A qualitative risk assessment of cleansing and disinfection requirements after an avian influenza outbreak in commercial poultry

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Abstract

1. During an Avian influenza (AI) outbreak in the United Kingdom the joint aim of the poultry industry and the Government is to eliminate and prevent the spread of infection, through control measures based on the current European Union (EU) Council Directive (2005/94/EC). An essential part of these measures is the cleansing and disinfection (C&D) of infected premises.
2. This risk assessment assessed the differences in risk of re-infection in a repopulated flock if the EU Directive is interpreted to permit secondary C&D to be undertaken either with or without dismantling complex equipment. The assessment estimated the probability of virus survival on different types of equipment in a depopulated contaminated poultry house before and after preliminary and secondary C&D procedures. A risk matrix spreadsheet tool was used to carry out the assessment and concluded that provided secondary C&D is carried out with due diligence (i.e. carried out to a defined code of practice as agreed by both industry and policy makers), the risk of re-infection from equipment is negligible both with and without dismantling complex equipment in all farm types considered.
3. By considering the equipment types individually, the assessment identified those areas of the house which may still contain viable virus post preliminary C&D and, therefore, on which attention should be focussed during secondary C&D. The generic risk pathway and risk matrix spreadsheet tool have the potential to be used for other pathogens and species given appropriate data.

Key words: disease; broilers; laying hens; influenza virus

Introduction

Poultry can be affected by a variety of diseases and parasites but Avian Influenza (AI) viruses and Newcastle disease (ND) viruses are the only avian diseases that must be notified to the competent authority by law if suspected in the United Kingdom (UK). Their notifiable status is due to the high mortality and morbidity experienced within an infected poultry population and the economic impacts from trading restrictions and embargoes placed on infected areas or countries (Aldous et al. 2010). During a notifiable avian disease (NAD) outbreak the Government's aim is to prevent the spread of infection through proportionate and evidence-based control measures based on the current European Union (EU) Council Avian Influenza Directive (2005/94/EC) (EU 2006). An essential part of these control measures is the cleansing and disinfection (C&D) of infected premises (IP) to remove virus from the IP before restocking can occur and movement/trade restrictions can be lifted. The efficiency and speed with which C&D is completed directly impacts the wider industry with economic implications. In the UK, following government funded preliminary C&D, a notice will be served on the owner/occupier of the IP requiring them to carry out secondary C&D at their own expense and to the satisfaction of a Government veterinary officer. Preliminary C&D essentially involves spraying all surfaces with disinfectant to 'damp down' any virus in the environment whilst secondary C&D involves cleansing to remove organic debris, degreasing and disinfecting and then repeating the process to provide a high level of confidence that any virus on the premises is eliminated.

The EU Directive states that during secondary C&D "washing and cleansing by careful brushing and scrubbing of the ground, floors, ramps and walls following the removal or dismantling, where possible, of equipment or installations otherwise impairing the effective cleansing and disinfection procedures" is required. The directive may be interpreted and implemented by necessitating all complex equipment or installations e.g. cages, egg belts etc., to be dismantled prior to secondary C&D. Dismantling and then reassembling the complex equipment is, however, time and labour intensive, leading to high costs to the individual producer and may result in an extended period before trade can re-commence for the wider industry.

This risk assessment assesses the differences in risk to a sentinel flock of poultry if the EC Directive was interpreted to permit secondary C&D to be undertaken either with or without dismantling all complex equipment that could otherwise be appropriately cleansed and disinfected. The results are presented as a qualitative assessment risk matrix tool based on a generic risk pathway with the potential to be used for other pathogens and species, given appropriate data. Worst case assumptions were made when no other data were available. The assessment estimated the probability of virus survival on different types of equipment in a depopulated contaminated poultry house before and after preliminary and secondary C&D procedures before deriving a probability of re-infection in a sentinel poultry flock.

Methods

Risk question

The following risk question was used as a basis for this assessment:

“What is the risk of re-infection with Avian Influenza in a layer breeder, broiler breeder, layer or broiler flock from complex equipment/installations, given the different interpretations and implementations of the EU directive with regards to C&D?”*

* *detailed in the following sections*

Throughout this report, poultry is taken to refer to the sectors being considered as outlined in the risk question for chickens *Gallus gallus* only.

Risk Pathway

The pathway, as shown in Figure 1, is generic for all poultry groups and premises type being considered in this assessment. Each step on the pathway considers a key stage of the process, in relation to either virus levels or risk mitigation. The pathway divides according to whether or not secondary C&D is carried out and, if it is carried out, whether or not dismantling of complex equipment occurs.

FIGURE 1 HERE

Risk assessment

There are three scenarios for which the risk was assessed:

1. Infection from complex equipment with preliminary C & D and no secondary C&D
2. Infection from complex equipment with preliminary C&D and secondary C&D without dismantling
3. Infection from complex equipment with preliminary C&D and secondary C&D with dismantling

For each step, the key outputs are probabilities of contamination and virus levels, and are defined as in Table 1.

TABLE 1 HERE

The risk assessment follows the guidelines and risk terminology as amended from the European Food Safety Authority (EFSA) (EFSA 2006) and the World Organisation for Animal Health (OIE) (OIE 2004). Briefly, the probabilities are expressed qualitatively as *negligible, very low, low, medium, high* and *very high* and defined as: *negligible*, so rare that it does not merit to be considered; *very low*, very rare but cannot be excluded; *low*, event is rare but does occur; *medium*, event occurs regularly; *high*, event occurs very often; and *very high*, event occurs almost certainly.

