1   **Risk assessment for recrudescence of avian influenza in caged layer houses**

2   **following depopulation: The effect of cleansing, disinfection and dismantling of**

3   **equipment.**

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18

19 **Abstract**

20 Following an outbreak of **highly pathogenic avian influenza virus (HPAIV)** in a poultry

21 house, control measures are put in place to prevent further spread. An essential part of the

22 control measures based on the European Commission Avian Influenza Directive

23 2005/94/EC is the **cleansing and disinfection (C&D)** of infected premises. C&D includes

24 both preliminary and secondary C&D and the dismantling of complex equipment during

25 secondary C&D is also required, which is both costly to the owner and also delays the

26 secondary cleansing process hence increasing the risk for onward spread. In this study a

27 quantitative risk assessment is presented to assess the risk of re-infection (recrudescence)

28 occurring in an enriched colony caged layer poultry house on restocking with chickens

29 after different C&D scenarios. The risk is expressed as the number of restocked poultry

30 houses expected before recrudescence occurs. Three C&D scenarios were considered

31 namely (i) preliminary C&D alone, (ii) preliminary C&D plus secondary C&D without

32 dismantling and (iii) preliminary C&D plus secondary C&D with dismantling. The source-

33 pathway-receptor framework was used to construct the model and parameterisation was

34 based on the three C&D scenarios. Two key operational variables in the model are (i) the

35 **time between depopulation of infected birds and restocking with new birds (TbDR)**

36 and (ii) the proportion of infected material that by-passes C&D, enabling virus to survive

37 the process. Probability distributions were used to describe these two parameters for

38 which there was recognised variability between premises in TbDR or uncertainty due to

39 lack of information in the fraction of by-pass. The risk assessment estimates that the

40 median (95% credible intervals) number of repopulated poultry houses before

41 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

42 2.9×10^8) under C&D scenarios (i), (ii) and (iii) respectively. Thus for HPAIV in caged layers

43 undertaking secondary C&D without dismantling reduces the risk by 16-fold compared to

44 preliminary C&D alone. Dismantling has an additional, although smaller, impact, reducing

45   the risk by a further six-fold and thus around 90 fold compared to preliminary C&D alone.

46   On the basis of the 95% credible intervals, the model demonstrates the importance of

47   secondary C&D (with or without dismantling) over preliminary C&D alone. However, the

48   extra protection afforded by dismantling may not be cost beneficial in the context of

49   reduced risk of onward spread.

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

51   **Implications**

52   Disease caused by highly pathogenic avian influenza virus (HPAIV) severely impacts on

53   the profitability of poultry farming. It is important to ensure that levels of residual HPAIV

54   infectivity in the poultry house are sufficiently reduced to ensure recrudescence does not

55   occur. The outputs of the work presented here have important benefits through supporting

56   reductions in both labour costs to the farmer and in the time to complete secondary

57   cleansing and disinfection by not having to dismantle and rebuild complex equipment. The

58   results of the risk assessment will help inform policy-makers and industry in their decision-

59   making and the risk assessment model could be applied to other avian pathogens such as

60   Newcastle disease virus using appropriate data.

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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

88   other poultry flocks through infection of wild birds or through fomite transmission. An

89   essential part of the control measures based on the European Commission Avian

90   Influenza Directive 2005/94/EC (EU, 2005) is the **cleansing and disinfection (C&D)** of

91   infected premises. Cleansing and disinfection includes preliminary and secondary C&D

92   and the dismantling of complex equipment during secondary C&D is also required.

93   Preliminary C&D is Government funded and involves spraying all parts of the premises

94   and any contaminated material remaining with disinfectant to 'damp down' any virus in the

95   environment. Secondary C&D is at the owner's expense and requires cleansing the

96   premises, including equipment and installations, to remove organic debris, degreasing and

97   disinfecting and then repeating the process.

