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HPAI PRO-POOR HPAI RISK REDUCTION

A quantitative risk assessment for the onward transmission of highly pathogenic avian influenza H5N1 from an infected small-scale broiler farm in Bogor, West Java, Indonesia

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Preface

Since its re-emergence, highly pathogenic avian influenza (HPAI) H5N1 has attracted considerable public and media attention because the viruses involved have been shown to be capable of producing fatal disease in humans. While there is fear that the virus may mutate into a strain capable of sustained human-to-human transmission, the greatest impact to date has been on the highly diverse poultry industries in affected countries. In response to this, HPAI control measures have so far focused on implementing prevention and eradication measures in poultry populations, with more than 175 million birds culled in Southeast Asia alone.

Until now, significantly less emphasis has been placed on assessing the efficacy of risk reduction measures, including their effects on the livelihoods of smallholder farmers and their families. In order to improve local and global capacity for evidence-based decision making on the control of HPAI (and other diseases with epidemic potential), which inevitably has major social and economic impacts, the United Kingdom (UK) Department for International Development (DFID) has agreed to fund a collaborative, multidisciplinary HPAI research project for Southeast Asia and Africa.

The specific purpose of the project is to aid decision makers in developing evidence-based, pro-poor HPAI control measures at national and international levels. These control measures should not only be cost-effective and efficient in reducing disease risk, but also protect and enhance livelihoods, particularly those of smallholder producers in developing countries, who are and will remain the majority of livestock producers in these countries for some time to come.

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Keywords

Avian Flu, H5N1, Highly Pathogenic Avian Influenza, HPAI, Indonesia, Quantitative risk assessment

More information

For more information about the project please refer to www.hpai-research.net

Acronyms

AIV	Avian influenza virus
CENTRAS	Centre for Tropical Veterinary Studies
CMU	Campaign Management Unit
DFID	Department for International Development
DGLS	Directorate General of Livestock Services
EPA	United States Environmental Protection Agency
EO	Expert opinion
FAO	Food and Agriculture Organization of the United Nations
HPAI	Highly pathogenic avian influenza
HPAIV	Highly pathogenic avian influenza virus
IFPRI	International Food Policy Research Institute
ILRI	International Livestock Research Institute
IPB	Bogor Agricultural Institute
LPAI	Low pathogenic avian influenza
LPAIV	Low pathogenic avian influenza virus
OIE	World Organization for Animal Health (*Office International des Epizooties*)
RVC	Royal Veterinary College
SEIR	Susceptible-Infected-Infectious-Removed
WAHID	World Animal Health Information Database
WHO	World Health Organization

Executive Summary

Background

Highly pathogenic avian influenza (HPAI) H5N1 is endemic in Indonesia, where it is an important cause of disease in commercial, semi-commercial and backyard poultry flocks. It is likely that small-scale broiler farms, which typically have poor biosecurity practices together with large populations of susceptible birds, play an important role in the spread of the disease in the country.

This report describes a quantitative risk assessment that was conducted to describe the risk of transmission of the HPAI virus (HPAIV):

a) between small-scale broiler farms, and
b) between small-scale broiler farms and backyard poultry flocks.

For this study we focus in particular on the District and Municipality of Bogor, West Java, Indonesia.

Approach

The risk assessment considers two general risk pathways: those arising from environmental contamination, and therefore indirect contact, and those arising from direct bird-to-bird contact. The model begins by simulating an outbreak of HPAI H5N1 on a small-scale broiler farm. The release and exposure assessment are then composed of 3 sections considering 1) mechanical transmission via fomites, 2) water-borne transmission, and 3) bird-bird transmission. For the risks associated with environmental contamination, we specifically consider the probability of virus contamination (P_c), the probability of virus survival (P_s) and the probability of infection given exposure to defined virus dose (P_i).

Where possible, this risk assessment has used a stochastic second-order modelling approach, whereby the variability and uncertainty in probabilistic model parameters are disaggregated and the model structured around the variability, with uncertainty overlaid. The probability distributions of variables used in the model were derived from survey data, estimated from available literature or developed based on expert opinion.

Results

2.1.1 Mechanical transmission

Collectors

Poultry collectors may play an important role in the spread of HPAIV H5N1 between small-scale broiler flocks in Indonesia. We estimated that, on average, the probability of transmission from an infected to a susceptible flock via collectors was between 3.2×10^{-2} and 1.3×10^{-1}, or, on 3 to 13 out of every 100 occasions in which a broiler farm is visited by collectors who have first visited a farm on which an outbreak is occurring, on average we would expect that at least one bird in the flock will become infected. This estimate relates to the *first* susceptible farm visited by collectors following the

visit to the infected farm; subsequent visits would be associated with lower risks as the quantity of contaminating virus decreases.

The impacts of risk mitigation on the likelihood of disease transmission via this route were estimated. We predict that a mandatory delay period of 24 hours between farm visits can reduce the probability of transmission to very low levels (9.5×10^{-4} to 4.5×10^{-3}) and to almost negligible levels after 48 hours (7.7×10^{-6} to 9.1×10^{-5}). Moreover, effective biosecurity and sanitation practices with a 2 to 3 log reduction in the amount of virus contaminating collectors and their equipment would be expected to result in a reduction of risk to very low levels (9.5×10^{-4} and 9.6×10^{-5}, respectively).

We assessed the impact of incentives for early disease reporting, for example through compensation, and estimate that the situation in which all farms report disease at the first suspicion was associated with a reduction in the average probability of onward transmission via collectors to between 2.8×10^{-3} and 1.6×10^{-2}. Some risk of onward transmission exists even in the presence of immediate reporting and movement bans as HPAI H5N1 can circulate undetected on an infected broiler farm for a short period.

Animal health workers

The risk associated with handling poultry with HPAIV H5N1 contaminated hands was assessed, considering the specific example of animal health workers who may handle infected poultry in the course of disease investigation on an infected farm and subsequently handle birds on a susceptible farm. We predict that birds handled in such a scenario have an average probability of becoming infected of between 2.9×10^{-2} to 9.5×10^{-2}. Effective hand sanitation, for example thorough washing with soap and water and a subsequent 2 to 3 log reduction in virus titre, resulted in a reduction in the average risk to very low levels (6.9×10^{-4} to 9.9×10^{-4} and 4.9×10^{-5} to 2.0×10^{-4}, respectively).

In a separate module, the risks associated with the transfer of infected material from contaminated animal health worker's shoes into the litter of a susceptible broiler house were assessed and the average probability of transmission was estimated to be between 3.9×10^{-2} to 1.5×10^{-1}. We demonstrate that a mandatory delay period of 24 hours between farm visits is likely to reduce this risk to very low levels (6.4×10^{-4} to 3.4×10^{-3}).

2.1.2 Water-borne transmission

The risks of HPAI transmission via water were assessed by considering the specific scenario in which a proportion of HPAIV H5N1 contaminated carcasses from a farm undergoing a disease outbreak is discarded into a river. We predict that the average probability that at least one bird within a random small-scale broiler flock in Bogor District or Municipality becomes infected following exposure to untreated river water is between 1.2×10^{-1} to 3.1×10^{-1} when the source river is 'small', 7.6×10^{-3} to 3.3×10^{-2} when the source river is 'medium'-sized, and 7.8×10^{-4} to 3.7×10^{-3} when the source river is 'large'. The majority of broiler producers in Bogor District chlorinate drinking water, and provided chlorine concentrations are sufficient to ensure a greater than 4 to 5 log reduction in virus titres, we expect that the risk of infection in a broiler flock from contaminated river water can be reduced to low levels.

The average probability that at least one bird within a household flock becomes infected following consumption of HPAIV H5N1 contaminated river water was estimated to be 4.6×10^{-4} to 2.3×10^{-3}

when the source river is small, 1.8×10^{-5} to 9.5×10^{-5} for water from medium-sized rivers and 1.7×10^{-6} to 9.1×10^{-6} for water from large rivers. Whilst individual household estimates are very low, if all backyard poultry within a *desa* [village] are exposed to contaminated river water we predict that the average probability that at least one bird within the *desa* becomes infected is between 1.3×10^{-1} to 3.3×10^{-1} following *desa*-wide consumption of H5N1 contaminated water from small rivers, 1.1×10^{-2} to 4.4×10^{-2} from medium sized rivers and 1.3×10^{-3} to 5.8×10^{-3} from large rivers.

2.1.3 Direct flock-to-flock transmission

We considered the specific risks associated with contact between free-ranging poultry flocks and a broiler farm on which an outbreak is occurring. Based on the spatial distribution of small-scale broiler farms in Bogor District and their proximity to surrounding households, the average contact rate was estimated to be 2.3 flocks entering the boundary of the broiler farm per day, with a 5^{th} percentile of 0 and a 95^{th} percentile of 7 free-ranging poultry flocks. By incorporating a range of possible values for the probability of disease transmission given contact, estimates of the number of backyard flocks that might be expected to become infected given an outbreak on a small-scale broiler farm were estimated.

Summary

This quantitative risk assessment has used current knowledge in order to predict the risk of spread of HPAIV H5N1 to domestic poultry along a variety of potential transmission routes from an initial outbreak on a small-scale broiler farm. The development of the model has allowed potential risk mitigation strategies to be tested.

The model output indicates that the environmental transmission route may play an important role in the spread of HPAIV H5N1 between broiler farms in the District and Municipality of Bogor, and in Indonesia more generally. Hence, relatively simple disease prevention strategies, such as sanitation and disinfection of visitors and their equipment, or the adequate treatment of drinking water for poultry, can potentially impact on the degree to which HPAIV H5N1 is able to spread from an infected small-scale broiler farm to the susceptible farms around it. Policy measures, such as the provision of incentives for producers to report HPAI H5N1 outbreaks in their flocks or changes in the behaviour and practices of poultry collectors would also be expected to impact on this risk.

It is necessary that the outputs from this study are interpreted in the light of several key areas data deficiency and uncertainty and therefore the need to make several large assumptions. However by demonstrating some of the routes by which HPAIV H5N1 can be spread between farms, and the degree to which the risk of spread can be minimized, the outputs can assist in risk communication to those people working in the poultry sector as well as contributing evidence to policy decisions.

1 Introduction

1.1 Background

Highly pathogenic avian influenza (**HPAI**) H5N1 was first officially reported in Indonesia in January 2004, but by that time the disease had spread throughout the country and caused the death of several thousand domestic poultry (Perry et al. 2009). The response to the epidemic involved a synchronized campaign of mass vaccination and the culling of infected and in-contact premises (Sumiarto and Arifin 2008). Despite these efforts, which involved a reported 131,846,470 birds vaccinated and 7,269,582 birds culled, it was reported that there were some 4,591,880 cases of HPAI H5N1 in Indonesia in 2004 alone (OIE [World Organization for Animal Health] 2004). The epidemic continued throughout 2005, spreading to 23 provinces, involving 151 districts or cities (Perry et al. 2009), and was officially reported to be endemic in the Indonesian poultry population in 2006 (WAHID [World Animal Health Information Database] 2006). By June 2009, 31 of the country's 33 provinces had had a least one outbreak of HPAI H5N1 in poultry, with the majority of these occurring on the Islands of Java, Sumatra, Sulawesi and Bali (Perry et al. 2009). As well as untold losses to the poultry industry, by the end of December 2009 HPAI virus (**HPAIV**) H5N1 had been the cause of at least 161 human cases in Indonesia, of which 134 were fatal (WHO [World Health Organization] 2009).

The seriousness of avian influenza in Indonesia is a reflection not only of its zoonotic potential, but also of the economic and social importance of the country's poultry industry. The sector is highly diverse, and incorporates backyard poultry producers rearing birds as a hobby or for subsistence in close proximity to semi-commercial producers rearing several hundred or thousand broiler or layer chickens (and more rarely ducks). In addition, Indonesia has a substantial commercial sector, characterized by farms rearing tens of thousands of birds. The poultry population is estimated at 1.522 billion birds of which 70.7% are broiler chickens, 19.1% are village chickens, 7.7% are layers and 2.4% are ducks (Perry et al. 2009).

1.1.1 The spread of avian influenza

The poultry sector in many Southeast Asian countries, including Indonesia, can be classified into sector 1, 2, 3 and 4 farms (Table 1). Sector 4 poultry have often been implicated as contributing to the maintenance of transmission of HPAIV H5N1 in affected areas (Tiensin et al. 2005; Koch and Elbers 2006; Songserm et al. 2006b), and indeed this is the sector that has been worst affected in Indonesia. Small-scale outbreaks and sustained spread between sector 4 farms may act as a source of infection for rarer outbreaks in sector 3, 2, and 1 farms (Iqbal 2009). Despite a probable role for backyard flocks in the maintenance of the disease, sector 3 farms are also increasingly being implicated. These small-scale commercial farms typically lack adequate biosecurity practices and the potential for introduction of HPAIV H5N1, and other infectious agents, is potentially high. The same inadequate biosecurity measures that allow HPAIV H5N1 to enter a flock are also likely to impact on the containment of the virus in the event of an outbreak. Hence, an outbreak in a semi-commercial flock, which may contain several thousand birds within a single shed, may present a substantial risk of onward spread to those poultry flocks that are in direct or indirect contact. Moreover, and given that the majority of the outputs from small-scale broiler production enter the live-bird marketing

system, there is also a potentially large public health concern as a result of HPAI H5N1 outbreaks in sector 3 farms.

Table 1: The Food and Agriculture Organization of the United Nation (FAO) poultry sector classification system

Sector 1	Industrial integrated system with high-level biosecurity and birds/products marketed commercially (e.g. farms that are part of an integrated broiler production enterprise with clearly defined and implemented standard operating procedures for biosecurity).
Sector 2	Commercial poultry production system with moderate to high biosecurity and birds/products usually marketed commercially (e.g. farms with birds kept indoors continuously; strictly preventing contact with other poultry or wildlife).
Sector 3	Commercial poultry production system with low to minimal biosecurity and birds/products entering live-bird markets (e.g. a caged layer farm with birds in open sheds; a farm with poultry spending time outside the shed; a farm producing chickens and waterfowl).
Sector 4	Village or backyard production with minimal biosecurity and birds/products consumed locally.

Source: FAO (2007)

Semi-commercial farms, and in particular small-scale broiler producers, have recently become the focus of much disease prevention activity in Indonesia, for example the Community Based Avian Influenza Control Project funded by the United States Agency for International Development (USAID) and the 'Cost-effective biosecurity for non-industrial commercial poultry operations in Indonesia' project funded by the Australian Centre for International Agricultural Research (ACIAR).

1.1.2 Risk assessment

Risk can be formally defined as the likelihood and magnitude of the occurrence of an adverse event (Ahl et al. 1993). The aim of a risk assessment is therefore to estimate, in a systematic manner, both these elements so as to provide inputs to an underlying decision problem (Stärk and Salman 2001). The methodology used will depend on the question being addressed, but methods can generally be grouped into four broad steps: hazard identification, release assessment, exposure assessment and consequence assessment (Covello and Merkhofer 1993). Such steps are considered appropriate for the development of risk assessments for animal diseases (Wooldridge 1996).

1.1.3 Risk assessment for HPAI

In the context of HPAIV H5N1, the majority of risk assessments conducted to date have considered the risk of disease introduction into the poultry production sector of a disease free country, for example the United Kingdom (Sabirovic et al. 2007) or Spain (Martínez et al. 2007). Quantitative microbial risk assessments have also been conducted to assess the risk posed to people as a result of exposure to contaminated poultry products or water (Pharo 2003; Schijven et al. 2005b; Greiner et al. 2007). However, there are relatively few published studies in which a formal risk assessment approach has been applied to predict the risk of spread of HPAIV H5N1 between farms, i.e. the transmission of the virus from one farm to another within an affected region. Recently, and as part of the Pro-Poor HPAI Risk Reduction project, this approach has been applied more widely to predict the risk of spread of HPAIV H5N1 within affected countries, for example Thailand (Kasemsuwan et al.