The following assumptions were made:

- Low temperature environmental conditions mirroring historical winter AI outbreaks in Europe. Barns will normally reduce to external ambient temperature during C&D and downtime; the speed at which this happens will depend on time of year and the particular system (and internal temperatures prior to depletion).
- Heating to high temperatures for a number of days to kill the virus is not carried out (although this technique has sometimes been used to kill red mites and may be approved as an option for notifiable disease control in the future)
- No water based products would be used in below freezing temperatures
- Viral load and survival within different organic matrices were based on values from the literature. When data was not available worst case assumptions were adopted using expert opinion. (See the Supplementary material for details). For example, in some cases proxy data, in particular, the use of *Salmonella* studies, was used to assess probabilities. Data on the number of bacteria pre and post C&D can help to indicate those areas where organic material is concentrated and those that are difficult to clean thoroughly whilst acknowledging that there will be differences between viral and bacterial environmental survival characteristics and susceptibility to C&D. Approved dilution rates for statutory use of Virkon S for 'diseases of poultry order and the avian influenza and influenza of avian origin order' which uses ND virus as the target organism is 2.8 X greater than that for general orders which uses *Salmonella* Enteritidis as the target organism. AI is less robust than ND so could therefore be considered very susceptible to disinfectants. It is also possible for bacteria to multiply in suitable conditions after C&D has been carried out whereas viruses will continue to be subject to natural decay over time depending on the environmental conditions.
- Highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) treated as one generic virus with the same parameters e.g. titres in organic matrices, survival times (due to variability among strains within these groupings and insufficient data to assess the viruses independently)
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- Time periods between C&D stages and repopulation (based on expert opinion and timescales from previous AI outbreaks (see Supplementary material)) with the exception of the post preliminary C&D which is an unrealistic scenario.
- Secondary C&D carried out with due diligence (i.e. according to a defined code of practice as agreed by both industry and policy makers)
- No risk mitigation strategies for outdoor paddocks in free range poultry houses

In terms of approach, for each poultry species and premises type combination (referred to hereafter as farm-type), there are different types of equipment and matrices in which the virus may be present. The four organic matrices considered were dust, feathers, faecal material (cloacal) and oropharyngeal deposits. Each matrix can vary in relation to the extent to which it contributes to the risk of infection for the different poultry houses and different items of equipment. For example, whilst oropharyngeal deposits can contain high levels of virus, there is very little organic material protecting the virus which makes it exposed to the effects of disinfectants unless it is in a hard to access area. Avian influenza virus is known to survive for up to 120 days in feathers (Yamamoto et al. 2010), however, direct environmental contamination from these contaminated feathers may be limited to a local area because of the nature of the material (Yamamoto et al. 2010).

For the assessment, each combination of equipment and matrices has its own set of probabilities along the pathway and therefore its own overall estimate of probability of infection (see Supplementary material). Due to the fact that these overall probabilities are a product of the conditional probabilities, each is therefore determined by the lowest of the pathway estimates (Gale et al. 2010). Thus, for a particular piece of equipment and matrix, if there is a negligible or very low probability present in the pathway, the risk from that equipment will be negligible or very low (at most). Clear definitions were allocated to each qualitative rating and agreed by the project board. Risk assessors then used these ratings with evidence from peer reviewed literature. Initial ratings were subsequently presented and discussed with disease experts and the poultry industry (see acknowledgements) and revised where necessary.

The risk assessment process maps all of the individual probabilities and pathways to identify any types of equipment which have a non-negligible risk using a risk matrix approach. Exposure via contamination of a particular piece of equipment is determined after a period of time before restocking and includes natural virus decay. The risk matrix assessment, including exposure, is presented as a spreadsheet tool to assist in the visualisation of the relative risks for the equipment types, matrices, farm types and C&D scenarios.

Results

In the spreadsheet based risk matrix tool, qualitative estimates of risk are provided for possible combinations of, farm-type, equipment and organic matrix in which the organism may be present. Figure 2 illustrates the assessment tool with the pathway flowing from left to right. It begins with the level of pathogen in each matrix, accumulation of the matrices on individual items of equipment and through the different C&D scenarios. It assesses the probability of virus survival, viral load and probability of exposure to virus for a sentinel flock for each scenario. The use of different equipment, farm types and matrices can be examined in the spreadsheet by using the filter facility in the column header row. For example, Figure 3 illustrates the use of the tool filtered to show only results for enriched colony caged layers. This demonstrates how the estimates for probability of infection and viral loads differ between the scenarios as described in Table 1. It is estimated until the point where the probability of virus survival and any remaining viral load is not considered to be at a significant enough level to cause infection in a sentinel flock of birds.