98   In the absence of epidemiological evidence and data on how effective dismantling is in

99   preventing further outbreaks of HPAIV in a poultry house after C&D, a quantitative risk

100   assessment model is developed here to assess the probability that newly introduced

101   immunologically-naive chickens used to restock a poultry house become infected

102   (recrudescence) with HPAIV after C&D has taken place. Three C&D scenarios in a caged

103   layer house are assessed, namely preliminary alone, preliminary plus secondary without

104   dismantling and preliminary plus secondary with dismantling with data drawn primarily

105   from HPAIV H5N1 scenarios.

106   **Materials and methods**

107   *Risk analysis and risk assessment*

108   The terms risk analysis and risk assessment have different meanings. Risk analysis is the

109   complete process for handling a threat. Risk assessment is a defined stage of the risk

110   analysis process. Thus the risk analysis process is hazard identification followed by the

111   risk assessment itself and finally risk management with risk communication important for

112   all three stages (OIE, 2019). The risk assessment estimates the risks associated with the

113   hazard and may be qualitative or quantitative. It should be noted that hazard and risk are

114   different. The hazard is the pathogen, HPAIV in this case, while the risk is the probability of

115   an adverse event from the hazard occurring, namely recrudescence of HPAI in the

116   restocked poultry. Risk assessment is one of a number of tools to help manage and

117   prevent poultry diseases like HPAIV through predicting the risks of outbreaks and

118   assessing by how much various control processes reduce those risks. Other tools include

119   epidemiological case studies based on previous outbreaks to identify and rank those

120   factors which contribute to incursion, transmission and spread of such diseases. The

121   advantage of risk assessment is that it can used to predict the probability of outbreaks

122   occurring so that preventative actions may be implemented through risk management and

123   policy (Goddard et al., 2012), hopefully before an outbreak occurs.

124   The risk assessment here is based on quantifying the amount of infectivity that restocked

125   poultry (the receptor) are exposed to from infectious HPAIV remaining in the poultry house

126   (the source) through all the conceivable exposure pathways within the poultry house (the

127   pathway). Conceptually these risk assessments, known as "source-pathway-receptor"

128   models, are relatively simple mathematically although the pathways may be complex

129   depending on the system being studied. The structure of the risk assessment has to be

130   appropriate for the system and the hazard. Thus the source-pathway-receptor model is

131   well suited to environmental/process risk assessments involving a series of protective

132   barriers. Another risk assessment approach is the entry-exposure-consequence

133   assessment used for import risk assessment for exotic livestock diseases (OIE, 2019) and

134   is often qualitative as for example for importation of lumpy skin disease virus into the UK

135   through cattle hides (Gale et al., 2015). Qualitative assessment does not require

136   mathematical modelling skills to carry out and so is often the type of assessment used for

137   rapid, reactive, evidence-based decision making (Kelly et al., 2018).

138   The choice of qualitative or quantitative in risk assessment depends on the nature of the

139   available data and the complexity of the model and also the scope of the risk question as

140   set by the risk manager. Qualitative risk assessment can be applied in the absence of

141   sufficient numerical data but where there is at least some basic knowledge, expert opinion

142   or other understanding of the magnitude of the risks for each of the risk assessment steps.

143   The model here allows for by-pass of the C&D process and is too complex for qualitative

144   risk assessment. Also being a multiple barriers model (i.e. including removal of manure at

145   the poultry house, destruction of virus by C&D and decay with time) it is not necessarily

146   suited to combining multiple low qualitative conditional probabilities using a risk matrix

147   approach (Kelly et al., 2018). Furthermore adding qualitative probabilities from several

148   parallel streams as required here is not straight forward. The risk assessment approach

149   here is therefore quantitative and complements a previous qualitative assessment

150   (Horigan et al., 2019).

151   Once the basic mathematical model as defined by the equations relating levels of HPAIV

152   in the poultry house at point of culling to the risk of infection in the restocked poultry have

153   been set out, there are several different approaches for quantitative risk assessment

154   including deterministic and probabilistic. The deterministic approach calculates the

155   arithmetic mean for each step in the source-pathway-receptor model and tends to deal

156   with uncertainty by using worst case assumptions particularly where data are lacking

157   (Gale, 2004 and 2005). The probabilistic approach produces a distribution of risks to

158   accommodate the uncertainty and/or variation and thus naturally provides 95% credible

159   intervals in addition to the median probability. This is important because it allows the risk

160   manager to be 97.5% confident that the risk is not higher.