2009) and Indonesia (Idris et al. 2010). Here, the risk has been assessed in a qualitative manner, providing a summary of the literature and expert opinion in order to describe the risk of spread in descriptive terms (i.e. high, medium, low or negligible risk), with a qualitative estimate of the associated uncertainty. Such activities can form the basis for the development of quantitative risk assessments, where quantitative inputs are used to derive a mathematical statement to describe the probability of an adverse outcome from exposure to a hazard at a defined level (Covello and Merkhofer 1993).

1.2 A quantitative risk assessment for the spread of HPAI in Indonesia

1.2.1 Scope

In the context of HPAIV H5N1 transmission between poultry in Indonesia, a meeting of stakeholders held in Bogor, Indonesia in November 2008 agreed that a priority for a greater understanding of the risks of onward spread following outbreaks on small-scale broiler farms was a research priority. This led to the identification of a range of risk pathways for the transmission of HPAI between sector 3 broiler farms and the development of a qualitative risk assessment to address this specific risk question (Idris et al. 2010).

This report describes an extension of the work by Idris et al. (2010), and is intended to meet the following objectives:

1. Development of a quantitative risk assessment (QRA) model to describe the risk of transmission of HPAIV H5N1:

    a) between small-scale broiler farms(sector 3), and
    b) between small-scale broiler farms and household flocks (sector 4).

2. Identification of key areas of data deficiency to target future research.

3. Presentation of practical control policies and estimation of the possible reductions in risk achieved through the implementation of these policies.

This risk assessment focuses on the District and Municipality of Bogor. This region was chosen as being representative of an area of high broiler density in Indonesia, and also an area in which the poultry industry is relatively well characterized (Centre for Tropical Veterinary Studies [**CENTRAS**] 2008; FAO 2008b). Whilst region-specific data have been used for the development of the majority of the risk pathways, it is hoped that the outputs from this risk assessment can be considered relevant in describing some of the risks associated with onward transmission of HPAIV H5N1 from infected small-scale broiler farms throughout the major poultry producing areas of Indonesia.

1.2.2 Approach

The risk assessment model used is broadly structured around that described by Covello and Merkhofer (1993), and considers the risks of release of HPAIV H5N1 (the ‘hazard’) from an infected small-scale broiler farm and an assessment of the likelihood of infection on an exposed small-scale broiler farm, or in an exposed backyard poultry flock. We focus on the specific microbiological hazard, and therefore attempt to provide an estimate of the actual quantity of virus that may move

along a range of transmission pathways. The probability of infection given exposure to virus at these concentrations allows an estimation of the risk of transmission along the pathway. Such a quantitative microbial risk assessment approach has been very widely applied in the assessment of the risks of transmission of infection to people, particularly from food or water-borne contaminants (for full review, see Haas et al. [1999]), but more rarely in the veterinary context (French et al. 2002; Schijven et al. 2005a).

1.2.3 Data

A review of the large body of published literature on avian influenza viruses (**AIV**) was used in the development of the risk assessment model, and to parameterize many of the pathways involved. Where possible, we used data specific to HPAIV, and H5N1 in particular, however in some cases it was necessary to draw on evidence from studies involving low pathogenic avian influenza virus (**LPAIV**) strains.

There is a large amount of grey literature regarding HPAI H5N1 and poultry production in Indonesia. Much of this includes the results of questionnaire based surveys, and some of these have been incorporated into the model where possible. In addition, data were made available by the Directorate General for Livestock Services (**DGLS**), Ministry of Agriculture, Indonesia on surveillance activities conducted in Bogor District and Municipality. Where data or literature based estimates were not available, we have relied on expert opinion (**EO**). Consultation with a variety of experts from government, academia, industry and non-governmental organizations was conducted. These consultations were generally informal, although in some cases specific parameter estimates were requested using a formal expert elicitation approach. The range of experts consulted is listed in the acknowledgments section.

1.2.4 Dealing with uncertainty and variability

This quantitative risk assessment has used a stochastic approach, whereby input parameters are described by probability distributions. Probability distributions can be considered to describe either the variability in true value that a parameter can take, or the uncertainty in the true value of a parameter. The former is the inherent randomness in the system, and is irreducible by further study, whilst the latter is the level of ignorance regarding the parameter, and can be reduced through additional investigation. In addition, and in the absence of sufficient data, we may be uncertain about the extent of the variability in a parameter.

Where possible, we have attempted to disaggregate input parameters into variable and uncertain distributions. In these cases, the model was then structured around the variability, with uncertainty associated with the model parameters overlaid. This method is based upon that described by Vose (2008) and uses a two-dimensional Monte Carlo simulation where the range and distribution of risks for the population constitutes an ‘inner loop’, to which an ‘outer loop’ is added by running the simulation (i.e. the inner loop) over a number simulations, using randomly selected values of the uncertain parameters each time. The probability distributions of variables in the model were derived from survey data, expert opinion or estimated from available literature.

All risk modelling was conducted in Microsoft Excel using either the @Risk 5.5 extension (Palisade Corporation 2009) or Visual Basic for Applications (VBA, Microsoft Corp.). Presentation of some elements of the model structure in Microsoft Excel is given in the appendix.

1.2.5 Hazard identification

Highly pathogenic avian influenza virus subtype H5N1

Influenza A belongs to the *Orthomyxoviridae* family, a large group of negative strand RNA viruses. Virtually all influenza A subtypes have been isolated from birds, in which they tend towards a so-called low-pathogenic phenotype (**LPAI**), typically causing relatively mild disease in both wild and domestic bird species (Osterhaus et al. 2008). However, following introduction into domestic poultry species, some subtypes (namely H5 and H7) have undergone mutation into a highly pathogenic form (HPAIV), in which mortality may be as high as 100%. The HPAIV strain that is currently of greatest concern is H5N1, which has been the cause of serious outbreaks in both domestic and wild bird species throughout much the world. Since this strain first appeared in Hong Kong in 1997, outbreaks in poultry have been reported throughout Asia, including the Republic of Korea, Viet Nam, Japan, Thailand, Cambodia, Lao PDR, Indonesia, China, Malaysia, Myanmar, Bangladesh, Bhutan, Nepal, India, and Pakistan. Moreover, HPAI H5N1 has been reported in poultry in both Africa and Europe (OIE 2010).

Transmission of avian influenza

Transmission of AIV can occur along a variety of pathways, involving either direct bird-to-bird contact or indirect contact as a result of environmental contamination. The range of risk pathways for the onward spread of HPAIV from small-scale broiler farms to other sector 3 and 4 farms in the District of Bogor has been described by Idris et al. (2010). For that study, risk pathways were identified in consultation with a wide variety of stakeholders and experts involved with HPAI control or poultry production in Indonesia. The generalized pathways described by Idris et al. (2010) are presented in **Error! Reference source not found.**1 and 2.

Whilst contact between live birds undoubtedly plays a significant role in the spread of HPAIV H5N1 in Indonesia (Smith et al. 2006), the majority of pathways identified are associated with the contamination of the environment and the subsequent movement of fomites (i.e. contaminated people or equipment), animals, water or air between farms. The probable relevance of these transmission routes is also reflected in reports from the literature (Table 2).

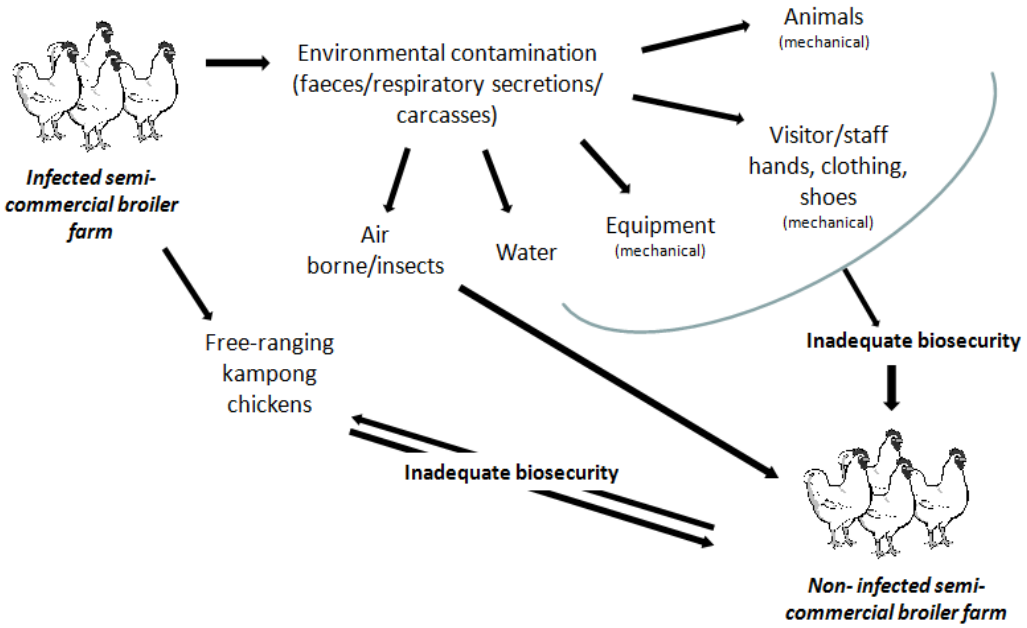

Figure 1: Simplified transmission pathway for the spread of HPAIV H5N1 from an infected small-scale broiler farm to a non-infected small-scale broiler farm in the District and Municipality of Bogor, Indonesia.

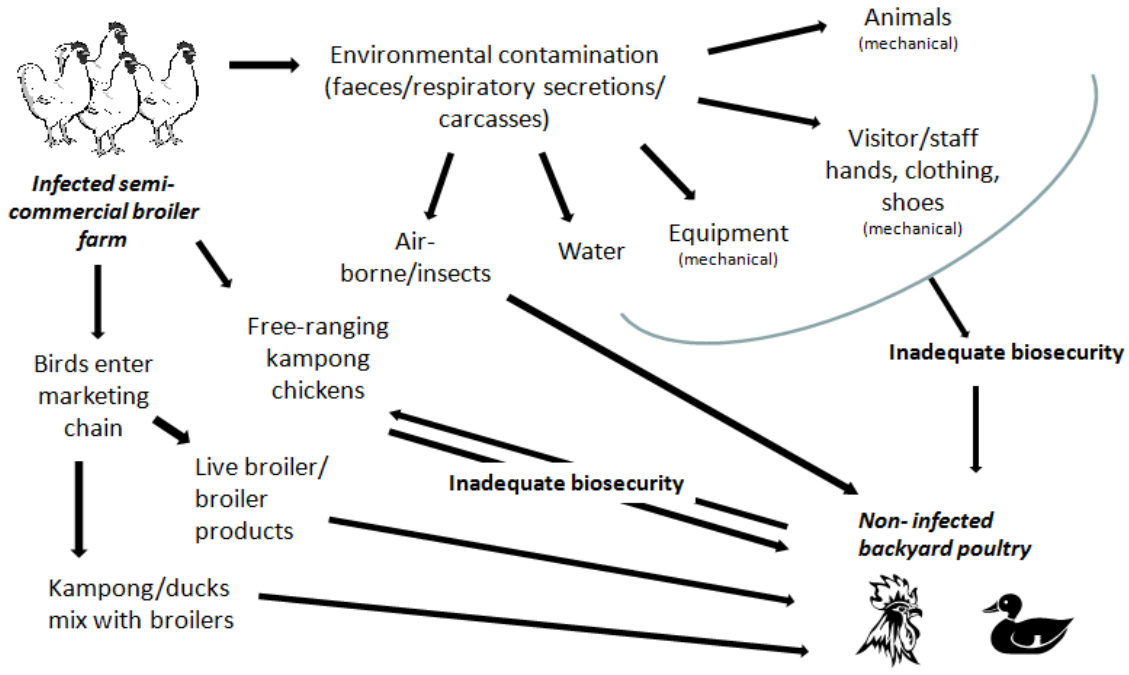

Figure 2: Simplified transmission pathway for the spread of HPAIV H5N1 from an infected small-scale broiler farm to a non-infected backyard poultry flock in the District and Municipality of Bogor, Indonesia.

Table 2: Potential transmission routes for the spread of HPAIV between holdings in Indonesia (including evidence from other countries, and evidence from LPAI and HPAIV virus strains)

Transmission route	Reference
Retail marketing of live poultry	(Panigrahy et al. 2002; Bulaga et al. 2003; Kung et al. 2003; Mullaney 2003; Wang et al. 2006; Sims et al. 2005; Cardona et al. 2009)
Movement of live birds between farms	(Kaoud 2007)
Shared equipment	(Halvorson et al. 1980; Capua and Marangon 2000; Thomas et al. 2005; Wee et al. 2006; Capua and Alexander 2007; Nishiguchi et al. 2007)
Transport vehicles	(Capua and Marangon 2000; McQuiston et al. 2005)
Human contacts	(Halvorson et al. 1980; Capua and Marangon 2000; McQuiston et al. 2005; Thomas et al. 2005; Capua and Alexander 2007; Nishiguchi et al. 2007; Henning et al. 2009; Vieira et al. 2009)
Air currents	(Brugh and Johnson 2003; Power 2008; Sedlmaier et al. 2009)
Water-borne	(Markwell and Shortridge 1982; Halvorson et al. 1985; Sivanandan et al. 1991; Laudert et al. 1993; Leung et al. 2007)
Wild birds, rodents, pets, insects	(Butler 2006; Kuiken et al. 2006; Sawabe et al. 2006; Sievert et al. 2006; Barbazan et al. 2008; Henning et al. 2009)
Scavenging poultry	(Henning et al. 2009)
Manure	(Kandun et al. 2010)*

*Reference refers to transmission to people.

1.3 General model framework

This risk assessment will consider the following specific release and exposure risk questions:

1.3.1 Release assessment

'What is the probability of viable HPAIV H5N1 spreading from an infected small-scale broiler farm to a non-infected small-scale broiler farm or non-infected backyard flock in the District and Municipality of Bogor, Indonesia, under current conditions and via high risk transmission pathways?'

The release assessment considers the transmission of HPAIV in the course of a single broiler chicken production cycle: in Indonesia this is typically 4 to 6 weeks.

1.3.2 Exposure assessment

'Given the *spread* of virus to a non-infected broiler farm, what is the probability that infection, transmission and establishment of the infection will occur on the farm.'

The risk assessment model will consider two general risk pathways; those arising from environmental contamination, and therefore indirect contact, and those arising from direct bird-to-bird contact. The risk assessment begins by modelling an outbreak of HPAI H5N1 on a small-scale broiler farm. The release and exposure assessments are then composed of 3 sections considering: 1) mechanical transmission via fomites; 2) water-borne transmission; 3) bird-to-bird transmission. For the risks associated with environmental contamination, we consider the probability of virus contamination (P_c), the probability of virus survival (P_s) and the probability of infection (P_i) (Zwieterin and van Gerwen 2000). The general model framework is presented in **Error! Reference source not found.**.