FIGURE 2 HERE

FIGURE 3 HERE

Based on evidence in the literature dust and faecal deposits were considered to contain a medium level of AI virus while oropharyngeal deposits and feathers were considered to contain high levels of virus (Yamamoto et al. 2008b, Yamamoto et al. 2008a, Pepin et al. 2014, Spekreijse 2013, Reis et al. 2012). Table 2 shows those items of equipment which give the highest predicted probability of infection for each of the three C&D scenarios for individual farm production types. The risk assessment predicts that, within any farm-type other than free range layers, the probability of infection in a sentinel flock from any equipment is negligible after secondary C&D, irrespective of whether or not dismantling occurs (Table 2). The ‘Medium’ probability results for preliminary C&D are assuming a sentinel flock is introduced directly after C&D has occurred. Whilst this is an unrealistic scenario, it demonstrates the probability of where residual virus may still be present within the poultry house at this time.

TABLE 2 HERE

For free range layers, the probability of infection from outdoor areas (which would not be affected by dismantling) was assessed as low (assuming no risk mitigation strategies have been applied to these areas), with the risk from all types of equipment being negligible; this is assuming a time period of ~37 days between culling and restocking and low temperature conditions. By considering the equipment types individually, the assessment identifies those areas of the house which may still contain viable virus post preliminary C&D to which sentinel birds may have access and where attention should be focussed during secondary C&D. Considering all poultry production types, these areas are drinking nipples, floor, outdoor areas, nest box liner and autonests, perches, slatted areas and enrichments.

Figure 4 shows the relative risk of infection across the different types of equipment, within a particular farm-type, for the preliminary C&D scenario, demonstrating the areas of highest risk. The two secondary C&D scenarios are not shown graphically because the risk from all equipment types was predicted to be negligible with the exception of the outdoor areas (risk was considered ‘Low’) for both scenarios. It is stressed that the ordinal scales used to produce Figure 4 are not quantitative values and are used only to illustrate qualitative relative risk.

FIGURE 4 HERE

The risk assessment found a negligible risk of re-infection in sentinel chickens resulting from contact with any equipment in enriched colony caged systems for both the secondary C&D scenarios. This is the poultry sector with the most complex equipment involving numerous cages and hard to access areas such as manure belts and nest boxes. A very low probability of virus survival was associated with faecal deposits on the nest box liners and scratching mats. At this stage of the C&D procedure, however, taking into account natural virus decay, the viral load was considered to be negligible as was the probability of a bird being exposed to a high enough level of virus to constitute an infectious dose. This is based on experimental minimum infectious dose data (Aldous et al. 2010) and the assumption that the birds would not come into direct contact with virus within the nest boxes. Nest box liners can be perforated to allow all the dust and muck to fall away, however, they can still become soiled by faeces and have been found to be more heavily soiled than wired areas due to droppings stuck in the mats (Guinebretiere et al. 2012). It is considered that a thorough C&D procedure would eliminate the majority of the organic matter and that the blades on the artificial turf mats, which prevent eggs from coming into direct contact with the droppings trapped within the blades, would also reduce the risk of the bird accessing any remaining virus.

A very low probability of virus survival and estimate of viral load was also found for dust and faecal deposits on the manure belts of colony caged systems. Due to the slow movement of these belts resulting in negligible dispersal of residual dried organic matter it was assumed that there would be negligible probability of the birds being exposed to an infectious dose of virus present on the belt.

For those poultry sectors with less complex equipment such as barn and free range layers and broiler breeders and rearers, the floor was found to have a very low probability of viral survival for both secondary C&D scenarios. The viral load at the time of restocking, however, was considered to be negligible as was the probability of infection in a sentinel flock. A very low probability of virus survival in faecal deposits on the nest box lining was also found after secondary C&D for barn and free range layers and broiler breeders but the risk of infection was reduced to negligible for the same reasons as colony caged birds.

For barn and free range layers, broiler breeder and the broiler rearer sector a medium risk of infection immediately after preliminary C&D was predicted for nipples, drinkers, the floor area, nest box liners, perches, enrichments, and slatted areas. Again these are the items of equipment that are most difficult to access during the spraying of disinfectant during preliminary C&D and which the chickens have close access to. The majority of the risk, for those items other than nipples and drinkers, arises from faecal deposits whereby the organic matter of the deposits could protect the virus from the full effect of the disinfectant applied during preliminary C&D thereby reducing its efficacy.

Discussion

This risk assessment concluded that provided secondary C&D is carried out with due diligence (i.e. carried out to a defined code of practice as agreed by both industry and policy makers) the risk of recrudescence of infection of AI viruses is negligible both with and without dismantling complex equipment in all farm types considered. The correct application of secondary C&D combined with the period of time between depletion and restocking allowing for viral decay are key components in the negligible risk rating for both scenarios. The few items of equipment which still had a very low probability of contamination were generally not in contact with the birds thereby reducing the risk of re-infection.

A low risk after secondary C&D was predicted for the outside paddocks of free range poultry houses but this is assuming that no risk mitigation activities take place. The outdoor areas or paddocks are unique to free range poultry sectors whereby C&D will have very little effect on any virus present. Virus here, however, will be subject to UV effect, in addition to natural decay as a result of temperature and other environmental factors. Outdoor survival of virus in wet, puddled cold paddocks, could be longer than in the poultry house but by the time the house has been cleaned and disinfected, restocked and the birds trained to use nest boxes before release to range, the natural decay of virus should result in a negligible risk at restocking. Additional interventions for outdoor areas include: scraping off heavy faecal load close to pop-holes, cutting pasture short to allow drying and exposure to the sun, use of products to 'dress' pasture, absorbing moisture and containing an anti-viral disinfectant or a heavy lime application to reduce pathogen growth.

