&D (Table 2) compared to preliminary C&D. Dismantling further reduces f_{bypass} compared to not dismantling for the equipment streams. Again the fractions for by-pass are based on

expert opinion of what is achievable in practice rather than experimental data. Two secondary C&D scenarios are considered, namely without and with dismantling. Higher percentage by-pass is assumed without dismantling (Table 2).

Exposure through inhalation of dust and ingestion by the restocked poultry

As for the by-pass fractions, the **fractions of the infective material remaining after C&D that are inhaled (f_{inhale}) and ingested (f_{ingest}) by the restocked poultry** as out in Table 1 for each of the streams are based on expert opinion and assumptions in the absence of data. Although there is considerable uncertainty in these estimates, it is considered they are worst case assumptions. It is assumed that moving parts convert 10% of any remaining infectivity into dust which is inhaled by the restocked of birds. Similarly 10% of any material remaining on the floor is suspended into the air through the disturbance by people walking through the poultry house. It is assumed that only 0.01% of any material left in the metal troughs is actually inhaled by the birds. It is assumed that 50% of any material left in the metal troughs, moving hoppers and chains is ingested by the birds, while the birds have no access to any material on the floors and only limited access to the waste streams.

Calculation of exposures to restocked poultry

The **infectivity ingested by the restocked poultry through each stream ($EID_{50\_ingest\_stream}$)** was calculated as

$$EID_{50\_ingest\_stream} = EID_{50\_source\_stream} \times 10^{-\frac{TbDR}{D_t}} \times f_{survive\_stream} \times f_{ingest}$$

where **$EID_{50\_source\_stream}$ is the source term infectivity in a given stream immediately after depopulation of the infected poultry** as calculated by Equation 1 and **$f_{survive\_stream}$ is the fraction of input pathogen surviving C&D for each stream** as calculated by Equation 2. Similarly the **infectivity inhaled by the restocked poultry through each stream ($EID_{50\_inhale\_stream}$)** was calculated as

$$EID_{50\_inhale\_stream} = EID_{50\_source\_stream} \times 10^{-\frac{TbDR}{D_t}} \times f_{survive\_stream} \times f_{inhale}$$

It should be noted that infective material present in the manure source term in Equation 1 may be converted to dust during the operation of equipment in the restocked poultry house and hence inhaled and thus it is appropriate to calculate $EID_{50\_inhale\_stream}$ from $EID_{50\_source\_stream}$ from Equation 1.

The **total poultry exposure ($Exposure\_EID_{50}$)** was calculated for each of the three C&D scenarios as.

$$Exposure\_EID_{50} = \sum_{All\ streams} EID_{50\_ingest\_stream} + \sum_{All\ streams} EID_{50\_inhale\_stream}$$

Receptor term: Using dose-response to estimate risk of infection for highly pathogenic avian influenza virus H5N1

While the EID_{50} is a useful assay to measure levels of live virus in manure components and airborne particulate, a dose-response is required to convert EID_{50} units into live chicken ID_{50} units, where one chicken ID_{50} is the amount of infectious virus which when given to a single chicken has a 50% probability of infecting that chicken. According to Aldous et al. (2010) there are $10^{3.4}$ EID_{50} units per chicken ID_{50} for H5N1 HPAIV (A/turkey/Turkey/1/05) in live chickens on challenge through both the intraocular (0.1 ml) and intranasal (0.1 ml) routes. Since H7N1 HPAIV is less infectious to chickens than H5N1 HPAIV with an ID_{50} of $10^{4.6}$ EID_{50} (Aldous et al., 2010), the H5N1 data are used here. Thus it is assumed that there are $10^{3.4}$ EID_{50}/chicken ID_{50} and the **number of chicken ID_{50}s ingested by the chicken flock as a whole within the poultry shed ($N_{Chicken\_ID50}$)** is given by

$$N_{Chicken\_ID50} = \frac{Exposure\_EID_{50}}{10^{3.4}}.$$

References

Aldous EW, Seekings JM, McNally A, Nili H, Fuller CM, Irvine RM, Alexander DJ and Brown IH 2010. Infection dynamics of highly pathogenic avian influenza and virulent avian paramyxovirus type 1 viruses in chickens, turkeys and ducks. Avian Pathology 39, 265-273.