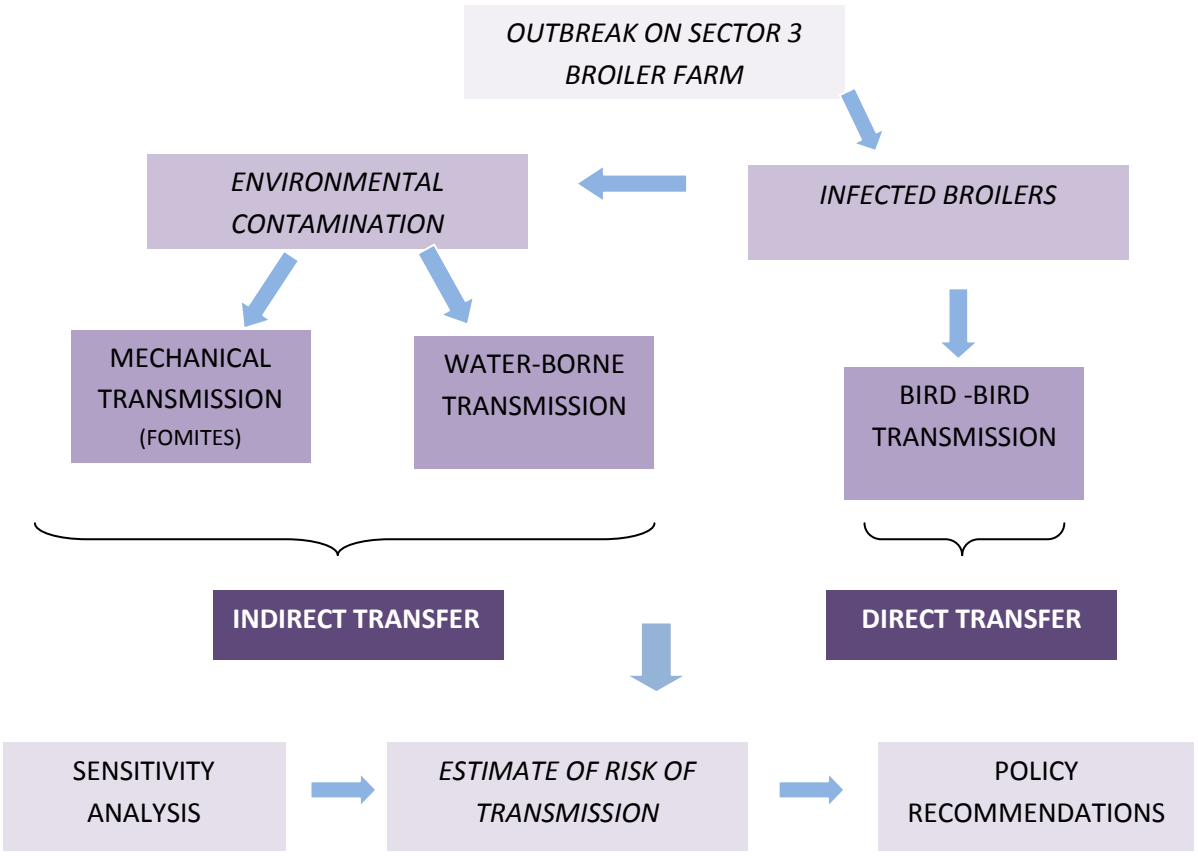

Figure 3: Risk assessment model framework.

2 Modelling an HPAIV H5N1 outbreak on a small-scale broiler farm

The degree to which HPAIV H5N1 spreads within a flock of broilers, and the number of birds infected over time, will influence the risk of onward transmission: as more birds become infected on an index farm, the probability of onward spread increases. Hence, between farm transmission will be influenced by the on-farm disease transmission dynamics (Stärk et al. 2000).

2.1 Estimating the within-flock prevalence

We considered an outbreak of HPAI H5N1 on an index farm and modelled the dynamics of infection using a simple susceptible-infected-infectious-removed (SEIR) model (Anderson et al. 1992). This forms the basis for the release assessment since it allows prediction of the number of birds that are infected within the index flock at any point in time (i.e. the within-flock prevalence, *wfp*), and therefore contributes to predictions of the probability of transmission given direct or indirect contact with the flock at that time.

The general structure of the model is shown in Figure 4, and describes the changes in the proportion of birds in each of the four compartments (susceptible, infected, infectious, and removed) with time (*t*) following the introduction of the virus into the flock. The movement of birds between these transition states is described by three parameters; the force of infection (λ), the disease rate (*f)* and the removal rate (*r)*. The force of infection (λ) reflects the rate at which an infectious agent is transmitted in a population of randomly mixing individuals based on the number of 'effective' contacts individuals make. 'Effective' contacts will allow the virus to be transmitted from an infectious bird to a susceptible bird at a rate of ßI_t, where the transmission rate parameter ß can be defined as the average number of secondary cases caused by one infectious bird per time unit in a susceptible population (Diekmann and Heesterbeek 2000). The disease rate (*f*) describes the rate at which infected individuals become infectious, whilst the removal rate (*r*) describes the rate at which infectious individuals die or become immune.

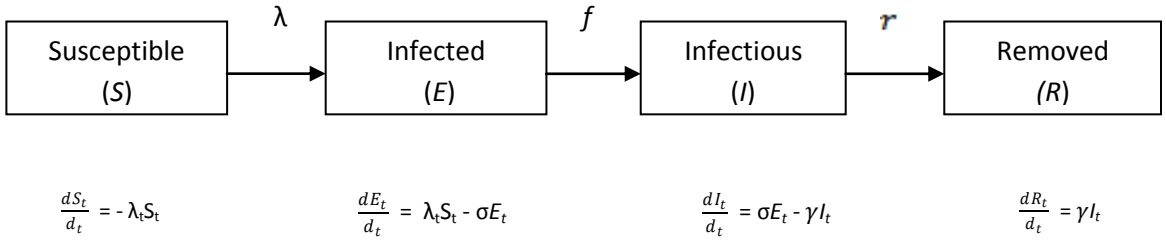

Figure 4: Structure of the SEIR model used to predict the within-flock prevalence.

The SEIR model adopted makes the important assumption that there is homogenous mixing within a flock of broilers (*A1*)[1]. Preston & Murphy (1989) demonstrated that birds within a flock of 18,200 broilers were highly mobile and were not confined in movement to areas occupied by a stable set of

1. *A1* indicates that this is the first assumption made. A full list of model assumptions is provided in the appendix

birds, suggesting that a population of ground-dwelling broiler chickens are relatively well mixed and that this assumption may be reasonable (Van Gerwen et al. 2005). The model also makes the assumption that each bird makes a fixed number of contacts per unit of time regardless of population size (A2). Such a frequency dependent assumption is thought to be appropriate when modelling disease in livestock populations with fixed stocking density (Bouma et al. 1995), and was used by Bouma et al. (2009) to model HPAI H5N1 outbreaks in a population of ground-dwelling birds. In support of this, Bos et al. (2009) showed poultry flock size did not influence the within-flock transmission rate for HPAIV H7N7.

2.2 Transmission model parameters

The SEIR model adopted was stochastic and model parameters were incorporated as probability distributions. Hence, each model iteration employed a randomly selected value for each parameter in order to define virus transmission dynamics.

Rate at which individuals become infectious (f) and rate at which they die (r)

Based on knowledge of the latent period for HPAIV H5N1 (i.e. time from infection to disease onset, δ) it is possible to estimate the rate at which infected individuals become infectious *(f)* as $1/\delta$. Similarly, the average infectious period, γ, can be used to inform the rate at which infectious individuals recover or die *(r)*, as $1/\gamma$.

There are relatively few data on the duration of either the latent period or infectious period for HPAIV H5N1 in infected chickens. As is shown in Table 3, chickens infected with HPAIV H5N1 will generally die as a result of infection, and this death will typically occur within 48 to 72 hours. Shedding from the cloaca and trachea has been detected 24 hours (Tumpey et al. 2002) and 28 hours post infection (Bublot et al. 2007a), and this is supported by evidence from other HPAIV strains (e.g. H5N2: van der Goot et al. 2003; Swayne and Beck 2005; H7N7: van der Goot et al. 2005).

Table 3: Mortality rates and mean time to death (MDT) for HPAIV H5N1 strains

Virus	Dose	Mortality	MDT[1]	Source
A/Environment/HongKong/437/99	$10^{6.0}$	100% (8/8)	5.5	(Cauthen et al. 2000)
A/chicken/Hong Kong	$10^{6.0}$	100%	2.1	(Shortridge et al. 1998)
A/chicken/HongKong/156/97	$10^{6.9}$	94% (15/16)	3–5	(Suarez et al. 1998)
A/chicken/HongKong/220/97	$10^{8.1}$	100% (16/16)	2	(Suarez et al. 1998)
A/chicken/Hong Kong/220/97	$10^{5.8}$ –$10^{6.2}$	100% (16/16)	1.5	(Perkins and Swayne 2001)
A/duck/Anyang/AVL-1/01	$10^{8.0}$	100% (8/8)	2.9	(Tumpey et al. 2002)
A/goose/Vietnam/113/01	$10^{6.0}$	100% (8/8)	2.6	(Nguyen et al. 2005)
A/goose/Vietnam/324/01	$10^{6.0}$	100% (8/8)	2.4	(Nguyen et al. 2005)
A/chicken/Korea/ES/03	$10^{5.9}$	100% (8/8)	2	(Lee et al. 2005)

[1]MDT: Mean time to death (days)

Bouma et al. (2009), using experimental data, predicted a relatively short latent period of 0.24 days (s.d. 0.043) and an infectious period of 2.1 days (s.d. 0.33). In the absence of additional data, we use these single study estimates in order to predict *f* and *r*, assuming that both latent period and infectious period are log-normally distributed (*A3*).

Force of infection (λ)

The force of infection within a flock can be derived based on the average number of secondary infectious cases resulting from the introduction of a primary infectious case into a totally susceptible population (i.e. the basic reproduction number, R_0). Estimates of R_0 can be used to define β, and therefore λ, as:

$$\beta = \frac{}{} \quad \text{and} \quad \lambda = \beta$$

where *n* is the susceptible population size and *γ* is the average duration of infection (i.e. the infectious period).

The stocking density for small-scale broiler production in Bogor is typically between 8 to 10 birds per m^2, with an average of 3,400 birds in a single shed (FAO 2008b). Caeco-oral transmission is considered to be the most important mechanism for spread of HPAIV H5N1 between chickens (Shortridge et al. 1998), hence as well as direct bird-to-bird contact, the contamination of feed and water sources is likely to play an important role in virus spread within a broiler house.

Given the high density of birds, together with the potential for indirect transmission, we expect the number of secondary cases resulting from the introduction of virus and the infection of at least one bird to be high. Modelling an outbreak of HPAIV in a flock of unvaccinated floor reared birds, Savill et al. (2006) estimated R_0 to be in the order of 66, whilst Truscott et al. (2007) used a value of 40. Bouma et al. (2009), using a limited number of birds in experimental transmission studies, projected an average R_0 for HPAIV H5N1 of only 1.6 (95% C.I. 0.9–2.5) whilst Poetri et al. (2009) estimated an R_0 of 12 (95% C.I. 4.7–28.7) following the experimental introduction of HPAIV H5N1 (A/chicken/Legok/2003) into a group of 20 chickens. Whilst these estimates are considerably lower than those used by Savill et al. (2006) and Truscott et al. (2007), it is difficult to extrapolate the observed findings to the field situation given that the experiments involved relatively small numbers of birds in low density situations. There remains considerable uncertainty around an estimate of R_0, and this parameter was incorporated into the transmission model as a uniform distribution between 20 and 40. The potential impact of values of R_0 outside this range on the risks of onward farm to farm spread of HPAIV H5N1 will be discussed in the exposure assessment.

Modelling an outbreak

The parameters and the probability distributions used for the transmission model are shown in Table 4. As farm size estimates are available for all broiler flocks in the District and Municipality of Bogor, an empirical distribution was constructed to describe the variability in flock size (*n*). It was decided at the initial project meeting (November 2008) that a 'small-scale' broiler farm could be defined as any commercial or semi-commercial farm with a capacity of 5000 broilers or less, hence the cut off for inclusion in this distribution was set at 5000. The resulting range was flock sizes between 400 and 5000 birds, with a mean of just over 3000 birds. It is generally held that there is little economic

incentive for small-scale broiler farmers to vaccinate their flocks against HPAI H5N1, and this is currently not Government policy; hence, we assumed that no small-scale broiler flock in Bogor was vaccinated and that each was equally susceptible to HPAIV H5N1 (*A4*).

Table 4. Parameters and probability distributions used for the modelling of bird-to-bird transmission of HPAIV H5N1 within a broiler house

Parameter	Distribution/fixed value	Source
Flock size (*n*)	Empirical distribution (Histogram)	FAO profiling study (FAO, 2008b)
Basic reproduction number (R_o)	Uniform(20,40)	*Estimated*
Latent period (δ) (days)	Lognormal (0.24, 0.043)	(Bouma et al. 2009)
Infectious period (γ) (days)	Lognormal (2.1, 0.33)	(Bouma et al. 2009)

The model was run over 5000 iterations, with random values drawn from the distributions representing n, δ, R_o, and γ at each iteration. Hence, the output was a variability distribution for the within-flock prevalence at any time, *t*.

2.3 Additional model considerations

The transmission model described allows estimation of the rate and the degree to which HPAIV H5N1 spreads within a susceptible flock of small-scale broiler. However, the likelihood of virus 'release' from an infected small scale-broiler farm will be influenced by a variety of other factors, including the stage at which the virus is introduced, the likelihood of detection and the actions of broiler producers in the event of detection. These additional sources of variability are summarized in 5, and the impact of each factor is discussed below.

2.3.1 Number of birds initially infected ***(N_{inf})***

For the baseline scenario, the initiation of infection within the flock results from the introduction of a single source of infection, and the subsequent infection of a single bird. This is a model simplification rather than a comment on the epidemiology of the disease. The presence of contaminated feed, water or litter might be expected to result in very widespread exposure and therefore the appearance of a large number of infected birds at or around the same time. The impact of this potentially important source of variability will be discussed in the exposure assessment.

2.3.2 Time of HPAIV H5N1 introduction ***(T_{inf})***

The stage at which the outbreak is initiated (i.e. infection of the primary case) will impact on the dynamics of infection. Clearly, infection in the early stages of production may allow a more sustained outbreak when compared to infection around the time of broiler depopulation. The risk of onward transmission may therefore also vary according to the time at which the outbreak is initiated.

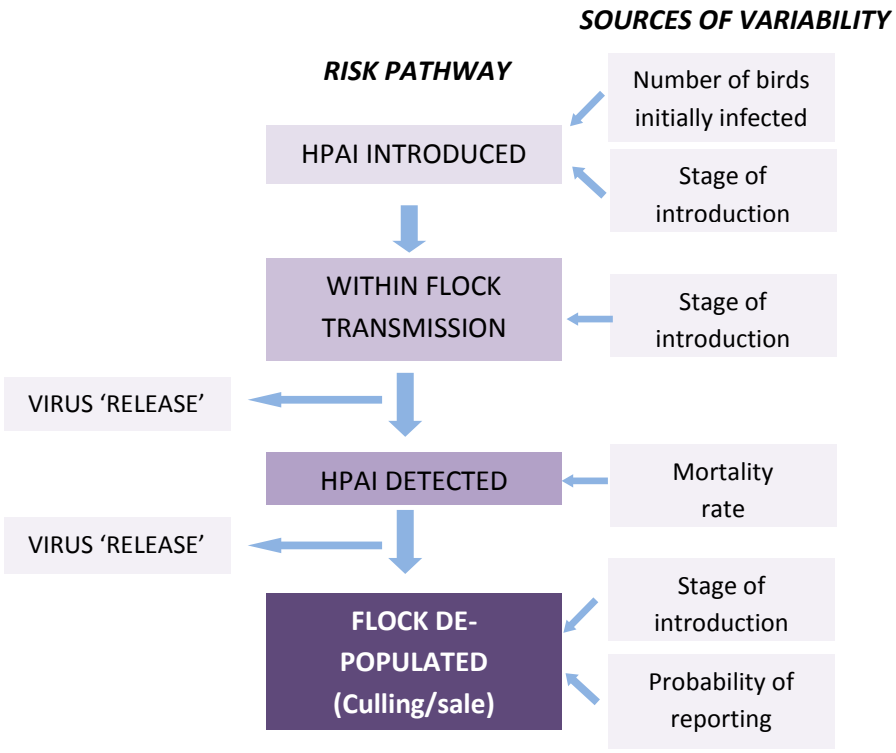

Figure 5. Risk pathway and sources of variability for the release of HPAIV H5N1 via fomites

The most common grow-out period for broilers in Bogor is 35 days, and in the majority of cases all birds will be removed from the broiler house by the 40th day of production. The variability in the time of virus introduction (T_{inf}) is characterized as a uniform distribution ranging from 0 to 40 days. There is some anecdotal evidence that HPAI H5N1 is rare in commercial and semi-commercial broiler flocks before the 2nd week of production, as is commonly reported for campylobacteriosis (Jacobs-Reitsma et al. 1994; Evans and Sayers 2000). The impact of time of virus introduction will be discussed in the exposure assessment.