By considering the equipment types individually, the assessment identifies those areas of the house which may still contain viable virus immediately post preliminary C&D and therefore on which attention should be focussed during secondary C&D e.g. those areas where feathers accumulate or there is a build-up of faecal material. It was assumed that in some areas of the house there will still be viable virus after preliminary C&D has taken place as it was assumed that organic matter will still be present when the disinfectant is applied. It should be stressed that the results are assuming a sentinel flock is introduced directly after preliminary C&D has occurred. Whilst this is an unrealistic scenario, it demonstrates the probability of where virus may still be residual within the poultry house at this time, and so helps prioritise areas for secondary C&D. For the colony caged layer sector a medium risk was found for nipples, nest box liners, perches and scratching mats. These areas of equipment could still potentially harbour significant viral loads after preliminary C&D and also be accessible to the birds so that they are exposed to a sufficient viral load to cause infection (Aldous et al. 2010).

In answering the risk question it was important to consider where the virus is likely to be present in the house, how it is affected by C&D and what access the birds have to those areas predicted to contain high enough levels of virus to constitute an infectious dose. Each poultry system has its own specific design and therefore the critical points for each housing system will differ. Some systems are easier to clean than others and when something is difficult to clean, the risk of it not being cleaned properly will be higher. This will be reflected in the efficacy of the disinfection because heavy organic soiling will influence the performance of the disinfection procedure negatively. In a study on C&D of different layer systems the necessity to pull the laying mats out of the nests and the extra attention spent on cleaning the dust and manure stuck between the tiered flooring were the two main reasons why the colony systems were more labour intensive in terms of cleaning. When this is done properly, however, the disinfection results should not be influenced by equipment type (Bossuyt K. 2012).

The length of time AI virus can remain infective in the environment, the specific conditions of the environment that increase persistence, and the infective dose required for primary transmission, have all been the subject of many experimental investigations. The majority of the data available for this assessment were based on laboratory conditions without factoring in ‘environmental realism’ (Dalziel et al. 2016). Persistence of AI viruses is dependent on many parameters such as time, temperature, pH, salinity, light (UV), desiccation and relative humidity (RH) (Stallknecht and Brown 2009) and the tenacity of AI viruses to physical and chemical factors also increases in the presence of organic material (Lu et al. 2003). In experimental conditions, multiple variables may be held constant (e.g., strain/isolate, pH, salinity, UV, and RH), while others are then varied (e.g., time and temperature). Although this helps isolate the effect of treatments, the interactions of treatments (Stallknecht and Brown 2009) may be missed and the results may therefore apply less well to field conditions. Considering the need for environmental realism to be applied to experimental data for survival of AI viruses within the poultry house environment, studies are currently underway to assess the survival of AI virus in a barn setting after C&D has been carried out and whether recrudescence of the virus in a sentinel flock occurs.

The infectivity of AI viruses at different temperatures is also variable from strain to strain (Paek et al. 2010). The lack of data of AI virus survival at low temperatures is particularly relevant, for example, nine out of the fourteen NAD outbreaks since 2006 in the UK occurred between November and February. Variation between viruses was most evident under cold water (4°C) conditions, with little variation observed at temperatures >28°C (Stallknecht and Brown 2009). Studies have also demonstrated that significant variability exists in the infection dynamics observed between individual virus strain, challenge dose and the specific host it infects (Aldous et al. 2010, Swayne and Slemons 2008a). It should therefore be acknowledged that extrapolation of data from a single virus strain across other avian species or for different strains should be viewed with caution. The application of a 'worst case scenario' for this assessment will have ensured that the risks have not been underestimated but, as such, there remains a medium level of uncertainty associated with the data used for these parameters.

Within this assessment LPAI and HPAI were treated as one virus. While there are likely to be differences between them in terms of persistence, infectious doses and shedding levels in the various matrices, it was considered that there was insufficient data available to assess the viruses independently, although where possible, virus specific data are presented. HPAI viruses are typically found in both the faeces and respiratory secretions of experimentally infected chickens (Spickler, Trampel, and Roth 2008). LPAI viruses have also been detected in both these secretions of experimentally infected chickens but the findings are less consistent than with HPAI (Spickler, Trampel, and Roth 2008). Naïve wild bird mediated introduction of LPAI viruses are often more likely shed via the cloaca but once the virus moves through a Galliforme host, shedding via the respiratory tract becomes more common. There have been no published studies on shedding of LPAI virus in feathers although the mechanism for virus presence in the follicle is unclear. Data also suggests that LPAI viruses require higher infectious doses to cause infection but that the broad variation in susceptibility of poultry species makes the probability of infection occurring unpredictable (Swayne and Slemons 2008a, Jones and Swayne 2004, Pantin-Jackwood et al. 2017, Van der Goot et al. 2003). HPAI virus has been found to be more persistent than LPAI virus in faeces and bedding material (Hauck et al. 2017), although this persistence may be related to the initial higher viral load deposition and degree of contamination with HPAI viruses. Thus, a lower infectious dose and high virus shedding (Aldous et al. 2010), along with greater environmental persistence, likely increase the risk of infection for HPAI compared to LPAI when there is an exposure event. The results are therefore presented for one generic virus acknowledging that this is likely to be a worst cases scenario for LPAI The generic nature does, however, mean that should more data become available, the assessment can be re-parameterised and rerun to obtain pathogen specific results.