161   *Model overview*

162   The quantitative model is based on observations made during a site visit to a laying house

163   which housed 129 000 chickens in enriched colony cages. The model is based on three

164   parts of the feed stream (namely the metal trough, the moving hopper and the moving

165   chain) and on three parts of the waste stream (namely the manure belt, the cross-

166   conveyor, and the manure air drying equipment). In addition the floor is included. Colony

167   cages are not specifically considered, but are included as part of the manure belt which

168   runs directly underneath the cages. Moving parts were included because of their capacity

169   to generate dusts, although poultry are unlikely to have direct contact with moving chains,

170   for example.

171   The approach uses the "source-pathway-receptor" model developed previously for

172   assessing the infection risks from pathogens through environmental routes involving

173   treatment processes such as composting and sewage sludge processing followed by

174   pathogen decay in the environment (Gale, 2004 and 2005). The source term is the amount

175   of infectivity in the poultry house at the point of culling and removal of the infected birds.

176   The receptor in this model is the whole chicken flock used for restocking the poultry house

177   after the given C&D scenario. By assuming that the dose-response is linear such that just

178   a single HPAI virion is able initiate infection in a poultry host, albeit with low probability, it

179   does not matter whether one chicken in the restocked flock ingests the whole dose (and all

180   the other chickens are not exposed) or whether each and every chicken has an equal

181   portion of the dose. This approach is equivalent to calculating an arithmetic mean

182   individual bird exposure as has been used previously for environmental source-pathway-

183   receptor risk assessments (Gale, 2004 and 2005) and avoids the need to estimate the

184   exact dose ingested by each and every one of the individual restocked birds. Furthermore

185   by assuming that a certain fraction of the residual infectivity is inhaled or ingested by the

186   incoming flock, the total number of restocked birds is not required in the exposure

187   calculation.The whole flock exposure is then used to calculate the **probability of at least**

188 **one chicken becoming infected in the poultry house ($p_{outbreak}$)**, since it would only

189 need one bird to be infected for the entire restocked population to succumb. The

190 probability $p_{outbreak}$ is thus in effect the probability of recrudescence in that poultry house,

191 and its inverse represents the average number of similar poultry houses deploying the

192 C&D scenario before one had a recrudescence.

193 *The source term: Virus loadings in the poultry house*

194 The quantitative model is based on a large layer poultry house with 129 000 chickens

195 (reflecting a site visit made in December 2016). It is assumed that 50% (i.e. 64 500) of the

196 birds are infected and shedding HPAIV at the point of culling. The unit of infectivity in the

197 exposure assessment is the **egg infectious dose 50% (EID$_{50}$)** which is the dose required

198 to infect 50% of inoculated embryonated fowls eggs (when given to each and every egg in

199 the group) in laboratory assay. The viral titre contributions to the source term expressed as

200 EID$_{50}$ units are estimated as described in Supplementary Material S1 for:-

201   1. The **total HPAIV infectivity from the three bird matrices namely feathers,**

202   **faeces and oropharyngeal secretions which goes into the "manure"**

203   **(EID_{50_manure})** calculated from data in Yamamoto *et al.* (2008) and Scottish

204   Government (2016) together with unpublished data from the **Animal and Plant**

205   **Health Agency (APHA)**; and

206   2. The **airborne particulate HPAIV infectivity which settles as dust ($EID_{50\_airborne}$)**

207   calculated from data of Spekreijse et al. (2011).

208 *Distribution of mass fractions of infected material to different feed and waste streams.* The

209 source term considers three waste and three feed streams within the poultry house,

210 together with the floor, as shown in Figure 1 for enriched colony caged layer houses. It is

211 assumed that 99.9% of the manure produced is removed daily during normal operation of

212 the poultry house and that this would have been removed in the 24 hours prior to culling.