2.3.3 Probability of disease detection *(P_d)*

Whilst clinical HPAI H5N1 may be associated with a wide variety of clinical signs (Swayne 2007), it is common that the only sign observed in chickens is sudden death (Nakatani et al. 2005; Tsukamoto et al. 2007); Nakamura et al. 2008). Hence, HPAI H5N1 reporting typically relies on producer recognition of increased mortality within a flock (Elbers et al. 2007). Given that the disease is endemic in Indonesia, together with extensive government and non-governmental organization education campaigns, it is assumed that broiler producers in Indonesia will include HPAI H5N1 as a differential diagnosis if the mortality level in their flock increases beyond the background level *(A5).* There is, however, likely to be a degree of variability in the stage at which the disease is detected, which may depend on a producer's age and experience, and previous HPAI H5N1 history, although the true extent of this variability is uncertain in the absence of survey data.

In highly industrialized production systems, for example in the UK and USA, typical mortality levels in 'normal' broiler flocks are around 0.05 to 0.1% per day, although this may vary by stage of production (McMullin 2006; Vieira et al. 2009). Whilst largely unknown in Indonesia, background mortality rates would generally be expected to be higher, particularly in small-scale flocks, given

higher endemic disease pressures together with relatively poorer control over the broiler house environment. On the island of Bali, Simmons (2006) reported that the total mortality rate of broilers under normal conditions (i.e. without AI) at the finisher stage varied from 0 to 20%, averaging 5%. If we can assume the finisher stage lasts for around 12 days (Mohlapo et al. 2009), this would equate to an average daily mortality rate of approximately 0.4%. For the purposes of this study we use a relatively high detection threshold of between 1 and 2% daily mortality in the flock (ε); government veterinary services in Indonesia typically use 1% as the threshold for investigation of disease outbreaks in an unvaccinated semi-commercial poultry flock. It is assumed that all small-scale broiler producers in Indonesia will include avian influenza as a differential diagnosis if the mortality level in their flock increases beyond this fixed threshold (A5). The expected variability in the estimate of ε was incorporated into the model as a uniform distribution between 1 and 2%.

By running the baseline transmission model with time of introduction randomly selected from the variability distribution representing time of virus introduction (T_{inf}), we estimated the mortality rate at the end of each 24 hour period from the start of the outbreak. The probability of detection for each day of the outbreak was then estimated as the proportion of the stochastic model iterations that resulted in mortality above the defined detection threshold (ε), which was also selected randomly from its variability distribution. By multiplying this value by the probability that the disease remains undetected for each of the preceding days, we estimated the probability that an outbreak would be *first* detected for each day since its initiation. The simulation results are presented in **Error! Reference source not found.**, and indicate that for over 84% of the model iterations (i.e. 5000 simulated outbreaks, each with random values of n, δ, $R_{o,}$γ, T_{inf} and ε), we would expect that the disease will be detected at the end of the second day (i.e. 48 hours from the initiation of the outbreak) but that that there is a 14% probability of detection at the end of the first day and 2% probability of detection on the 3rd day. Based on the ranges of model parameters used, we would expect that all outbreaks would be detected by the 4th day.

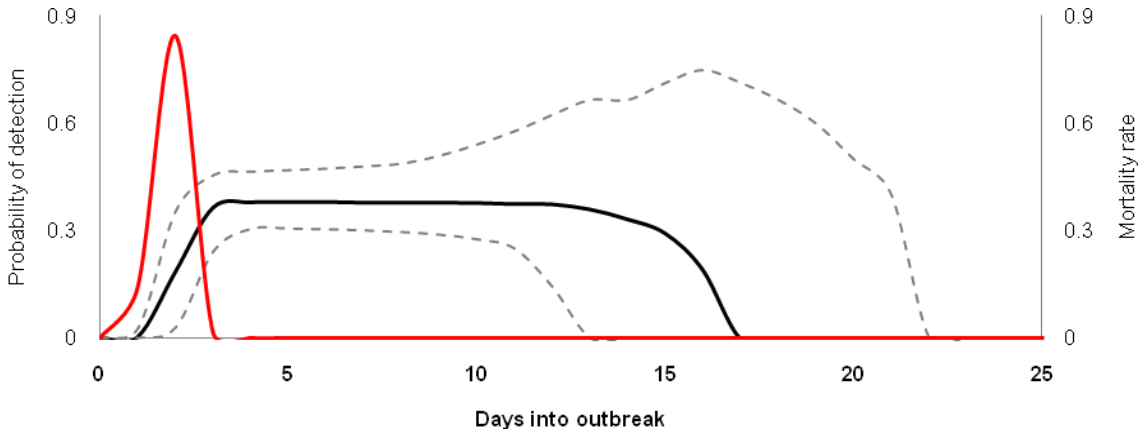

Figure 6. Predicted daily mortality due to HPAIV H5N1 as the proportion of all birds that die during the course of the day. The mean estimate (black solid line) and 5th and 95th percentiles (dashed lines) are shown. The probability that the HPAI H5N1 outbreak is first detected for each day of the outbreak is also shown (red line). Based on 5000 model iterations.

The impacts of different values of R_0 on the probability of detection are presented in Figure 7. The impact is relatively small for values of R_0 between 20 and 40. Unsurprisingly, at a higher reproduction number of 40, the mortality rate increases more rapidly (than an R_0 of 30 and 20) and therefore more outbreaks are detected at the end of the first day (37%, compared with 14% and 1%, respectively). At lower values of R_0 (i.e. less than those within the modelled range, but of the order predicted by Bouma et al. 2009 and Poetri et al. 2009), the day at which an outbreak is most likely to be detected decreases from day 3 (68% of iterations) for a R_0 of 10, to day 4 (40% of iterations) for an R_0 of 5 and day 11 (18% of iterations) for an R_0 of 2 (Figure 8). The time until the outbreak is detected could be expected to affect the probability of onward transmission and will be discussed in the exposure assessment.

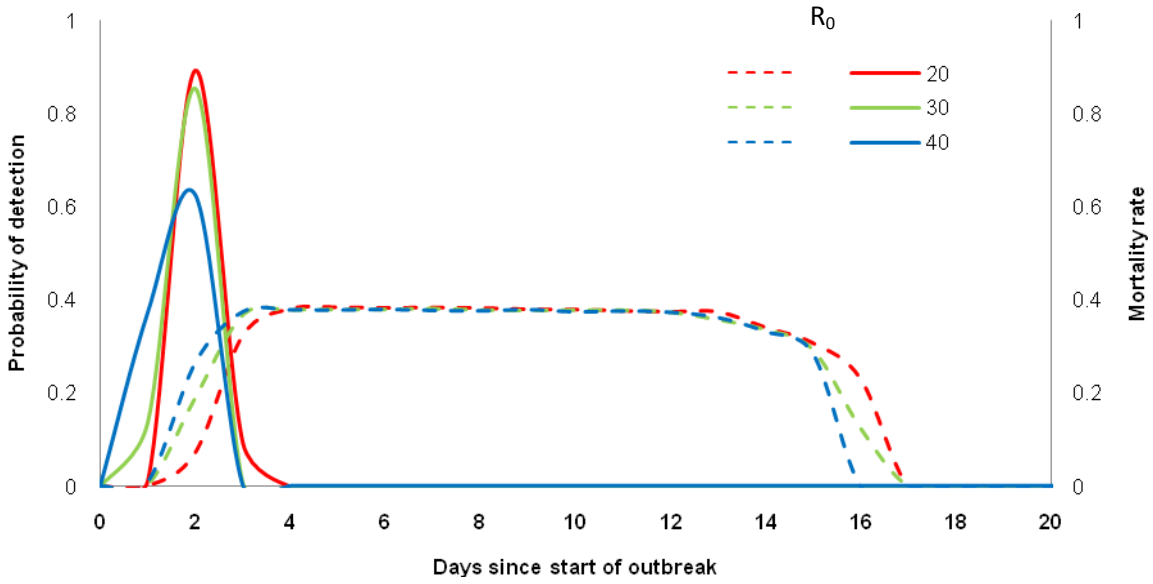

Figure 7. Predicted daily mortality rates (dashed line) due to HPAI H5N1 and probability of detection at a range of fixed R_0 values, with all other model values (n, δ, γ, T_{inf}) drawn at random from their respective variability distributions. Based on 5000 model iterations

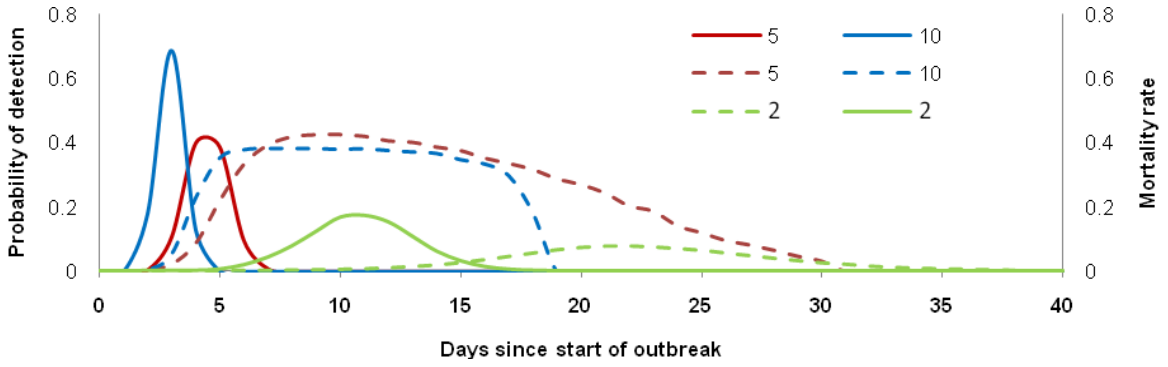

Figure 8. Predicted daily mortality rates (dashed line) due to HPAI H5N1 and probability of detection at a range of fixed R_0 values, with all other model values (n, δ , γ, T_{inf}) drawn at random from their respective variability distributions. Based on 5000 model iterations

2.3.4 Response to an outbreak

With some exceptions, districts and provinces in Indonesia do not offer compensation for birds that are culled as part of disease control activities, and this is typically the case for commercial poultry producers in Bogor. Hence, there may not be substantial incentives for producers to report suspicion of HPAI in their flocks.

We assumed that all broiler producers will modify their behaviour in response to a suspected outbreak of HPAI H5N1 in their flocks (*A6*), and simplified this behaviour into two general responses:

1) Producers report suspicion to government services which investigate within a defined period and initiate disease control activities on the farm if HPAI H5N1 is confirmed.
2) Producers continue with the production cycle, selling any remaining live chickens at the first possible opportunity.

Out of 14 broiler farmers with flock sizes ranging from 1000 to 100,000, three producers, when interviewed, suggested that they would report an HPAI-like disease in their flock to a government office (CENTRAS 2008). Whilst there may be considerable response bias in these estimates, by extrapolating from this extremely small sample size we could approximate that around 21% of producers in Bogor might report a high mortality event in their flock. However, in the absence of compensation, it is anecdotally reported that the majority of producers will sell live birds in the face of an outbreak. Such an approach will minimize economic losses in the absence of compensation, but will clearly also present a potential risk to public health, and provide numerous occasions for the onward transmission of HPAIV H5N1.

The focus of this risk assessment will be on the onward transmission of virus from those producers who do not inform government services if they suspect disease in their flocks, but the risks associated with this practice will be compared with the presumed lower risk strategy of disease reporting.

3 Release assessment: Transfer of HPAIV H5N1 via fomites

There are three main sources of environmental contamination that may present a risk for the onward spread of HPAIV H5N1 from an infected broiler flock: faeces, respiratory secretions, and contaminated carcasses.

Fomites can be defined as any object, animate or in-animate, that can become contaminated with an infectious organism and allow the mechanical transfer of that organism to a susceptible individual or flock.

HPAIV H5N1 contamination of fomites

Fomites can become contaminated through direct contact with infected birds, or indirectly through contact with litter or faecal material that has previously been contaminated by respiratory or faecal secretions from infected birds. Hence, the likelihood that a fomite becomes contaminated with infectious virus depends on the quantity of virus shed by infectious birds (*V*), the prevalence of infection birds within the flock *(wfp),* and the extent to which the virus is able to survive in the broiler house (*k*) (Figure 9).

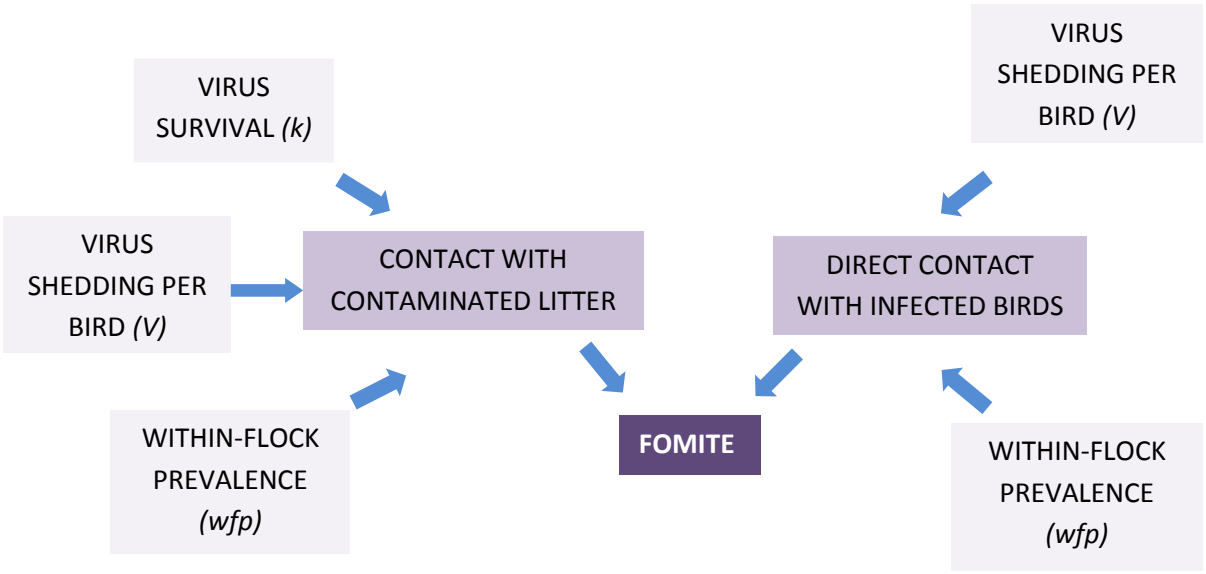

Figure 9. Contamination of fomites with HPAIV H5N1

3.1 Probability of contamination: contact with contaminated litter

Any person who enters a broiler house during the course of an HPAI outbreak can expect to come into contact with litter that has been contaminated by virus shed by infected birds.