C&D is a costly and laborious task and its success in eliminating virus from the houses depends not only on the choice and correct application of disinfectants but specifically upon attention to detail to remove organic matter from those areas identified as still capable of causing infection directly after preliminary C&D. In the UK, reference should be made to the Defra approved disinfectant list which provides a list of products that can be used in case of an AI outbreak and the concentration they must be used at. Consideration should be given to the efficacy of disinfection at different temperatures and in the presence of organic matter whilst minimising corrosion of metal surfaces, the pitted nature of which could harbour virus and protect it from the disinfectant. Laying houses are difficult to clean thoroughly because of their intrinsically complicated structures, which are even more complex in the case of cage laying houses (Wales, Breslin, and Davies 2006). Access to cage interiors, feeders and muck belts is very difficult unless effort and time is invested. It would appear that in these circumstances a large amount of residual organic matter is expected after a standard disinfection procedure (Carrique-Mas et al. 2009). Removal of equipment in on-floor houses which were cleaned separately resulted in a high standard of cleaning. However, this was during routine C&D between flocks so does not allow for the natural decay of the virus over the time taken between culling and re-population that is accounted for in this assessment. Whilst minimal virus decay is likely take place in light of the assumption of low temperature environmental conditions, the time period of 37 days used in this assessment falls within the bounds of viral decay in faeces reported in some studies (Webster et al. 1978, Beard 1984, Lu et al. 2003).

Two of the main uncertainties within this assessment are the infection dynamics and survival of AI viruses and the virus strain variability which may influence these data. There is a need to fully understand the complexity of the large number of potential interacting variables that can affect virus survival within the poultry house environment. Survival of virus on fomites constructed from different materials is important at the interface of the equipment with the deposit containing the virus (Wood et al. 2010, Greatorex et al. 2011, Tiwari et al. 2006, Noyce, Michels, and Keevil 2007, Sakaguchi 2010, Bean et al. 1982, McDevitt et al. 2010) but more studies are required to determine viral decay within the organic matrix itself.

Based on these conclusions, recommendations for improving the efficacy of secondary C&D could include the improvement in equipment design to allow better access to those items of equipment with which a higher risk was associated e.g. muck belts and nest boxes. Specific

C&D guidelines for higher risk equipment such as this could be outlined in the C&D procedure (Huneau-Salaun et al. 2010). Design of new poultry sheds could include the requirement to eliminate horizontal surfaces that collect dust, with smooth surface finishes and level concrete floors to facilitate cleaning. The height of new sheds should be tall enough to allow the use of a vehicle fitted with an enclosed, ventilated cab with filtered air intakes to clean the whole of the floor (HSE 2012).

Overall, the risk pathway and matrix tool used for this assessment are generic in nature and can be applied to other pathogens and species to compare scenarios where appropriate data exists. The risk assessment matrix 'tool' complements the pathway and is a novel application which allows the probability of infection from individual items of equipment to be compared taking into consideration probability of virus survival, viral load and probability of exposure throughout the pathway. In this assessment, the pathway and tool provide a framework for effective application of C&D in a way which can lead to reduction of costs to industry and mitigating some delays in recovering country freedom.

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Table 1: Probability definitions for the generic risk pathway derived for individual types of equipment

Scenario	Steps and Outputs
No Secondary C&D	Initial contamination on Complex Equipment
	P_C: Probability virus present on complex equipment at time of preliminary C&D
	V_H: Viral load on complex equipment at time of preliminary C&D
	Preliminary C&D
	P_P: Probability virus present on complex equipment after preliminary C&D
	V_P: Viral load on complex equipment after preliminary C&D
	Exposure
	P_E: Probability birds exposed to virus on complex equipment
	V_E: Viral load on complex equipment to which birds are exposed
	Consequence (Infection)
	P_I: Probability of infection, given exposure and viral load to which birds are exposed (dose-response)
Secondary C&D without Dismantling	Initial contamination on Complex Equipment
	P_C: Probability virus present on complex equipment at time of preliminary C&D
	V_H: Viral load on complex equipment at time of preliminary C&D
	Preliminary C&D
	P_P: Probability virus present on complex equipment after preliminary C&D
	V_P: Viral load on complex equipment after preliminary C&D
	Secondary C&D without Dismantling
	P_{SND}: Probability virus present on complex equipment after secondary C&D without dismantling
	V_{SND}: Viral load on complex equipment after secondary C&D without dismantling
	Exposure
	P_E: Probability birds exposed to virus on complex equipment
	V_E: Viral load on complex equipment to which birds are exposed
	Consequence (Infection)
	P_I: Probability of infection, given exposure and viral load to which birds are exposed (dose-response)
Secondary C&D with Dismantling	Initial contamination on Complex Equipment
	P_C: Probability virus present on complex equipment at time of preliminary C&D
	V_C: Viral load on complex equipment at time of preliminary C&D
	Preliminary C&D
	P_P: Probability virus present on complex equipment after preliminary C&D
	V_P: Viral load on complex equipment after preliminary C&D
	Secondary C&D with Dismantling
	P_{SD}: Probability virus present on complex equipment after secondary C&D with dismantling
	V_{SD}: Viral load on complex equipment after secondary C&D with dismantling
	Exposure from Complex Equipment
	P_E: Probability birds exposed to virus on complex equipment
	V_E: Viral load on complex equipment to which birds are exposed
	Consequence (Infection)
	P_I: Probability of infection, given exposure and viral load to which birds are exposed (dose-response)