213   Therefore 0.1% of the manure is still present in the poultry house after culling and removal

214   of the infected poultry. It is assumed that all of the airborne fraction settles as dust after

215   removal of the poultry. The **fractions of manure (f_{stream_manure})** and the **fractions of**

216   **airborne particulate ($f_{stream\_airborne}$)** assumed to be entering each of the three feed

217   streams and the three waste streams, together with the floor are set out in Table 1. These

218   are estimated on the basis of the site visit. The **source term infectivity in a given stream**

219   **immediately after depopulation of the infected poultry ($EID_{50\_source\_stream}$)** is given by:-

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

221     $EID_{50\_airborne} \times f_{stream\_airborne}$

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

223   The pathway from residual infectivity in the poultry house at the point of depopulation of

224   the infected flocks to the restocking with the new poultry flock is set out in Figure 2. The

225   pathway is used to calculate the total exposure to the receptor in terms of EID_{50} units and

226   sets out the barriers which act to decrease the exposure to the receptor. These include

227   natural decay in addition to destruction of virus by the C&D process.

228   *Modelling virus decay during the period between depopulation and restocking.* Viruses

229   cannot multiply outside the host and undergo natural decay once outside the host.

230   The **decimal reduction time (D_t)** is the time for a 10-fold decrease (i.e. 1 $\log_{10}$) or 90%

231   decrease in the virus loading. D_t times for HPAIV H7N1 A/ostrich/Italy/984/2000 and H5N1

232   A/turkey/Turkey/1/2005 in chicken faeces were 3.33 days and 12.05 days at 4°C and 0.83

233   days and 4.41 days at 20°C, respectively (C. Warren, personal communication). As is well

234   known from other studies of virus inactivation, decay is more rapid at the higher

235   temperature of 20°C compared to 4°C. The D_t time used for decay of HPAIV in this risk

236   assessment is 10 days. Although the D_t for H5N1 at 4°C is >10 days at 12.05 days, the

237 value of 10 days takes into account that temperatures may exceed 4°C even during the

238 winter period (particularly in 2016). Furthermore the temperature in the shed with birds

239 present is higher, although the temperature will fall after depopulation. Using D_t times for

240 chicken faeces as in the models here is a worst case scenario because the D_t times

241 measured in poultry litter were much shorter at <5 min and <10 min for H7N1 and H5N1

242 respectively at both 4°C and 20°C (C. Warren, personal communication). For the purpose

243 of risk assessment, decay is assumed to occur over the **time period between**

244 **depopulation of the infected poultry and restocking with the new birds (*TbDR*)**.

245 Minimum and maximum values of 40 and 90 days respectively were used to define a

246 uniform distribution for *TbDR* (see Supplementary Material S2).

247 *Modelling virus inactivation by cleansing and disinfection.* In this risk assessment, the

248 overall inactivation of HPAIV by C&D is modelled by summing the titres surviving in two

249 separate 'portions':-

250   1. The bulk phase, which undergoes efficient cleansing and disinfection; and

251   2. The by-pass phase, which misses efficient cleansing and disinfection altogether so

252   that no pathogen inactivation takes place.

253 This is based on the method developed by Gale (2004) for removal of pathogens by

254 composting of catering waste and simplifies the risk assessment methodology into

255 estimating:-

256   1. The **fraction of pathogen surviving in the properly cleansed and disinfectant-**

257   **treated bulk phase portion (γ)**; and

258   2. The **fraction of debris and organic material (and hence associated viruses) in**

259   **those parts within each stream where C&D cannot reach and which therefore**

260   **by-passes the bulk phase and effective C&D (f_{bypass})**.

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

262   thus calculated as:-

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

264   Values of γ are allocated in Table 1 for each of the six streams and the floor on the basis of

265   the measured decrease in total aerobic bacteria counts in the most closely related

266   equipment during C&D of an operational poultry house as reported by Lucyckx et al.

267   (2015). This is described in Supplementary Material S2. Minimum and maximum values of

268   f_{bypass} to represent the proportions of organic material (and hence associated viruses)

269   which by-pass C&D within each stream for preliminary and secondary C&D with and

270   without dismantling are set out in Table 2. These were used to define a uniform distribution

271   for f_{bypass} and were based on what is thought to be operationally achievable as set out in

272   Supplementary Material S2.

273   *Calculation of exposures to restocked poultry through inhalation of dust and ingestion*. The

274   **fractions inhaled (f_{inhale})** of the remaining infective material (after conversion to dust

275   through moving parts in the equipment or other disturbance in the restocked poultry

276   house) by the restocked poultry are set out in Table 1 for each of the streams together with

277   the **fractions ingested (f_{ingest})** through feeding and pecking. In the absence of data, these

278   are based on expert opinion and assumptions as set out in Supplementary Material S2.