3.1.1 Quantifying HPAIV H5N1 shedding by infected birds

The main routes of shedding of HPAIV H5N1 by infected chickens are in respiratory secretions via the oropharynx and gastrointestinal excretions via the cloaca (Subbarao et al. 2003; Lee et al. 2005; Swayne and Beck 2005; Das et al. 2008;).

There have been a number of studies that are able to provide an estimate of the amount of virus shed in respiratory or faecal secretions (Table 5). Typically, the experimental population involved has been small and, in the majority of cases, an estimate of the average virus production is provided without any indication of the range of values detected. In the light of these limitations, estimates were combined in order to determine the mean value for faecal and respiratory virus shedding. It was assumed that each of the individual estimates were Poisson distributed (Haas et al. 1999) (*A8*), and we used a parametric bootstrap to generate uncertainty about the true mean number of virus particles per gram or millilitre of faecal and respiratory secretion from an infected bird. The resulting output had a mean faecal titre ( ) of 4.92 $\log_{10}$ EID_{50} (embryo infectious dose 50) (s.d. 0.56) and a mean respiratory titre ( ) of 5.57 $\log_{10}$ EID_{50} (s.d. 0.63).

3.1.2 Amount of HPAIV H5N1 shed per infected bird

Faeces

There are relatively few data on the faecal output of broiler chickens, but the United States Environment Protection Agency (**EPA**) (EPA 2001) estimate that a 25,000 bird broiler unit may produce up to 1 tonne of droppings every day. Hence, a typical small-scale broiler farm with around 5000 birds might be expected to produce 200 kg per day, or 40 g of faeces per bird per day. We attempted to incorporate the potential (although uncertain) range of values around this estimate of faecal production per bird per day as a Betapert distribution with a minimum value of 20 g, a maximum of 50 g and most likely value of 40 g for a bird of 1250 g.

If it is assumed that an average day old chick is 20 g, reaches a weight of 1000 g by 25 days and a weight of 1250 g by 45 days (i.e. generalized production targets for broilers in Indonesia) (A7), a polynomial equation can be fit to predict the weight of birds at each stage of production. Given this predicted daily weight, the individual bird daily faecal output was estimated by sampling from the variability distribution describing faecal output per gram of broiler weight (i.e. Betapert (30,40,50)/ 1250), F_{day}). Combining these estimates with the distribution describing mean virus faecal titre ( ) per gram and multiplying by the number of birds in the infectious compartment of the SEIR model, allows an estimation of the amount of virus entering the house at time *t* through faecal excretion.

Respiratory secretions

Respiratory output per day (R_{day}) was modelled as a Betapert distribution with a minimum of 0 ml, a maximum of 20 ml and most likely value of 5 ml per day for a bird of 1250 g, based on the authors' opinion. Whilst respiratory secretions are known to increase as a result of HPAIV H5N1 infection (Swayne, 2007), it has not been possible to derive quantitative estimates of secretion, hence there remains uncertainty about the true range of these estimates.

We assume that both respiratory and faecal excretions find their way to the ground litter. Here, we make the additional simplifying assumption that the movement of birds within the broiler house will

result in complete mixing of virus within the litter, so that the virus per gram of faeces contained within litter in the house can be described by a Poisson process (*A9*).

Table 5. Shedding of HPAIV H5N1 from the cloaca and oropharynx

Virus	Dose (log10 EID_{50})	Route	Sample time (hrs)	No. chicken	Age (wks)	Cloaca (log10 EID_{50}/ml)	*Oropharynx* (log10 EID_{50}/ml)	Reference
A/chicken/ South Korea/ES/03	5.9	*i.n.*	72	4	4	6.0[1]	5.8[1]	(Lee et al. 2005)
A/chicken/ South Korea/ES/03	5.9	*i.n.*	72	4	4	6.1[1]	6.7[1]	(Lee et al. 2005)
A/chicken/HK/ 491/97	6.0	*i.n.*	72	8	4	4.6	4.6	(Subbarao et al. 2003)
A/chicken/ Yamaguchi/7/04	4.0	*i.n.*	48	1	12	<3.8	<3.5	(Imai et al. 2007)
A/chicken/ Yamaguchi/7/04	4.0	*i.n.*	48	1	12	5.5	<4.2	(Imai et al. 2007)
A/chicken/ Yamaguchi/7/04	4.0	*i.n.*	48	1	12	>5.8	>5.8	(Imai et al. 2007)
A/chicken/ Yamaguchi/7/04	4.0	*i.n.*	48	1	12	5.2	4.2	(Imai et al. 2007)
A/Goose/ Guangdong/1/96	6.0	*i.n.*	72	10	6	3.2 ± 0.9	4.4 ± 1.6	(Li et al. 2008)
A/Goose/ Qinghai/3/ 05	6.0	*i.n.*	72	10	6	4.2 ± 0.2	5.2 ± 0.3	(Li et al. 2008)
A/chicken/ Fujian/1/07	6.0	*i.n.*	72	10	6	3.5 ± 0.5	4.7 ± 0.6	(Li et al. 2008)
A/chicken/ South Korea/ES/03	3.5	*i.n.*	48	10	3	3.4 ± 2.4[1]	5.0 ± 2.0	(Bublot et al. 2007b)
A/chicken/ South Korea/ES/03	5.0	*i.n.*	48	10	3	5.3 ± 1.2[1]	6.2 ± 1.5[1]	(Bublot et al. 2007b)
A/chicken/ South Korea/ES/03	6.5	*i.n.*	48	10	3	5.7 ± 0.6[1]	6.9 ± 0.5 [1]	(Bublot et al. 2007b)
A/chicken/ South Korea/ES/03	8.0	*i.n.*	48	10	3	5.65 ± 0.4[1]	6.9 ± 0.4 [1]	(Bublot et al. 2007b)
A/chicken/ Vietnam/8/04	4.5	*i.n.*	48	5	3	5.7 ± 0.5	–	(Bublot et al. 2007b)
A/chicken/ Supranburi/2/04	6.0	*i.n.*	28	20	7	3.9	5.5	(Bublot et al. 2007a)
A/chicken/ Supranburi/2/04	–	*DC*	48	10	7	5.0	5.7	(Bublot et al. 2007a)
A/chicken/ Indonesia/7/03	6.0	*i.n.*	48	10	6	5.82	6.16	(Swayne, 2006)
A/duck/ Anyang/ AVL-1/01	6.0	*i.n*	24 – 72	8	4	1.1 – 3.1	4.4 – 4.8	(Tumpey et al. 2002)
A/chicken/ Yamaguchi/7/04	7.0[2]	*i.n/DC*				2.0 – 5.0	3.5 – 5.0	(Tsukamoto et al. 2007)

i.n. = intra-nasal. DC = direct contact, [1] Estimated from figures (i.e. actual numbers not provided in the text), [2] Experiment describes shedding following intranasal infection and shedding by birds in direct contact with inoculated birds; the inoculating dose is only known for i.n. infected birds

3.1.3 Estimating HPAIV H5N1 survival in the broiler house

In order to estimate the maximum likelihood term for the average HPAIV H5N1 titre per gram of litter ( ) at time *t*, it is necessary to consider the survival of virus excreted by infected birds in the proceeding time steps (t_0), and therefore the cumulative titre of virus in the litter.

Predictions of HPAIV H5N1 survival in faeces

The survival of avian influenza viruses in the environment is influenced by environmental temperature. Investigating survival of LPAIV H5N2, Beard et al. (1984) found that virus in dried faeces could be recovered for 35 days when stored at 4°C, but for only 7 days when stored at 20°C. Similarly, Lu et al. (2003) found that the infectivity of H7N2 was abolished after 24 hours at 30–37°C but persisted for 2 days at 15–20°C. In Thailand, Chumpolbanchorn et al. (2006) demonstrated that HPAIV H5N1 in chicken manure lost its infectivity after 24 hours at temperatures of 25°C, but after only 15 minutes when the temperatures were 40°C. High environmental temperatures would therefore appear to militate against HPAIV H5N1 survival in faeces.

Humidity may also impact on virus survival. It is generally agreed that high relative or absolute humidity results in a more rapid decline of airborne influenza A virus (Lowen et al. 2007; Shaman and Kohn 2009). In contrast, Songserm et al. (2006a) demonstrated that AIV contained in a substrate with higher moisture content (i.e. fresh faeces) persisted for longer than virus in dried faeces at equivalent temperatures. Similarly, Shortridge et al. (1998) found that virus within dry faeces at room temperature (25 °C) had undetectable levels by day 1, whilst infectious virus was still detectable in wet faeces held at 25 °C after 4 days. Fresh faecal droppings have a moisture content of at least 60 % (Chumpolbanchorn et al. 2006), and it seems likely that higher humidity would allow the moisture content of faeces to remain higher for longer, permitting prolonged survival. This effect was further demonstrated in a study conducted by the United States EPA (EPA 2009), where HPAIV H5N1 mixed with faeces and stored at between 22 and 23°C in an environment with a relative humidity of 40 %, was detectable at only very low levels after one day and zero infectious virus could be recovered after 2 days. The same experiment, but with a higher relative humidity of around 80 %, allowed the recovery of 7.7×10^4 EID_{50} of virus after 1 day and 3.2×10^3 after 2 days.

In contrast to these estimates, Kandun et al. (2010), in Indonesia, were able to identify HPAIV H5N1 through RT-PCR in a bag of chicken manure that was at least 2 weeks old. The authors did not conduct infectivity trials, therefore it is not clear if the identified viral material remained infectious after this period, however they suggest that the manure was the most likely source of infection for a human case of HPAI H5N1.

The broiler house environment: temperature and humidity

The majority of small-scale broiler farms in Bogor, and indeed throughout Indonesia, use traditional open houses, typically with poor insulation. Few, if any, producers in this sector make use of mechanical ventilation. In naturally ventilated houses and particularly those that are open and without roof insulation, the internal environment (i.e. temperature and relative humidity) is typically close to external conditions (Chepete and Tsheko 2006; Czarick and Fairchild 2008). The internal broiler environment can therefore be predicted based on external conditions.

The average daily temperatures and relative humidity were downloaded from the closest weather station to Bogor District (Curug/Budiarto, around 35 km from Bogor Municipality) for 2009 (Weather Underground 2009). These data revealed an average temperature of 27.5 °C (± 1.2 °C), with some small, but significant, differences between mean daily temperature by month (F=6.91, p=<0.01) (Table 6). The average relative humidity for 2009 was around 77 % (s.d. 7.74), with relatively small, but significant, differences between daily humidity on a monthly basis (F=15.47, p=<0.01).

Table 6. Estimated monthly temperature and humidity information for Bogor District for 2009

Month	Mean temperature (°C)	Std. Dev.	Mean relative humidity (%)	Std. Dev.
January	26.58	1.18	81.74	6.87
February	26.56	1.09	82.46	5.78
March	27.71	0.86	78.03	7.09
April	27.70	0.79	79.87	4.56
May	27.63	1.07	78.67	6.71
June	27.67	0.71	79.13	4.85
July	27.48	1.12	70.87	6.71
August	27.68	0.94	71.19	4.50
September	28.43	1.10	67.83	7.12
October	28.06	1.21	75.77	8.09
November	27.50	1.46	79.27	7.73
December	27.42	1.34	79.06	6.17
Total	**27.54**	**1.19**	**76.95**	**7.74**

Source: Weather Underground (2009)

3.1.4 Estimating the virus transformation ratio

The duration that an infectious organism can persist in the environment can be described as the product of the rate of transformation (*k*) as:

$$\frac{dV}{dt} = -kV$$

Where *V* is the quantity of virus and *k* is the decay constant. Hence, the quantity of virus surviving at time *t* (V_t), is:

$$Vt =$$

Despite the relative importance of this parameter, only two virus survival studies provide data of sufficient quality and quantity to adequately predict the survival of HPAIV H5N1 in faeces at the range of temperatures and humidity that could be expected within a broiler house in Bogor (i.e. based on Figure 100 and 11). These are the EPA (2009) study, describing the survival of HPAIV H5N1 (A/Vietnam/1203/04) at a temperature of 22 to 23 °C and a relative humidity of 89 % and the Shortridge et al. (1998) study, where survival of A/Hong Kong/156/97 H5N1 was assessed at a temperature of 35 °C and unknown humidity, although this was presumed to be high given that faeces were described as 'wet'. For these two environmental conditions, the best fitting value for *k* was determined using a one-phase exponential decay model in GraphPad Prism 5 (GraphPad Software, San Diego California USA, www.graphpad.com). Given the small number of studies used for this estimation, these values are inevitably associated with uncertainty in the true range of values of *k*. Moreover, the environmental conditions described are fixed, whilst the environmental conditions within the broiler house are not and rates of decay may therefore vary according to both time of day or year. Although several studies have been conducted in Southeast Asia investigating virus survival (Chumpolbanchorn et al. 2006; Songserm et al. 2006a), additional work is urgently required to fully characterize the survival of HPAIV H5N1 at the range of environmental conditions observed in these regions, and, critically, estimates of virus titre should be made at more regular intervals in order that the virus decay curve can be fully parameterized.

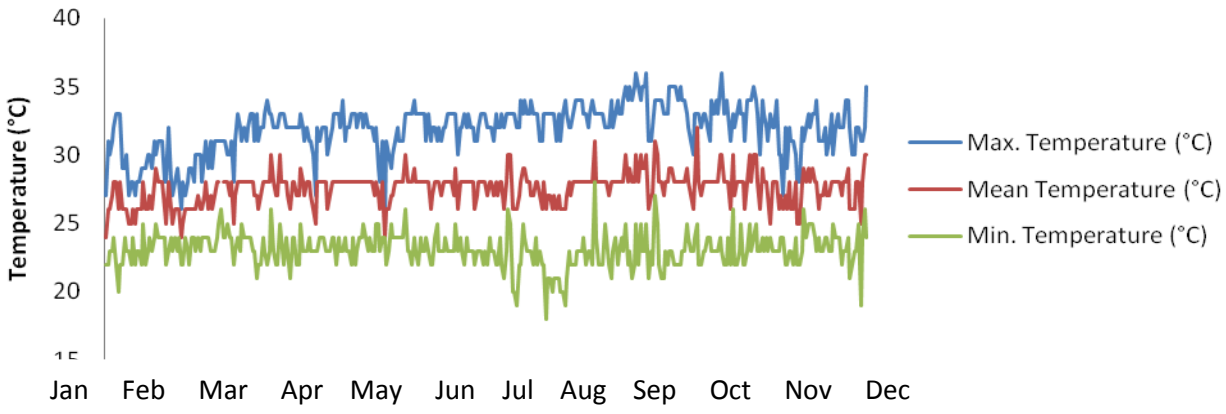

Figure 10. Predicted mean, maximum and minimum temperatures for the District of Bogor in 2009.

Source: Weather Underground (2009)

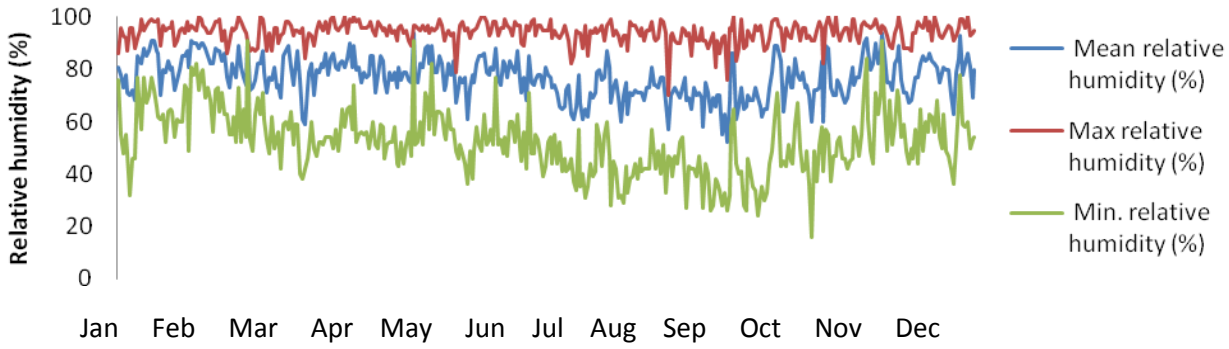

Figure 11. Predicted mean, maximum and minimum daily relative humidity for the District of Bogor in 2009.