EU 2005. EU Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC Article 49. Retrieved on 20 January 2017 from http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32005L0094.

Hernndez A, Martro E, Andreu LM, Martin M and Ausina V 2000. Assessment of in-vitro efficacy of 1% VirkonS against bacteria, fungi, viruses and spores by means of AFNOR guidelines. Journal of Hospital Infection 46, 203-209.

Lucyckx KY, Weyenberg SV, Dewulf J, Herman L, Zoons J, Vervaet E, Heyndrickx M and De Reu K 2015. On-farm comparisons of different cleaning protocols in broiler houses. Poultry Science 94, 1986-1993.

Supplementary Material S3

***animal*. The international journal of animal biosciences.**

Risk assessment for recrudescence of avian influenza in caged layer houses following depopulation: The effect of cleansing, disinfection and dismantling of equipment.

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R code

```
#---
#  run: "R-studio desktop"
#  written: "R version 3.4.1"
#---
### Model ----
Risk_Calc <- function(Mass, EID50, Number_Birds, p_Remain, days_of_shedding,
                      Scenario, Parameters1, Parameters2,
                      ns, min, max, min_TbDR, max_TbDR,
                      Parameters4, Parameters5,
                      EID50_oralID50, CV)
          {
            Source_term_calculation <-function(Mass, EID50, Number_Birds, p_Remain, days_of_shedding)
                  {
```

```
            Cloacal=Mass*EID50[1]*Number_Birds*0.5*p_Remain
            GAL=EID50[2]*Number_Birds*0.5*days_of_shedding
            I=cbind(Cloacal,GAL)
            rownames(I)="Infectivity"
            return(Source_term_calculation=I)
            }
# Estimating the uniform distributions for the fraction by-passing the bulk phase
        simul<-function(ns, min, max, min_TbDR, max_TbDR)
             {
              l1=as.numeric(dim(min)[1])
              l2=as.numeric(dim(min)[2])
              y<-array(dim=c(l1,l2,ns))
              y1<-c()
              y2<-array(dim=c(l1+1,l2,ns))
              for (j in 1:ns){
                for (i in 1:l1){
                  for (k in 1:l2){
                    y[i,k,j]<-runif(1, min[i,k], max[i,k])
                  }#k
                }#i
                y1<-runif(1, min_TbDR, max_TbDR)
                y2[,,j]<-array(rbind(y[,,j],y1))
                }#j
              return(Parameters3=y2)
              }#fun
  Source_term<-Source_term_calculation (Mass, EID50, Number_Birds,
                                p_Remain, days_of_shedding)
# Initial infection on Equipment
```

```
  DIF=array(dim=c(7,2))
  dimnames(DIF)<-list(c("Metal trough","Moving hopper",        "Moving chain",
                        "Manure belt",        "Cross conveyer",    "Air drying eqp",
                        "Floor"),
                      c("Manure","Airborne particulate"))
  for (i in 1:length(Source_term)) {
    DIF[,i]=Source_term[i]*(Parameters1[,i])
  }
# Decay Rate as a costant

  Parameters3<-simul(ns, min, max, min_TbDR, max_TbDR)
  nc=as.numeric(dim(Parameters3)[2])
  nit=as.numeric(dim(Parameters3)[3])
  nr=as.numeric(dim(Parameters3)[1])-1
  eff_decay<-array(dim=c(7,2))

  if (Scenario==1) {
    DIF4=array(dim=c(1,nit))
    mod="Preliminary disinfection"
  }      else if (Scenario==2)
  {
    DIF4=array(dim=c(nit,nit))
    mod="Secondary: By-pass rate without dismantling"
  }  else if (Scenario==3)
  {
    DIF4=array(dim=c(nit,nit))
    mod="Secondary: By-pass rate with dismantling"
  }
```

```
  for(ii in 1:nit){
    # print (ii)