279   Exposures (in EID_{50} units) to the restocked poultry through ingestion and inhalation were

280   calculated for each of the seven streams from $EID_{50\_source\_stream}$ (Equation 1) allowing for

281   decay of HPAIV according to D_t over the *TbDR* period in the fraction, $f_{survive\_stream}$, (from

282   Equation 2) of HPAIV surviving C&D in each stream. The equations are set out in

283   Supplementary Material S2. The **total poultry exposure ($Exposure\_EID_{50}$)** was

284   calculated as the sums of the exposures through the ingestion and inhalation routes for

285   each of the seven streams using equations set out in Supplementary Material S2. This

286   represents the total exposure to the poultry in a given poultry house. The units are "EID_{50}

287   in total poultry population per poultry house". Because the model assumes that a fixed

288   proportion of the remaining infectivity is ingested or inhaled (according to f_{ingest} and f_{inhale} in

289   Table 1) by the poultry flock as a whole, the risk assessment is not dependent on the

290   number of restocked poultry. This is realistic for poultry houses with large numbers of birds

291   where a steady state is likely to be reached over a few days, but would be less appropriate

292   for houses with only a few birds. This avoids a more complex calculation involving the

293   estimation of how much debris each of 129 000 chickens ingests each day and the

294   number of days over which this could occur.

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

296   The **number of chicken ID_{50}s ingested by the chicken flock as a whole within the**

297   **poultry shed ($N_{Chicken\_ID50}$)** is calculated from the total poultry exposure (*Exposure_EID_{50}*)

298   using the poultry infectivity data for HPAIV H5N1 of Aldous et al. (2010) as described in

299   Supplementary Material S2. The probability of at least one infected chicken in the poultry

300   house, and hence the probability of an outbreak in the poultry house, $p_{outbreak}$, is then given

301   by:-

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

303   where p_{50} is the risk of infection from a single chicken ID_{50} when given to a chicken (i.e.

304   0.5). The inverse of $p_{outbreak}$, is the number of infected poultry houses cleansed according

305   to the given C&D procedure before recrudescence in the restocked poultry is expected to

306   occur in one. The number of infected chickens in the poultry house could be calculated

307   from $N_{Chicken\_ID50}$ as done for livestock grazing on land to which composted catering waste

308   had been applied (Gale, 2004) and would be greater than one for high values of

309     $N_{Chicken\_ID50}$. However, the number of infected chickens in the poultry house is of little

310   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

336   respectively as expected and reducing the percentage of infected birds in the source term

337   from 50% to 5% increased the predicted average number of houses before a

338   recrudescence by 10-fold as expected. As part of face validity (Sargent, 2011),

339   representatives of poultry industry agreed the conceptual model represented in Figure 1

340   and Figure 2 was correct and that the model's input-output relationships are reasonable

341   (Gale et al., 2018).

342   **Results**

343   *Highly pathogenic avian influenza virus loadings in a poultry house at point of culling and*

344   *removal (depopulation) of infected poultry*

345   The total HPAIV infectivity in the poultry house at the end of depopulation and after

346   removal of 99.9% of the manure is 3.89 10^7 $EID_{50}s$ (Supplementary Table S1). This is

347   mainly from cloacal/oropharyngeal secretions and feathers in the remaining manure, with

348   settling of airborne particulate making only a small contribution. By apportioning the

349   infectivity according to the fractions, f_{stream_manure} and $f_{stream\_airborne}$ from Table 1 in Equation

350   1, the amounts of infectivity in each of the feed and waste streams and on the floor at the

351   point of depopulation are calculated (Table 3).