Source: Weather Underground (2009)

The best fitting value for *k* based on the EPA (2009) study was 0.1739 (95% C.I. 0.1604 to 0.1875), with a half life of 4 hours (95% C.I. 3.70 – 4.32). The value for *k* at the higher temperature of the 35°C in the Shortridge et al. (1998) study was 0.1919 (95% C.I. 0.1866 to 0.1972) with a shorter half life of 3.17 hours (95% C.I. 3.52 – 3.72). It should be noted that the reliability of this estimate is somewhat questionable, given that it is not clear how faeces were maintained in a 'wet' state.

In order to incorporate the uncertainty around the estimate of *k*, we characterized the decay constant as a uniform distribution, defined by the uniform range of values between to the lower 2.5th percentile from the EPA (2009) study and the upper 97.5th percentile of the Shortridge et al. (1998) study.

3.1.5 Estimating HPAIV H5N1 concentration in litter

The cumulative viral output from respiratory and faecal excretion from all infected birds throughout the course of the outbreak was adjusted based on the effect of this virus decay constant (i.e. e^{-kt}) and divided by the cumulative faecal output by all birds in the flock. Based on 5000 iterations, an estimate of the cumulative virus titre produced in the broiler house per day of the outbreak was derived and, from this, the mean HPAIV H5N1 concentration per gram of faeces ( ) calculated. The resulting estimates are presented in Figure 12.

3.1.6 Contamination of footwear

Any person who enters a broiler house can expect to receive a typically large amount of faecal contamination on their footwear. Estimates of at the time the visitor enters the broiler house can be combined with an estimate of the faecal contamination of footwear (C_{shoes}) in order to predict the amount of virus that may be present on the shoes of visitors following contact with contaminated litter.

We assumed that possible values of C_{shoes} could range from a minimum contamination of 30 g to a maximum of 100 g (for both shoes), and the variability around these estimates was modelled as a uniform distribution.

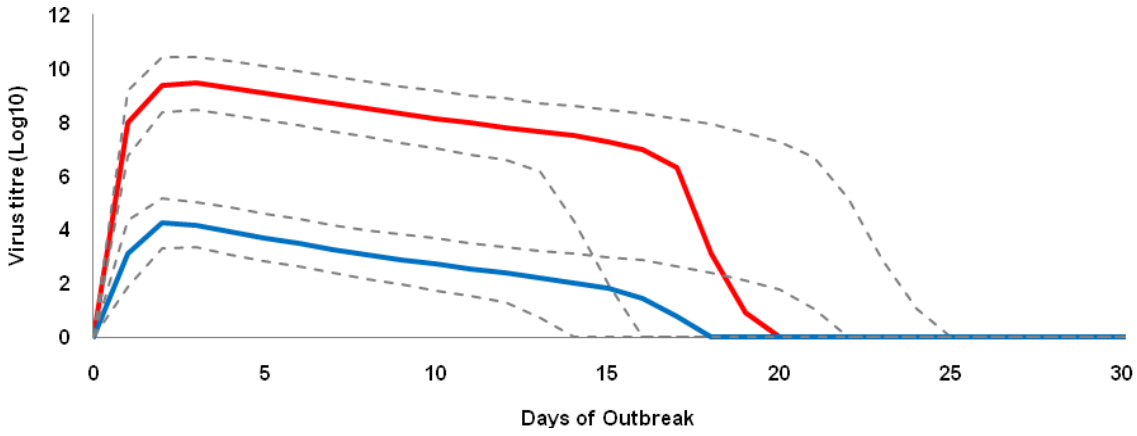

Figure 12. Estimates of per day (blue line) and mean cumulative HPAIV H5N1 production through respiratory and faecal excretion per day (red line). The 5th and 95th percentiles for each estimate are shown as dashed lines.

3.2 Probability of contamination: direct contact with infected birds

3.2.1 Contamination of hands

Any person handling broilers could be expected to receive faecal contamination on their hands, either directly from faeces produced by the handled bird or from general contamination of the bird's body. In order to estimate the faecal contamination remaining once gross contamination is removed (H_f), we use the data provided by Finley et al. (1994), who showed that adherence of soil to skin can be modelled as a lognormal distribution with an arithmetic mean of 0.52 (s.d. 0.9) mg/cm^2 of in-contact skin. This was combined with a fixed estimate of hand surface area (Lee et al. 2007) to predict residual faecal contamination resulting from handling chickens. For this, we make the untested assumption that faecal adherence is equivalent to soil adherence, and that an estimate of virus concentration within the remaining faeces is an adequate estimate of viral contamination of hands following handling (*A10*). Moreover, for the baseline scenario, we assume that any person handling a single infected broiler chicken will be subject to the same degree of faecal adherence, and therefore virus concentration, as someone handling a number of infected broilers (i.e. faecal adherence to hands, amounting to between 0.017 to 0.8 g per hand, would be the same regardless of the number of birds handled) (*A11*).

3.3 Probability of virus survival on fomites

Environmental persistence is a key parameter in microbial risk assessments, since it can place strict limits on the impact of a transmission pathway (Haas et al. 1999). Despite its probable relevance, the issue of environmental persistence on fomites and its impact on the transmission of avian influenza, and particularly HPAIV H5N1, has remained a relatively neglected topic (Weber and Stilianakis 2008). Those estimates that are available tend to be highly variable in the way in which abiotic factors such as temperature, humidity, pH and solar UV are controlled. Hence, variability in survival estimates may be a reflection of the different experimental methods used rather than a reflection of true variability in the population.

3.3.1 Duration of virus survival on fomites

Virus survival on fomites is influenced by a variety of intrinsic factors, which include fomite properties or virus characteristics, and extrinsic factors, including environmental temperature and humidity (Boone and Gerba 2005; Kramer et al. 2006). It has been shown that influenza A and B viruses can survive on metallic surfaces for 72 hours (Bean et al. 1982), and contact with similar surfaces was expected to be responsible for the transmission of influenza infection to people (Barker et al. 2001; Morens and Rash 1995). Virus decay has been shown to be more rapid on plastic surfaces, with a maximum survival of 48 hours and a T_{90} (i.e. 90 % reduction in infectivity) of 4 hours. Influenza A on porous surfaces such as clothes had the fastest rate of inactivation, with detectable virus surviving for only 24 hours and a T_{90} of 3 hours (Bean et al. 1982).

In the most extensive study of AIV survival on fomites, Tiwari et al. (2006) applied H13N7 to a variety of porous and non-porous fomites and measured survival over time. We fitted a one-phase exponential model to a selection of results from this study (Table 7). The resultant rate of decay was considerably slower than those already discussed for faecal material: only in the case of virus decay on plastic do confidence intervals overlap with those for virus decay in faeces exposed to a similar temperature (i.e. from the EPA [2009] study). This is unexpected, particularly given that virus contained within a protective organic substrate, such as faeces, would be expected to survive for longer than a solution of virus spread onto a fomite. The differences may be explained to some extent by variation between strains (Songserm et al. 2006a; Terregino et al. 2009), however these results further highlight the need for more extensive studies investigating the survival of AIV in the environment.

Table 7. Survival of AIV (H13N7) on a selection of fomites

Titre of virus recovered ($TCID_{50}$/ml) after indicated time of storage*					***k***
Fomite	**0 hr**	**24 hr**	**48 hr**	**72 hr**	***(95% C.I)***
Steel	8.7×10^{3}	1.9×10^{3}	0.8×10^{2}	0.9×10^{2}	0.0661 (0.045 – 0.087)
Gumboot	3.4×10^{3}	3.1×10^{3}	2.5×10^{2}	2.5×10^{2}	0.02628 (0 – 0.089)
Cotton fabric	8.9×10^{1}	5.0×10^{1}	–	–	0.02403
Plastic	2.0×10^{3}	7.8×10^{1}	5.0×10^{1}	1.4×10^{1}	0.1331 (0.058 – 0.209)

*Initial virus titre of 6.3×10^{6} TCID50/ml
Source: Tiwari et al. (2006)

Given that the most likely material contaminating fomites is faeces, we assume that the rate of virus decay on fomites is the same as that in the broiler house, and use the previously defined uncertainty distribution for *k (A12).*

3.3.2 Estimating travelling time between farms

In order to predict the probability that infectious virus can spread between small-scale broiler farms, it was necessary to estimate the time *(t)* taken for the contaminated fomite to move between farms, and therefore the time over which virus might undergo decay.

In order to estimate the average travelling time between broiler farms (t_f), the location of all small-scale broiler farms in Bogor was obtained (FAO 2008b) and plotted using ArcMap 9.3 (ESRI, Redlands) (Figure 13). The Hawth's tool for ArcGIS extension (Beyer, 2004) was used to estimate the distance of each individual broiler farm from all other farms in the District and Municipality of Bogor, accounting for duplication. A continuous non-parametric empirical distribution was then fitted to the distance matrix derived. These data were used to determine the average journey time between farms by assuming that visitors using a vehicle may travel between farms at a rate between 10 to 50 km/hr, with the variability modelled as a uniform distribution. The resulting distribution had an average travelling time between farms, (t_f), of 56 minutes (5th percentile: 5, 95th percentile: 149).

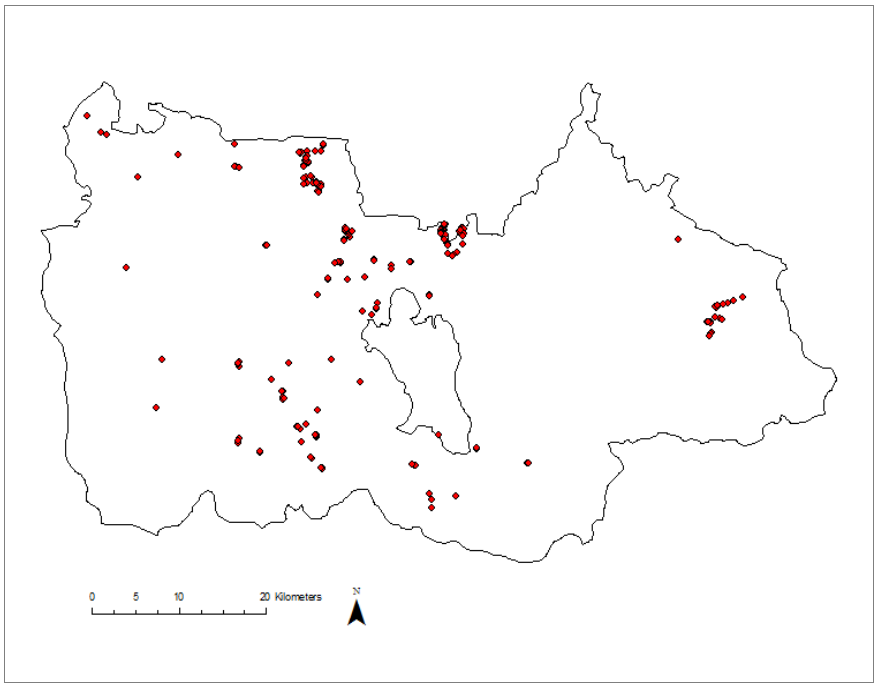

Figure 13: Distribution of small-scale broiler farms in the District and Municipality of Bogor.

In the absence of detailed road data for Bogor, all distances were measured as Euclidean distances between points which is likely to underestimate the resulting distances. Moreover, the estimate does not consider stop-overs and therefore predicts the range of travelling times from farm to farm, without stops in between. The impact of altering travelling time between farms is discussed in the exposure assessment.

3.4 Transfer of HPAIV H5N1 via fomites: specific release pathways

There are a very large number of potential fomites that may act as transmitters of infection between small-scale broiler farms. For the purposes of this risk assessment, we focus on the specific risks

associated with visits to an infected broiler farm by poultry collectors and animal health workers. These pathways were highlighted as being particularly important in the transmission of HPAIV H5N1 between farms in Indonesia by Idris et al. (2010).

3.4.1 Collectors

The majority of small-scale broiler producers in Bogor make use of poultry collectors at the end of the grow-out period. In most cases, collected birds from small-scale broiler farms are initially housed at a collecting facility, which may be linked directly to a slaughter point or birds may be sold live in markets (CENTRAS 2008). Given that poultry collectors may move between a large number of farms, and can come into direct contact with birds on each of these, they have been implicated in the transmission of a variety of poultry diseases between farms (Ramabu et al. 2004; Allen et al. 2008).

In Bogor, collectors typically operate using either a pick-up truck with a capacity of around 200 birds or a dedicated collection truck with a maximum capacity of around 1250 birds. Hence, at depopulation, even a moderately sized broiler flock would be expected to receive numerous visits from collectors. Collecting yard holding facilities have a limited capacity, typically between 1000 to 2000 birds (CENTRAS 2008), and therefore broiler producers may use a number of different collectors in the course of depopulation.

Flock depopulation typically occurs over the course of two to three days, depending on the prices available at market, which can be highly variable, even on a day to day basis. The average harvest point is around 35 days in Indonesia, and it is unusual that birds would remain on farm beyond 40 days. Whilst collectors generally aim to fill their trucks in a single visit, it is not uncommon for vehicles to visit numerous farms before capacity is met. This is particularly the case at the end of depopulation, when the remaining birds in a house are 'mopped-up'. Moreover, partial depopulation, in which a small proportion of the early market-weight attaining broilers are sold at an age of 25 to 30 days, followed by complete depopulation one week later, is also common practice in Bogor, and indeed in many broiler producing areas of the world (Allen et al. 2008; Hald et al. 2000; Russa et al. 2005).

Such staggered depopulation, either intentionally to meet a specific market demand or unintentionally through limited vehicle capacity, may allow HPAIV H5N1 to be introduced to a susceptible farm, and result in an outbreak, before the shed is entirely depopulated. Indeed, it is widely held that multiple depopulations increase the risk of bacterial infections such as *Campylobacter* in poultry flocks when compared to those flocks with single depopulations (Berndtson et al. 1996; Hald et al. 2000; Jacobs-Reitsma et al. 1994; Wedderkopp et al. 2000).

Probability collector visits in the event of an outbreak

As described previously, in broiler flocks in which an HPAI outbreak has been detected but not reported, we assume that the producer will seek the services of collectors at the earliest opportunity following disease detection. In Indonesia, there is a market for relatively small broilers of around 1 kg. This weight is typically achieved at around 25 days, and this will be assumed to be the earliest marketable age and therefore the earliest point at which collectors will visit the farm. Hence, if a producer suspects HPAI H5N1 before the 25th day of production, we make the assumption that collectors will visit the farm on the 25th day (*A13*). On those farms on which an outbreak is detected on or beyond the 25th day, we assume collectors will visit the farm 24 hours after detection, but that

production will cease by the 40th day (*A14*), and therefore that all collectors will have visited by this point.

Collectors may also visit the farm before the disease has been detected. For example, virus may be introduced into the flock on the 30th day of production, and collectors visit on the 31st day, before the mortality rate in the flock has increased to sufficient levels that an outbreak is detected. Hence, we used a Betapert distribution to describe collector visiting practice, where the most likely visiting day was assumed to be the 35th, with a minimum of 25 and a maximum of 40. Collectors then visited the simulated farm on a day drawn at random from this distribution, if the day chosen was earlier than the day of disease detection.