Table 2: Probability of infection in a sentinel flock for the three scenarios included in the risk pathway (equipment in brackets are those items with the highest risk at that stage)

Farm Type	**Preliminary C&D only (R_P)**	**Secondary C&D without dismantling (R_{SND})**	**Secondary C&D with Dismantling (R_{SD})**
Enriched Colony Caged	Medium (nipples; nest box liner; perches, scratching mat)	Negligible (All equipment)	Negligible (All equipment)
Free range layer	Medium (nipples; floor; outdoor areas; nest box liner; perches; slatted areas; enrichments)	Low (outdoor areas)	Low (outdoor areas)
Barn layer	Medium (nipples; floor; nest box liner; perches; slatted areas; enrichments)	Negligible (All equipment)	Negligible (All equipment)
Broiler breeder	Medium (nipples; floor; nest box liner; autonest; slatted areas; enrichments)	Negligible (All equipment)	Negligible (All equipment)
Broiler rearer	Medium (nipples; floor; slatted areas; enrichments)	Negligible (All equipment)	Negligible (All equipment)

Figure Captions:

Figure 1: Generic risk pathway considering key stages of the C&D process and illustrating the three scenarios for which the risk of re-infection is assessed. The different stages of the pathway will incorporate more detail including, for example how the virus survives over time. The variables are defined in table 1.

Figure 2: Risk Matrix qualitative assessment tool: example output

Figure 3: Risk matrix qualitative assessment tool for enriched colony caged layers showing results for Scenario 1 (P_1) and the 'Negligible' probability of virus survival on most bits of equipment after secondary C&D without any dismantling

Figure 4: Comparison of the combined risk for items of equipment from all four organic matrices in the different poultry sectors immediately after preliminary C&D. The ordinal scales are not quantitative values and are used only to illustrate qualitative relative risk. The results for preliminary C&D are assuming a sentinel flock is introduced directly after C&D has occurred. Whilst this is an unrealistic scenario, it demonstrates the probability of where virus may still be residual within the poultry house at this time.