352   *Predicted exposures and risks of recrudescence to restocked poultry*

353   The estimated median HPAIV exposures to the restocked poultry in terms of $EID_{50}s$ per

354   poultry house are presented in Table 4. Secondary C&D (without dismantling) decreases

355   the median exposure by 15-fold compared to just preliminary C&D alone. When

356   dismantling is applied, the median exposures are decreased by a further 6-fold, and the

357   overall decrease in exposure compared to preliminary C&D alone is over 88-fold. These

358   decreases in exposure directly reduce the risks of recrudescence reflecting the linear

359   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

436   and Equation 3 tends to $p_{outbreak} = 0.69 \times N_{Chicken\_ID50}$ at low values of $N_{Chicken\_ID50}$ (Gale,

437   2004). A recent attempt to develop a mechanistic dose-response model for viruses (Gale,

438   2018) has indicated a theoretical mechanism for a threshold effect where the virus dose

439   needs to be sufficiently high to overwhelm the host innate defences (e.g. mucins in

440   mucus), although this has not be proven experimentally. Clearly allowing for a **minimum**

441   **infectious dose (MID)** greater than one virion in the model would greatly diminish the

442   risks predicted here to the re-stocked poultry, depending on the magnitude of that MID.

443   This is because a single virion alone could not cause infection and those individual birds

444   exposed to doses below the MID would not be infected in reality but according to the

445   model here are at risk of infection. However, the exposure assessment would have to be

446   modified to predict the actual exposure to each and every one of the 129 000 chickens

447   taking into account all sources of variation in the source and pathway terms so as to

448   predict how many chickens are exposed to doses above the MID. A further consideration

449   is whether the exposure to individual poultry in the restocked birds is in one single

450   exposure or repeated over several days or weeks. Thus, exposure to small amounts of

451   virus distributed over a longer time might influence the virus inactivation by the immune

452   system resulting in a higher resistance against infection than in case of exposure to the

453   whole dose at once (Pujol et al., 2009; Marois et al., 2012). The risk assessment here

454   takes a worst case and ignores this possibility. Indeed, it is likely that the highest

455   exposures would occur early on for the restocked birds.

456   The predicted values of $p_{outbreak}$ in Figure 3 vary over some six orders of magnitude mainly

457   reflecting the large range for *TbDR* in the uniform distribution and the assumption of log-

458   linear decay of HPAIV over the *TbDR*. This together with more information on the degree

459   of by-pass highlights areas of the model for which additional field data would be of use.

460   Overall, the probability of recrudescence of HPAI disease in caged layers following

461   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.

493   **Declaration of interest**

494   None declared.

495   **Ethics committee**

496   Not relevant.

497   **Software and data repository resources**

498   The model was not deposited in an official repository.

499   **References**

500   Alarcon P, Brouwer A, Venkatesh D, Duncan D, Dovas CI, Georgiades G, Monne I, Fusaro A, Dan

501   A, Smietanka K, Ragias V, Breed AC, Chassalevris T, Goujgoulova G, Hjulsager CK, Ryan E,

502   Sanchez A, Niqueux E, Tammiranta N, Zohari S, Stroud D, Savic V, Lewis NS and Brown IH 2018.

503   Comparison of 2016–17 and previous epizootics of highly pathogenic avian influenza H5

504   Guangdong lineage in Europe. Emerging Infectious Diseases 24, 2270-2283.

505   Aldous EW, Seekings JM, McNally A, Nili H, Fuller CM, Irvine RM, Alexander DJ and Brown IH

506   2010. Infection dynamics of highly pathogenic avian influenza and virulent avian paramyxovirus

507   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.

532 Horigan V, Gale P, Adkin A, Brown I, Clark J and Kelly L 2019. A qualitative risk

533 assessment of cleansing and disinfection requirements after an avian influenza outbreak in

534 commercial poultry. British Poultry Science 60, 691-699.

535 Kelly L, Kosmider R, Gale P and Snary E 2018. Qualitative import risk assessment: A

536 proposed method for estimating the aggregated probability of entry of infection. Microbial

537 Risk Analysis 9, 33-37.

538 Lucyckx KY, Weyenberg SV, Dewulf J, Herman L, Zoons J, Vervaet E, Heyndrickx M and De Reu

539 K 2015. On-farm comparisons of different cleaning protocols in broiler houses. Poultry Science 94,

540 1986-1993.