By running the stochastic transmission model over 5000 model iterations, where for each iteration a random value was drawn from the distribution representing each of the transmission model parameters, we estimated that the probability a collector will visit the farm in the course of an outbreak was 0.63 (i.e. the collector visited the farm on 63% of the 5000 simulated outbreaks). Hence, for 37% of outbreaks we would expect there to be insufficient birds for collection after the 25th day; the minimum value for the number of birds remaining on the farm to warrant a visit by collectors was assumed to be between 30 and 50 birds, and was incorporated into the model as a uniform distribution. The probability that a collector will visit the farm before the disease had been detected was 0.096 (9.6% of outbreaks), and 15.2% of all collector visitations were made before the disease had been detected.

Contamination of collectors and their equipment

In order to incorporate the impact of travelling time on virus concentration, we make the simplifying assumption that all collectors visiting the farm will travel to collecting yards in Bogor before moving to the next farm (*A15*). The empirical distribution describing travel times between farms in the District and Municipality of Bogor (t_f), was therefore updated to incorporate the Euclidean distance between each individual farm and the City of Bogor (t_b). By combining the distance to Bogor City for each individual farm with the distance to Bogor City for every other small-scale broiler farm, an empirical distribution describing total travel distance between farms, via Bogor city, was produced (t_{cy}). It is assumed that collectors will stop-over in Bogor for between 0.5 and 2 hours, and the variability in this estimate (t_{stop}) was modelled as a uniform distribution. Hence, by combining a randomly drawn value from the empirical distribution representing travel distance, travel speed, and stop over duration, the length of time between farm visits ($t_{collector}$) was estimated (Figure 144). The mean travelling time for collectors, incorporating a stop-over in Bogor, was 2.87 hours (5th percentile: 1.56, 95th percentile: 4.86).

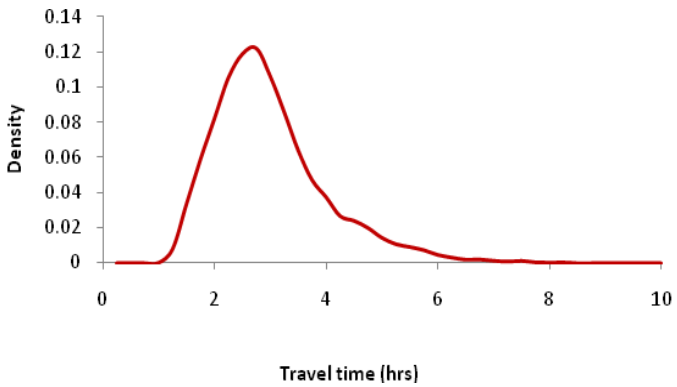

Figure 14: Variability distribution representing travel time between small-scale broiler farms, via Bogor city and incorporating a stop-over ($t_{collector}$).

Contamination of footwear

In order to predict the amount of virus present in faecal material contaminating the shoes of collectors upon arrival to a susceptible farm, we randomly selected values from the distribution representing C_{shoes} (i.e. viral contamination of shoes), and the empirical distribution representing t_{cy} and combined these with a fixed value drawn from the uncertainty distribution representing the rate of virus decay (k).

Faecal contamination of carry crates

The stress associated with transport may increase faecal production in poultry, and reduce the consistency of faeces (Mulder 1995). These factors make it highly likely that both birds and transport crates will receive potentially large amounts of faecal contamination en-route to the collecting yard (Buhr et al. 2000; Corry et al. 2002; Slader et al. 2002; Ramesh et al. 2003). This contamination, and the associated microbial risk, has been implicated in the infection of birds transported in crates previously contaminated by *Campylobacter* infected birds (Berrang et al. 2003; Hansson et al. 2005). Carrying crates bought onto a farm may also allow the transfer of infectious material, particularly if these are insufficiently cleaned and disinfected before entry (Ramabu et al. 2004).

Virus contamination of carry crates

We assume that each carry crate leaving an infected farm may contains up to 25 birds (*A16*). Crates are loaded onto a truck in 7 rows and 7 columns (hence a maximum of 49 crates, or 1225 birds), whilst a pick-up truck can contain 2 rows, each with 4 crates (hence a maximum of 8 crates, or 200 birds). The degree of viral contamination each crate receives will be determined by the number of infectious birds it contains, and on its relative position in the truck (Hartnett 2001). Given that collection crates are slatted, allowing the majority of faecal material to move away from birds, the crate on the bottom layer of a truck may be contaminated by faeces, and potentially virus, from birds in each of the 6 carriers above it.

The expected number of infected birds within each crate was determined using a simple binomial process in which we assumed the probability that each of the 25 birds entering the crate was infected was equal to the *wfp* at the time of collection. This was modelled as a set of separate scenarios, corresponding to the number of crates on the truck or pick-up truck (i.e. 49 or 8 separate crate contamination scenarios, respectively). In order to estimate the amount of faeces produced per crate, we combined an estimate of journey time to collecting yards in Bogor (t_b) with the

expected faecal output per unit time. Given that faecal output increases as a result of the stress of transport, we doubled the estimate of faecal output per day (F_{day}). We made the assumption that between 1 and 5% of the faecal material produced by chickens within a crate will remain in the crate, and this was modelled as a separate uniform distribution for each crate contamination scenario. The expected concentration of virus per crate was predicted by sampling from the distribution representing mean virus titre per gram ( ) and the faecal output per infected bird per crate. We allowed faeces and virus from the crates in the layers above to mix with the faecal material produced within each crate, hence a specific crate did not necessarily have to have infectious birds in it in order for it to receive viral contamination.

Hence, over 5000 iterations, random samples were drawn from the distributions representing faecal output per bird (F_{day}), travelling time to Bogor (t_b), the proportion of faecal output remaining in the crate, the number of birds that are infected (i.e. variable *wfp* from the stochastic transmission model), and the faecal titre per gram ( ). In order to account for the decay of virus as a result of travelling time, the resultant estimate of total virus contamination per crate was combined with an estimate of *k* (the virus transformation constant) and the travelling time between farms, via Bogor (t_{cy}). This was repeated over 50 simulations, where for each simulation a fixed value was selected from the uncertainty distribution for *k*. The full model is presented in the appendix.

Northcutt and Jones (2004), in the US, found that only 28.4% of the survey respondents washed and sanitized transportation cages and trucks between each use. The extent to which collectors in Bogor perform such activities is unknown, although virtually all collecting yards (94.9%) surveyed in the DKI Jakarta Province reported cleaning collecting crates on a routine basis (CIVAS 2007), although the nature of this routine, or the extent of the disinfection, is not clear. Hence, for the baseline scenario we will assume that cages are not washed between farm visits. The potential impact of collecting crate sanitization will be discussed in the exposure assessment.

Contamination of hands

Whilst collectors can expect to receive substantial amounts of faecal, and potentially viral, contamination of their hands as a result of handling birds, we do not consider the specific risks associated with contamination of collectors hands. Birds that are handled by collectors with contaminated hands will, as a matter of course, be removed from the farm on which they are handled. Hence, although the handling of susceptible a bird by collectors with contaminated hands may result in infection of that bird, its removal from the farm means such a transmission route is beyond the scope of this risk assessment.

3.4.2 Animal health providers

Poultry producers in Bogor make use of a variety of external service providers in the course of a single production cycle, some of which may enter the broiler house and therefore act as a potential route for the onward transmission of HPAIV H5N1.

In the event of an HPAI outbreak, and once mortality within the flock has increased beyond the presumed threshold level (ε), animal health providers, such as veterinarians or pharmaceutical technical officers, may be called to the farm in order to investigate the high mortality. Any person who enters the broiler house may receive contamination of their footwear, and potentially hands if birds are handled in the course of the investigation. Whilst such workers are undoubtedly aware of

the importance of sanitation and disinfection between farm visits, it is anecdotally reported that these measures are infrequently applied. In such circumstances, contamination of animal health workers investigating the early stages of an HPAI outbreak may constitute a route for the onward transmission of HPAIV H5N1.

Contamination of shoes

An estimate of the amount of virus expected to contaminate shoes was derived using the same approach as that for collectors, however, for each iteration of the simulated model, we assumed animal health workers would visit the farm 6 hours following detection (i.e. mid-way through the day following detection) (*A17*).

Contamination of hands

Those persons handling infected broilers may present a risk for the onward transmission of the virus if susceptible birds are subsequently handled on a non-infected farm. We combined the distributional estimates for H_f with estimates of to predict the total viral contamination of animal health workers hands at the point of leaving the farm on which the outbreak is occurring. Although the within-flock prevalence could be considered to influence the likelihood that a bird handled by a visitor is infected, we assume that any birds handled by animal health providers are those exhibiting clinical signs, and are therefore very likely to be infected on a farm on which an HPAI outbreak is occurring. Hence, the within-flock prevalence was not included in the assessment of hand contamination.

Considering both viral contamination of shoes and hands, we used values of t_f (travelling time between farms), rather than $t_{collector}$ (travelling time between farms, via Bogor and incorporating a stop-over) to determine the impact of virus decay (*k*) on the eventual virus concentration on shoes and hands arriving on a susceptible farm. Hence the output is a second-order distribution for the concentration of virus present on shoes ( ) and per cm^2 of contaminated hands ( ).

Release via fomites: summary

There is a very large number of potential fomites that may allow HPAIV H5N1 to be mechanically transmitted from infected to susceptible birds, or from an infected farm to a susceptible farm. This risk assessment considers two specific examples, namely the contamination of the shoes and equipment of collectors, and the contamination of shoes and hands of animal health workers. The probability of infection given exposure to the virus released via these pathways is considered in part B, the exposure assessment.

4 Release assessment: Transfer of HPAIV H5N1 via water

Water-borne transmission is likely to play an important part in the maintenance of avian influenza viruses (**AIV**) in free-living waterfowl populations (Breban et al. 2009; Roche et al. 2009; Rohani et al. 2009). The water-borne route has also been identified as a source of infection during LPAI outbreaks in domestic ducks (Halvorson et al. 1985; Markwell and Shortridge 1982) and turkeys (Halvorson et al. 1985; Sivanandan et al. 1991; Laudert et al. 1993). In each case, it is likely that the virus originated from faecal contamination of water sources by wild birds. However, and as well as contamination of water by wild birds, there is a risk that waste products from poultry farms may spread to water bodies via surface run-off or enter groundwater through inadequate disposal of waste products on poultry farms (WHO 2006). The potential routes for contamination of rivers (or other surface water bodies) and ground water are presented in Figure 1515.

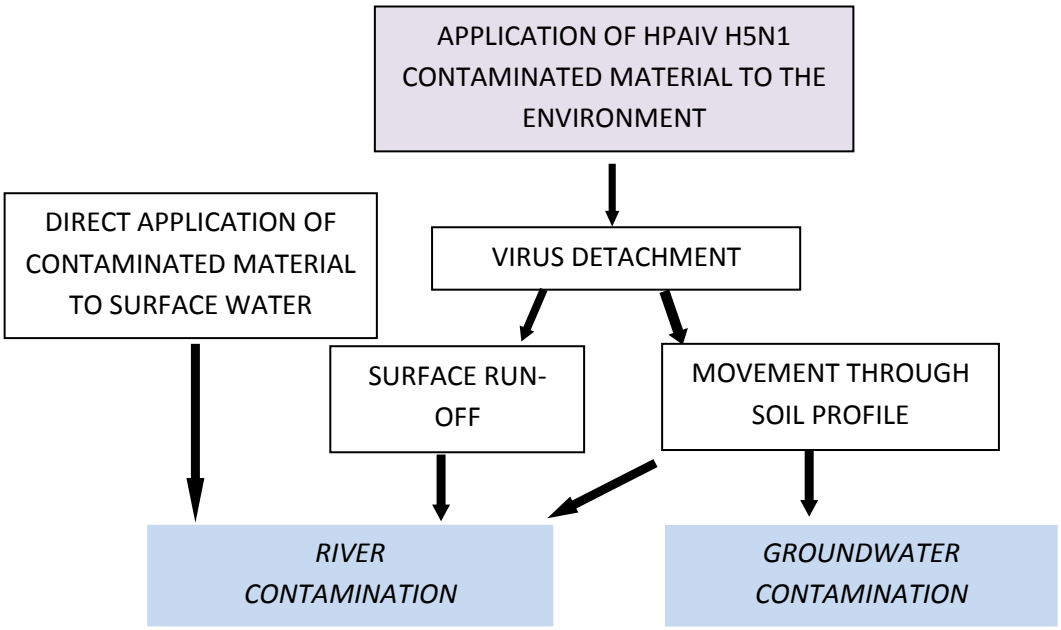

Figure 15: Pathways for the transmission of H5N1 to water.

In a survey conducted in the 9 districts surrounding Jakarta, 4.9% of broiler producers reported throwing dead birds directly into a river (FAO 2008b). Given that around 4% of households in Indonesia rely on rivers as their main water supply (RandCorporation 2007), direct deposition of contaminated material into surface water may constitute a serious risk to poultry flocks downstream, as well as being of public health concern (de Jong et al. 2005).

The potential for virus movement through the soil profile and subsequent groundwater contamination with HPAIV H5N1 is, to our knowledge, entirely unknown. Historically, groundwater contamination has been responsible for the majority of waterborne diseases in people, including viral infections (Beller et al. 1997; Brassard et al. 2005; de Roda Husman and Bartram 2007) and the use of untreated groundwater for domestic poultry may therefore constitute a potential route for the transmission of HPAIV H5N1 between flocks. The movement of infectious organisms through the

soil profile depends on a variety of physical factors, including rainfall, temperature, soil structure, and pH (Sobsey et al. 1986). Moreover, virus specific factors, such as capsid diameter and isoelectric point, may have a large influence (Dowd et al. 1998).

In order to assess the risks posed by transmission via water, we considered the specific scenario of direct deposition of poultry carcasses contaminated by HPAIV H5N1 into a water course. Given the current lack of information, the potential for groundwater contamination will not be considered further in this risk assessment.

4.1 Virus contamination of river water following deposition of carcasses

Titres of HPAIV H5N1 in chicken thigh and breast meat have been reported to be in the range $10^{6.8}$–$10^{8.0}$ and $10^{5.5}$–$10^{7.9}$EID/g, respectively (Swayne and Beck 2005; Swayne et al. 2006). Based on these estimates, a 1 kg poultry carcass might be expected to contain up to $10^{8.3}$ to $10^{10.3}$ EID_{50} of HPAIV H5N1 in skeletal muscle alone. Infection of chickens with AIV results in invasion of the blood, brain, bone marrow, heart, spleen, and lungs, as well as skeletal muscle (Lee et al. 2005), hence contaminated carcasses can potentially contain even greater quantities of virus. However, the degree to which virus particles are able to detach from a carcass when in river water is unknown. It is reasonable to assume that the minimum amount of virus leaching from a contaminated carcasses will be equivalent to the amount present within the cloaca and respiratory tract at the time of the bird's death, although it may be considerably more. Hence, we assumed that the amount of faecal and respiratory secretions available for contamination of a river was equivalent to one day's faecal and respiratory output per bird, and assumed that this is leached over the course of a single day (*A18)*. Hence, those distributions representing faecal and respiratory output per day (F_{day}, R_{day}) were combined with estimates of virus titre per gram of faeces ( ) and per millilitre of respiratory secretion ( ). In order to account for the expected delay between a bird's death from HPAI H5N1 and it entering the river, we combined these estimates with fixed values from the uncertainty distribution representing virus decay in faeces (i.e. the distribution for *k*; we assumed that the rate of virus decay in carcasses is equivalent to decay in faeces (*A19*), where time between death and entering the river was modelled as uniform distribution between 1 and 12 hours. Hence, the output was a second-order distribution for the amount of virus that would be expected to leach per carcass entering the river, μ_c.