    eff_decay<-DIF/10^((Parameters3[nr+1,1,ii])/10)
# Effect of C&D
# Viral loadings after cleansing and disinfection
    By_pass_preliminary=array(dim=c(7,2))
    By_pass_secondary_without_dismantling=array(dim=c(7,2))
    By_pass_secondary_with_dismantling=array(dim=c(7,2))
    dimnames(By_pass_preliminary)=list(c("Metal trough","Moving hopper", "Moving chain",
                                         "Manure belt",  "Cross conveyer",    "Air drying eqp",
                                         "Floor"),
                                       c("Manure","Airborne particulate"))
    By_pass_preliminary=(1-Parameters3[-(nr+1),1,ii])*Parameters2+Parameters3[-
(nr+1),1,ii]
    DIF1=eff_decay*By_pass_preliminary
# Different scenarios
    if (Scenario==1) {
      DIF2=DIF1
      DIF3=array(dim=c(7,2))
      dimnames(DIF3)=list(c("Metal trough","Moving hopper",   "Moving chain",
                            "Manure belt",         "Cross conveyer",    "Air drying eqp",
                            "Floor"),
                          c("Manure","Airborne particulate"))
      for (j in 1:2) {
        DIF3[,j]=DIF2[,j]*(Parameters4[,j]+Parameters5[,j])
                  }
      DIF4[1,ii]=sum(DIF3, na.rm=TRUE)
            }  else if (Scenario==2)
        {
```

```
for (jj in 1:nit) {
  By_pass_secondary_without_dismantling=(1-Parameters3[-(nr+1),2,jj])*
                                  Parameters2+Parameters3[-(nr+1),2,jj]
  DIF2=DIF1*By_pass_secondary_without_dismantling
  DIF3=array(dim=c(7,2,nit))
  dimnames(DIF3)=list(c("Metal trough","Moving hopper", "Moving chain",
                        "Manure belt", "Cross conveyer",    "Air drying eqp",
                        "Floor"),
                      c("Manure","Airborne particulate"))
  for (j in 1:2) {
    DIF3[,j,jj]=DIF2[,j]*(Parameters4[,j]+Parameters5[,j])
                }
  DIF4[jj,ii]=sum(DIF3, na.rm=TRUE)
                  }
    }  else if (Scenario==3)
        {
   for (jjj in 1:nit) {
     By_pass_secondary_with_dismantling=(1-Parameters3[-(nr+1),3,jjj])*
                                     Parameters2+Parameters3[-(nr+1),3,jjj]
     DIF2=DIF1*By_pass_secondary_with_dismantling
     DIF3=array(dim=c(7,2,nit))
     dimnames(DIF3)=list(c("Metal trough","Moving hopper",       "Moving chain",
                           "Manure belt",        "Cross conveyer",    "Air drying eqp",
                           "Floor"),
                         c("Manure","Airborne particulate"))
     for (j in 1:2) {
       DIF3[,j,jjj]=DIF2[,j]*(Parameters4[,j]+Parameters5[,j])
                   }
```

```
          DIF4[jjj,ii]=sum(DIF3, na.rm=TRUE)
                        }
            }
     }
# Predicted Risk
  PI=(DIF4/EID50_oralID50)*CV
  return(list(Scenario=mod,
              Infect=Source_term,
              PredictedRisk=PI,
              Infectivity=DIF4,
              Exposure_median=median(DIF4),
              Exposure_CI_low=quantile(DIF4, 0.025),
              Exposure_CI_high=quantile(DIF4, 0.975),
              Probability_CI_low=quantile(PI, 0.025),
              Probability_CI_high=quantile(PI, 0.975),
              Probability_median=median(PI)
  ))
}
### Defining parameters ----
# Mass
Mass <- 120
# EID_50
EID50<-c(Cloacal=5011.8723362727,
         GeneralAirborneLoading=1.7864041909)
# Number of birds
Number_Birds <- 129000
# Minimum and maximum values used to define the uniform distributiions by-passing
# the "bulk" phase (f_bypass)
```

```
min_preliminary<-c(0.05, 0.01,0.01,0.10,0.01,0.01,0.01)