541 Marois I, Cloutier A, Garneau E and Richter MV 2012. Initial infectious dose dictates the innate,

542 adaptive, and memory responses to influenza in the respiratory tract. Journal of Leukocyte Biology

543 92, 1–12.

544 OIE 2018. OIE situation report for highly pathogenic avian influenza. Last updated 31 August 2018.

545 Retrieved on 30 May 2019 from

546 http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/OIE_AI_situation_re

547 port/OIE_SituationReport_AI_August2018.pdf.

548 OIE 2019. Terrestrial animal health code. Section 2. Risk analysis. Last updated 28 June

549 2019. Retrieved on 29 November 2019 from

550 https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_import_ris

551 k_analysis.pdf.

552 Pujol JM, Eisenberg JE, Haas CN and Koopman JS 2009. The effect of ongoing exposure

553 dynamics in dose response relationships. PLOS Computational Biology 5, e1000399.

554 Roberts H, Gale P and Brown I 2018. Findings of H5N6 HPAI in wild birds in UK/Ireland and LPAI

555 in poultry in France. Retrieved on 30 May 2019 from

556 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/682151/avian-flu-

557 wild-birds-H5N6-180213.pdf.

558 Sargent RG 2011. Verification and validation of simulation models. In proceedings of the 2011

559 Winter Simulation Conference, pp. 183–198. Retrieved on 3 December 2019 from

560 https://www.informs-sim.org/wsc11papers/016.pdf.

561 Scottish Government 2016. Calculating the amount of poultry manure produced. Retrieved on 20

562 January 2017 from www.gov.scot/Resource/Doc/278281/0096546.doc.

563 Spekreijse D, Bouma A, Koch G and Stegeman JA 2011. Airborne transmission of a highly

564 pathogenic avian influenza virus strain H5N1 between groups of chickens quantified in an

565 experimental setting. Veterinary Microbiology 152, 88-95.

566 Yamamoto Y, Nakamura K, Okamatsu M, Miyazaki A, Yamada M and Mase M 2008. Detecting

567 avian influenza virus (H5N1) in domestic duck feathers. Emerging Infectious Diseases 14, 1671-

568 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.*

Infected components	Feed streams		Moving chain	Waste streams			
	Metal trough	Moving hopper		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

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.*

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

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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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600 **List of figure legends**

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602 **Figure 1: Source term contributions of highly pathogenic avian influenza virus as**

603 **egg infectious dose 50% (EID$_{50}$) units from infected chickens in manure (EID_{50_manure})**

604 **and as particulate matter in the air which settle as dust ($EID_{50\_airborne}$) at point of**

605 **depopulation of poultry house. The fractions of manure (f_{stream_manure} shown as**

606 **percentages in normal font) and the fractions of airborne particulate ($f_{stream\_airborne}$**

607 **shown as percentages in italic font) entering each stream are from Table 1.**

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610 **Figure 2: Pathway detailing the fate of highly pathogenic avian influenza virus**

611 **infectivity as egg infectious dose 50% (EID$_{50}$) units to calculate total exposure to**

612 **restocked chicken poultry flock (Receptor) after cleansing and disinfection (C&D)**

613 **and virus decay over the time between depopulation of infected chickens and**

614 **restocking with new chickens (TbDR).**

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617 **Figure 3: Frequency distribution for the values of the probability of recrudescence**

618 **per chicken poultry house ($p_{outbreak}$) as predicted by 1 000 iterations of the model for**

619 **a) preliminary cleansing and disinfection (C&D) alone; b) preliminary C&D followed**

620 **by secondary C&D without dismantling; and c) preliminary C&D followed by**

621 **secondary C&D with dismantling.**

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Supplementary Material S1

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 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)
               {
```

```r
          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
```

```r
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"
}
```

```r
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)

      {
```

```r
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])
              }
```

```r
        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)
```

```r
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))
```

```r
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 ----

```r
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 ----

```r
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 ----

```r
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))
```

```r
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)
```