606 Figure 1:

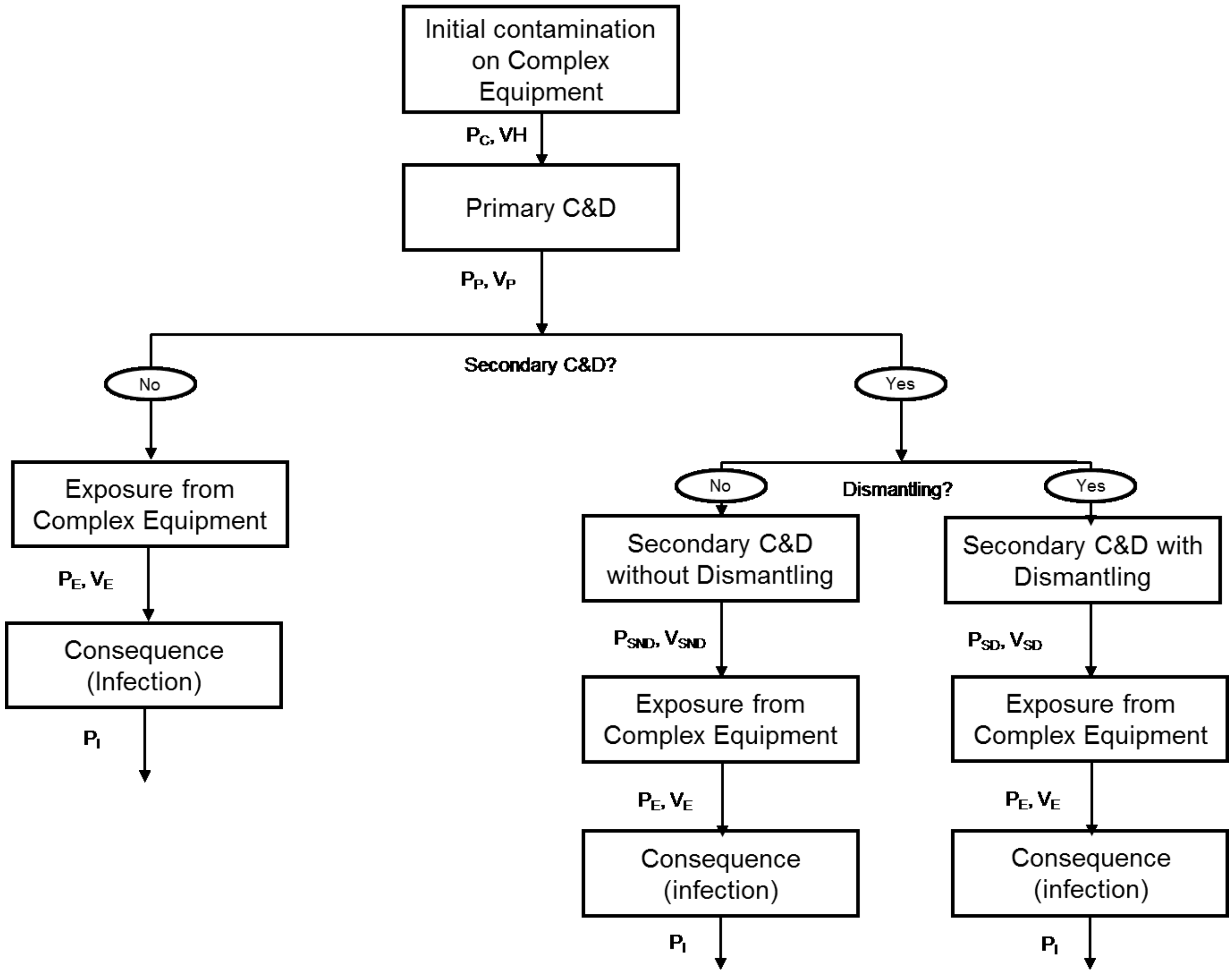

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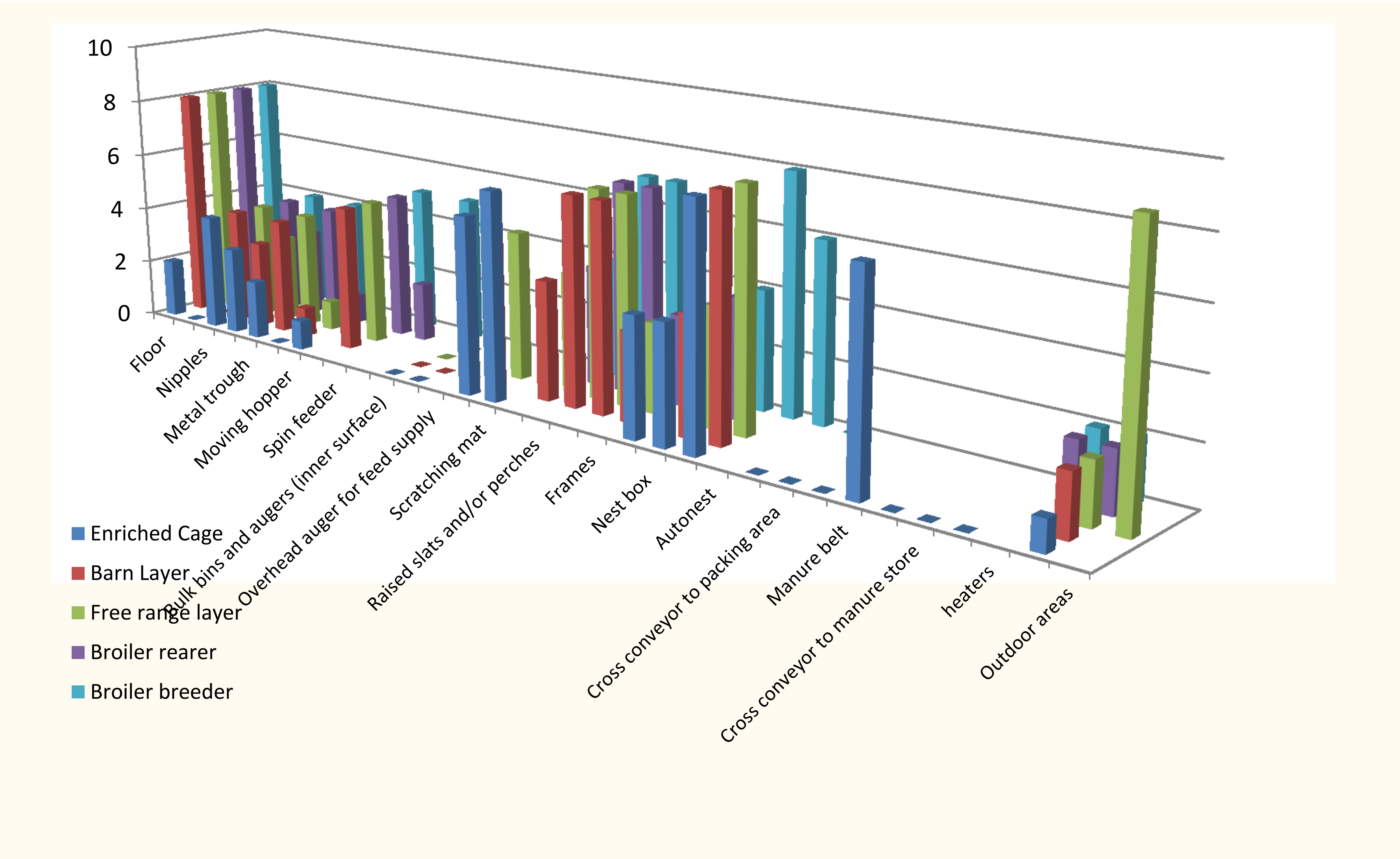
Figure 2:

Equipment	Farm Type	Matrix with which equipment can come in contact with	Level of pathogen in matrix	Rate of contact between equipment and matrix	Accumulation of matrix on equipment	Probability virus present in matrix on equipment after depopulation and before primary C&D (assume 2 days) (P_C)	Viral load in matrix on equipment at time of preliminary C&D (V_H)	Probability of virus survival in matrix on equipment after primary C&D (P_P)	Viral load in matrix on equipment after primary C&D and at time of restocking (V_P)	Probability birds exposed to virus in matrix on or from equipment (P_E)
scratching mat	Enriched Colony cages layers	Dust	Medium	High	Medium	Medium	Medium	Low	Low	Low
	Enriched Colony cages layers	Oropharyngeal	High	High	Medium	Medium	High	Low	Low	Low
	Enriched Colony cages layers	feathers	High	Low	Low	High	Low	high	Low	Very low
	Enriched Colony cages layers	Faecal (cloacal)	Medium	High	High	High	High	medium	Medium	Medium
Colony cages	Enriched Colony cages layers	Dust	Medium	High	Low	Medium	low	Low	Very Low	Very Low
	Enriched Colony cages layers	Oropharyngeal	High	High	Low	Medium	low	Low	Very Low	Very Low
	Enriched Colony cages layers	feathers	High	Low	Low	High	low	high	Low	Very low
	Enriched Colony cages layers	Faecal (cloacal)	Medium	High	Medium	High	Medium	medium	Medium	Low
Slatted area	Barn layers	Dust	Medium	High	Medium	Medium	Medium	Low	Low	Low
	Barn layers	Oropharyngeal	High	High	High	Medium	High	Low	Low	Low
	Barn layers	feathers	High	Medium	Low	High	low	high	Low	Very low
	Barn layers	Faecal (cloacal)	Medium	High	High	High	High	medium	Medium	Medium
	Free range layers	Dust	Medium	High	Medium	Medium	Medium	Low	Low	Low
	Free range layers	Oropharyngeal	High	High	High	Medium	High	Low	Low	Low
	Free range layers	feathers	High	Medium	low	High	low	high	Low	Very low
	Free range layers	Faecal (cloacal)	Medium	High	High	High	High	medium	Medium	Medium
	Broiler-rearer	Dust	Medium	High	Medium	Medium	Medium	Low	Low	Low
	Broiler-rearer	Oropharyngeal	High	High	High	Medium	High	Low	Low	Low
	Broiler-rearer	feathers	High	Medium	low	High	low	high	Low	Very low
	Broiler-rearer	Faecal (cloacal)	Medium	High	High	High	High	medium	Medium	Medium

Figure 3:

Equipment	Rate of contact between equipment and matrix	Accumulation of matrix on equipment	Probability virus present before primary C&D (P_C)	Viral load at time of preliminary C&D (V_H)	Probability of virus survival after primary C&D (P_P)	Viral load after primary C&D (V_P)	Probability birds exposed to virus (P_E)	Viral load to which birds are exposed (V_E)	Probability of infection in sentinel flock after preliminary C&D (P_I)	Probability of virus survival after secondary C&D (no dismantling) (P_{SND})
Metal trough	High	Medium	High	High	High	Very Low	Low	Very Low	Very Low	Negligible
Moving hopper	Medium	Low	High	Low	High	Very Low	Low	Very Low	Very Low	Negligible
Moving chain	Medium	Low	High	Low	High	Very Low	Negligible	Very Low	Negligible	Negligible
Bulk bins and augers	Low	Low	Medium	Very Low	Very Low	Negligible	Negligible	Negligible	Negligible	Negligible
Nipples	High	High	Medium	High	Medium	Medium	High	Medium	Medium	Negligible
Drinkers	High	High	Medium	High	Low	Low	Medium	Low	Low	Negligible
Nest box	Low	Low	High	Low	High	Low	Very Low	Very Low	Very Low	Negligible
Nest box liner	Medium	Medium	High	Medium	High	Medium	Medium	Medium	Medium	Very Low
Perches	High	High	High	High	High	Medium	Medium	Medium	Medium	Negligible
Scratching mat	High	High	High	High	High	Medium	Medium	Medium	Medium	Very Low
Colony cages	High	Medium	High	Medium	High	Medium	Low	Low	Low	Negligible
Ventilation	High	High	Medium	Medium	Medium	Medium	Very Low	Low	Very Low	Negligible
Egg belt	Medium	Low	High	Low	Low	Very Low	Very Low	Negligible	Negligible	Negligible
Cross conveyor (eggs)	Medium	Low	High	Low	Low	Very Low	Very Low	Negligible	Negligible	Negligible
Packing area	Low	Low	High	Low	Low	Very Low	Negligible	Negligible	Negligible	Negligible
Manure belt	High	High	High	High	High	Medium	Low	Medium	Low	Very Low
Cross conveyor (manure)	High	High	High	High	Medium	Medium	Negligible	Negligible	Negligible	Negligible
Manure air drying equipment	High	High	High	High	High	Medium	Negligible	Negligible	Negligible	Negligible
Manure store	High	High	High	High	Medium	Medium	Negligible	Negligible	Negligible	Negligible
Floors	Low	Low	High	Low	High	Very Low	Very Low	Very Low	Very Low	Negligible
Walls	Low	Low	Medium	Low	Low	Very Low	Very Low	Negligible	Negligible	Negligible

Figure 4: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Floor
Nipples
Metal trough
Moving hopper
Spin feeder
Bulk bins and augers (inner surface)
Overhead auger for feed supply
Scratching mat
Raised slats and/or perches
Frames
Nest box
Autonest
Cross conveyor to packing area
Manure belt
Cross conveyor to manure store
heaters
Outdoor areas
Enriched Cage
Barn Layer
Free range layer
Broiler rearer
Broiler breeder