max_preliminary<-c(0.20, 0.10,0.10,0.40,0.10,0.10,0.10)
min_secondary_without<-c(0.005, 0.005,0.01,0.025,0.005,0.005,0.005)
max_secondary_without<-c(0.02, 0.02,0.04,0.10,0.02,0.02,0.02)
min_secondary_with<-c(0.00025, 0.00025,0.005,0.005,0.00025,0.00025,0.005)
max_secondary_with<-c(0.01, 0.01,0.02,0.02,0.01,0.01,0.02)
min<-cbind(min_preliminary,min_secondary_without,min_secondary_with)
max=cbind(max_preliminary,max_secondary_without,max_secondary_with)
# Defining the time minimum and maximum values of the
# period between depopulation of infected poultry and
# restocking with the new birds
min_TbDR<-40
max_TbDR<-90
# Defining Number of simulation ----
ns <- 1000
# p_remain
p_remain <- 0.001
# Defining number of days of shedding
days_of_shedding <- 1.3
# Defining scenario:
# Preliminary disinfection = 1
# Secondary: By-pass rate without dismantling = 2
# Secondary: By-pass rate with dismantling = 3
scenario <- c(1:3)
### Defining Parameters1 = Fractions of infectivity entering the different streams ----
X=c(0.001, 0.001,    0.001, 0.897,          0.05, 0.05, NA,
    0.01, 0.01, 0.01, 0.2, 0.1, 0.05, 0.62)
Parameters1<-array(X, dim=c(7,2))
```

```
dimnames(Parameters1)<-list(c("Metal trough","Moving hopper",     "Moving chain",

                            "Manure belt", "Cross conveyer",    "Air drying eqp",

                            "Floor"),

                           c("Manure","Airborne particulate"))

### Defining Parameters2 = Fractions of infectivity surviving C&D through the different
streams ----

Y=c(0.0003162278, 7.94328234724282E-05,    7.94328234724282E-05,
        2.51188643150958E-05,    7.94328234724282E-05,    6.30957344480193E-05,
7.94328234724282E-05,

    0.0003162278,    7.94328234724282E-05,    7.94328234724282E-05,
        2.51188643150958E-05,    7.94328234724282E-05,    6.30957344480193E-05,
        7.94328234724282E-05)

Parameters2<-array(Y, dim=c(7,2))

dimnames(Parameters2)<-list(c("Metal trough","Moving hopper",     "Moving chain",

                            "Manure belt", "Cross conveyer",    "Air drying eqp",

                            "Floor"),

                           c("Manure","Airborne particulate"))

### Defining Parameters4 = Fractions of infectivity inhaled through the different streams --
--

K=c(0.0001,  0.1,    0.1,    0.1,    0.1,    0.1,    0.1,

    0.0001,     0.1,    0.1,    0.1,    0.1,    0.1,    0.1)

Parameters4<-array(K, dim=c(7,2))

dimnames(Parameters4)<-list(c("Metal trough","Moving hopper",     "Moving chain",

                            "Manure belt", "Cross conveyer",    "Air drying eqp",

                            "Floor"),

                           c("Manure","Airborne particulate"))

### Defining Parameters5 = Fractions of infectivity ingested through the different streams -
---

M<- c(0.5,     0.5,    0.5,    0.01,   0.01,   0.1,    0,

      0.5, 0.5, 0.5, 0.01,        0.01,   0.1, 0)

Parameters5=array(M, dim=c(7,2))
```

```
dimnames(Parameters5)<-list(c("Metal trough","Moving hopper",      "Moving chain",
                            "Manure belt",  "Cross conveyer",    "Air drying eqp",
                            "Floor"),
                           c("Manure","Airborne particulate"))
### Running the model ----
for (s in 1:(length(scenario))) {
results[[s]] <-Risk_Calc(Mass, EID50, Number_Birds, p_remain,
                         days_of_shedding, s, Parameters1, Parameters2,
                         ns,min,max,min_TbDR, max_TbDR, Parameters4, Parameters5,
                         10^3.4, 0.69)
```