4.1.1 Inactivation of HPAIV H5N1 in water

Webster (1978) showed that AIV were able to survive for over 32 days in river water at 4°C, but were undetectable after 7 days at 22°C. The impact of temperature on virus survival in river water has been further demonstrated by a variety of authors, and is summarized in Table 8.

Table 8. Inactivation rates of AIV in water at different temperatures. HPAIV H5N1 are highlighted in bold

Avian Influenza Virus	Experimental conditions	Temp. (°C)	Inactivation rate, μ (per day)	Estimated persistence (days)	Reference
AIV H6N2 (Chicken)	Reverse osmosis water (pH 8.2)	4	0.02	184 – 696	(Graiver et al. 2009)
		21	0.05	78–136	
		37	0.10	43 – 56	
MN/98 H5N2 (Mallard)	Distilled water (pH 7.4)	17	0.014	429	(Brown et al. 2009)
MN/00 H5N3 (Mallard)			0.019	316	
NJ/01 H5N7 (Turnstone)			0.026	231	
NJ/01 H5N8 (Turnstone)			0.021	286	
MN/98 H7N3 (Mallard)			0.028	214	
TX/02 H7N4 (Teal)			0.034	176	
DE/00 H7N3 (Gull)			0.009	667	
DE/02 H7N3 (Turnstone)			0.031	194	
Mongolia/05 H5N1 (Swan)			0.038	158	
Anyang/01 H5N1 (Duck)			0.064	94	
MN/98 H5N2 (Mallard)	Distilled water (pH 7.4)	28	0.051	118	
MN/00 H5N3 (Mallard)			0.071	85	
NJ/01 H5N7 (Turnstone)			0.114	53	
NJ/01 H5N8 (Turnstone)			0.167	36	
MN/98 H7N3 (Mallard)			0.086	74	
TX/02 H7N4 (Teal)			0.100	60	
DE/00 H7N3 (Gull)			0.090	67	
DE/02 H7N3 (Turnstone)			0.252	24	
Mongolia/05 H5N1 (Swan)			0.228	26	

Anyang/01 H5N1 (Duck)			0.203	30	
H6N2 (Mottled Duck)	Distilled water	17	0.11	54	(Stallknecht et al. 1990a)
	(pH 7.2)	28	0.13	46	
H3N8 (Gadwall)	Distilled water	17	0.031	194	(Stallknecht et al. 1990b)
H4N6 (Teal)	(pH 7.3)		0.028	214	
H6N2 (Mottled duck)			0.028	214	
H12N5 (Teal)			0.048	125	
H10N7 (Teal)			0.41	15	
H3N8 (Gadwall)	Distilled water	28	0.092	65	
H4N6 (Teal)	(pH 7.3)		0.075	80	
H6N2 (Mottled duck)			0.065	92	
H12N5 (Teal)			0.197	30	

In order to estimate the temperature and pH of rivers in Bogor District, we extracted historical data for these parameters for a variety of rivers within 150 kilometres of Kabupaten Bogor from the Gemstat database (http://www.gemstat.org/queryrgn.aspx) (Table 9). The inactivation rate constant in water, π, based on all virus strains in which the experiment was conducted at 28°C (Table 8) was found to be approximately log-normally distributed, with best fitting parameters of $\gamma = 0.043$, $\beta = 0.064$.

Brown et al. (2009) demonstrated that factors such as pH and salinity may also impact on virus stability, with AI viruses being most stable at low temperatures (<17°C) combined with a slightly basic pH (7.4–8.2), and fresh to brackish salinities (0–20,000 ppm) and had a much shorter persistence in warmer temperatures (>32°C) combined with acidic conditions (pH<6.6, >32°C and high salinity (>25,000 ppm). These findings were supported by earlier work by Slemon et al. (1978) who showed that AIV from ducks survived longest at 17°C, with low levels of salinity and a pH of 8.2 as well as the potential impacts of abiotic factors, Table 8 demonstrates that there may be quite substantial variability in survival between AIV strains.

4.1.2 Dilution of virus in river water

The approach used by Schijven et al. (2005a; 2005b) was used to simulate three generalized dilution scenarios for infectious particles in river water, considering a ‘small’, ‘medium’ and ‘large’ sized river (Table 10).

Table 9: Water temperature and pH for a selection of rivers within 150 km of Kab. Bogor

River	Date of sampling	Mean pH (s.d.)	Mean temp. °C (s.d.)	Distance from Kab. Bogor (km)*
Sunter	1985 – 1994	7.04 (0.30)	28.81 (1.25)	43
Ciliwung	1979 – 1981	7.30 (0.36)	28.16 (1.13)	33
Citarum - at Tanjungpura	1979 – 1981	7.63 (0.34)	27.76 (1.15)	60
Citarum - at Nanjung	1985 – 1994	7.31 (0.41)	25.42 (1.30)	100
Cimanuk	1979 – 1981	8.00 (0.35)	27.19 (1.48)	149

*Measured from recording station to the approximate centre of Kab. Bogor (6°30'32.34"S, 106°48'34.72"E)

Source: Gemstat (2010)

Table 10: Dimensions of rivers employed in the dilution scenarios

Size	F_r (m^3/day)†	Width (m)	Depth (m)	L_r(m)‡
Small	8.6×10^4	10	1.5	5.8×10^3
Medium	2.2×10^6	50	2.6	1.7×10^4
Large	2.3×10^7	125	3.8	4.8×10^4

†Flow rate

‡Characteristic length (1 day flow) of a river

Source: Schijven et al. (2005a, 2005b)

We assume that there is complete mixing of leached faecal and respiratory secretions, and complete detachment of virus allowing a relatively homogeneous suspension of virus contaminated river water (*A20*) (Schijven et al. 2005a).

We considered a single contamination scenario, in which a number of carcasses representing between 1 and 2% of the total flock size (i.e. the detection threshold for infection within a flock of broilers, ε) were discarded into either a 'small', 'medium' or 'large' sized river. Hence, estimation of the concentration of virus in surface water (C_r) was estimated as:

$$C_r = \text{———}$$

Where n is an estimate drawn at random from the empirical distribution describing small-scale broiler flock size in Bogor and is the flow rate for each of the three dilution scenarios (table 10). We considered the risk over a single day, hence was fixed at 1.

Estimates for the rate of virus decay in water (π) were considered to be uncertain. Hence, second-order estimates of the range of possible values of C_r were derived by running 5000 iterations where random values were drawn from the distributions representing the number of carcasses entering the river ( ) and amount of virus expected to leach per carcass, μ_c. This was repeated over 50 simulations, each simulation using a random value selected from the distributions representing π (and k). The resultant summarized distributional estimates of C_r are presented in Table 11: the estimates are presented as ranges given the uncertainty in both k and π.

Table 11: Estimates for concentration of virus particles (EID_{50}) per litre of river water based on three dilution scenarios

	Mean concentration (C_r) (virus/litre)	***5th percentile***	***50th percentile***	***95th Percentile***
Small	$1.1x10^{1}$–$1.7x10^{1}$	$2.5x10^{-2}$–$3.6x10^{-2}$	$2.7x10^{-1}$–$3.5x10^{-1}$	$4.0x10^{1}$– $5.2x10^{1}$
Medium	$4.2x10^{-2}$–$6.8x10^{-2}$	$9.7x10^{-4}$–$1.4x10^{-3}$	$1.1x10^{-2}$–$1.4x10^{-2}$	$1.6x10^{-1}$–$2.0x10^{-1}$
Large	$4.0x10^{-3}$–$6.5x10^{-3}$	$9.3x10^{-5}$–$1.3x10^{-4}$	$1.0x10^{-3}$–$1.3x10^{-3}$	$1.5x10^{-2}$–$2.0x10^{-2}$

Source: Schijven et al. (2005a)

The probability of infection in poultry given exposure to the predicted virus concentrations in river water is discussed in the exposure assessment.

5 Release assessment: Transfer of HPAIV H5N1 via live poultry

Typically, the only live birds that are brought onto a broiler farm are day old chicks direct from a breeder farm or via a middle-man supplier. Hence, the movement of infected broilers between farms is considered to be a negligible route for the transmission of HPAIV H5N1 in Bogor District and Municipality (Idris et al. 2010). The movement of free-ranging poultry between broiler farms, or between small-scale broiler and backyard producers, may, however, provide a potential route for the transmission of the virus. Based on the Indonesian Family Life survey (RandCorporation 2007), 47.7% of Indonesian households owned at least one poultry species as part of a sector 4 production system (i.e. a backyard flock). DGLS surveillance data reveal a wider range of estimates by *desa* [village]; in 25.8% of *desas* in Bogor District and Municipality, less than 25% of households own poultry, whilst in 51% of *desas* there is between 26 and 75% household poultry ownership, and in 23.5% of *desa*, more than 75% of households keep backyard poultry. In addition to poultry ownership being common, the majority of households are expected to allow their poultry to roam freely. Data from the Regency of Yogyakarta (approx. 400 km from Bogor) revealed that around 58.9% of households keeping poultry did not confine birds during the day (95% C.I. 57.4–63.3) (International Food Policy Research Institute [**IFPRI**] 2009).

5.1 Predicting contact between free-ranging poultry and broilers

The likelihood that a flock of free-ranging poultry will come into direct contact with broiler chickens, or, more likely, with their environment (i.e. foraging around the broiler house), will depend on the proximity of the flock to a broiler farm.

Whilst there would appear to be few data regarding the ranging behaviour of unconfined domestic poultry, Collias and Collias (1996) investigated the behaviour of groups of free-ranging red jungle fowl (*Gallus gallus*), the progenitor of the Indonesian Kampong chicken, in San Diego Zoo. The study revealed that these birds were confined to relatively small home ranges, ranging from approximately 5000 m^2 to 12,000 m^2. In the absence of quantitative data from domestic poultry species, the extent of the home ranges from Collias and Collias (1996) was used to predict the number of households from which free-ranging chickens might enter the boundary of small-scale broiler farms in Bogor.

Estimating contact rates per day

We used Google Earth (Google Inc., Mountain View, CA) to estimate the number and geographic location of all households within a distance of approximately 120 metres from a random selection of 50 small-scale broiler farms in Bogor District. This distance was chosen to represent the maximum diameter of the home ranges of free-ranging red jungle fowl from the Collias and Collias (1996) study. The Euclidean distance of each of the identified households to its respective broiler farm was estimated using the Spatial Analyst extension for ArcMap 9.3 (ESRI, Redlands, CA), and the output exported into @Risk for risk modelling. Here, distances were converted to a linear scale between 0 and 1 using the maximum score procedure, so that:

Where represents the Euclidean distance of each household from its nearest broiler farm, and was fixed at 120 metres, the maximum presumed ranging distance of backyard poultry. In order to account for the variability in the predicted ranging behaviour of poultry, the maximum score procedure was repeated for each household using an of 40 metres, the minimum diameter of home ranges from the Collias and Collias (1996) study.

We considered that a uniform distribution, defined by the upper and lower estimates from the maximum score procedure, represented the variability in the probability that a flock of chickens owned by a household would enter the environment of the broiler farm in the course of a single day ( ) (*A21*). Based on this assumption, those households that were closest to the broiler farm had the highest probability of entering the farm, decreasing linearly to a probability of 0 at a distance of 120 metres.

Only a proportion of households within the 120 metre buffer of each broiler farm could be expected to own poultry. This proportion, , was modelled using a Betapert distribution, with the most likely value taken as the proportion of households with poultry from the Indonesian Family Life survey (i.e. 48%) (RandCorporation 2007). We allowed this distribution to range from 1 to 100%, as indicated by the wide variation in DGLS estimates. The proportion of households that allowed their poultry to range freely during the day ( ) was estimated based on empirical data from the IFPRI survey in Yogyakarta (2009). This survey involved questioning between 2 and 69 people from 72 *desa*s from the Yogyakarta Regency on a variety of poultry related issues. We characterized the uncertainty around estimates of the proportion of households allowing poultry to range freely from each of the 72 *desa* using a Beta distribution, and used a parametric bootstrap to generate the uncertainty about the true value of .

By running a simple Binomial process with 300 iterations, we estimated the number of flocks in the buffer surrounding each of the 50 randomly selected broiler farms that would be expected to enter the farm boundary in the course of a single day. For each iteration, entry onto the broiler farm for each household was defined as 1 or 0 by the sum of separate Bernoulli processes where the probability of success for each process was defined by , and . By totalling the number of 'successes' for each iteration, the output over all iterations was thus an estimate of the range of possible values for the number of household flocks that could be expected to enter the environment of a broiler farm in the course of a single day, N_{hh}.

This estimation was run as a second-order model, where for each of the 300 iterations, a random value was drawn from the variability distribution for and fixed values selected from the uncertainty distributions for and . This process was repeated over 1000 simulations for each of the 50 randomly selected broiler farms in Bogor District.

Model output

The range of values of N_{hh} for each of the 50 randomly selected farms is shown in Figure 16, and the range of predicted values from the second-order model is presented in Table 12.

Table 12: Estimates of the number of free-ranging household flocks that are expected to enter the environment of a broiler farm in the course of a single day

	Mean	5th Percentile	50th Percentile	95th Percentile
N_{hh}	0.017 – 6.83	0 – 1	0 – 6	0 – 14

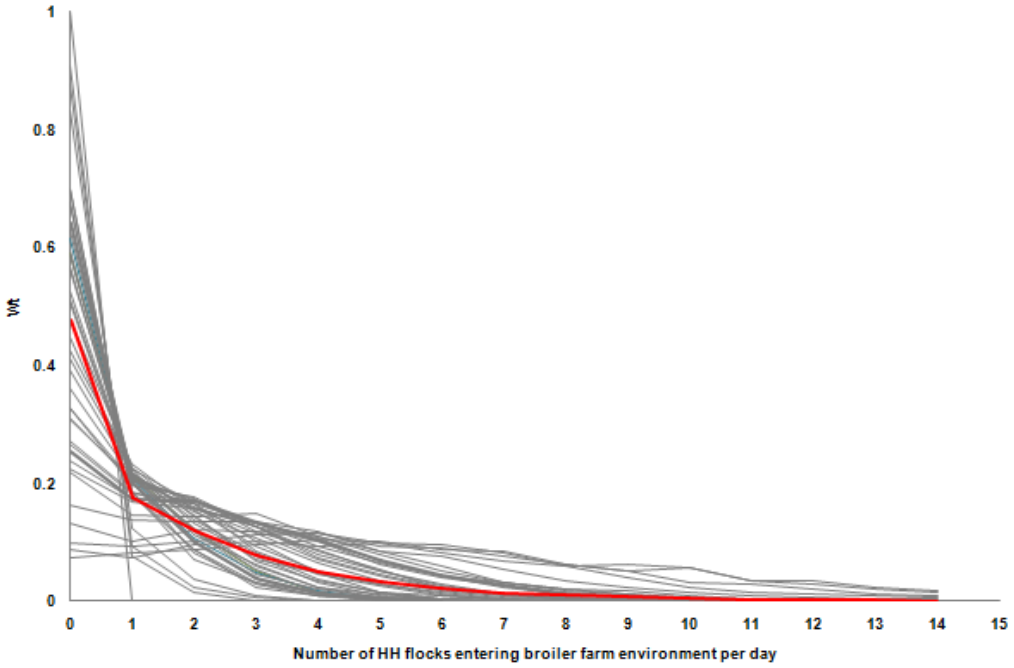

Figure 16: Density distribution for the predicted number of free-ranging chicken flocks that may enter the environment of 50 randomly selcted broiler farms per day. The red line representes the average for all 50 farms.

The mean value for all simulations is 2.3 flocks entering the broiler farm per day, with a 5th percentile for all simulations of 0, a 95th percentile of 7 and a median value of 2. It should be noted that the model structure is based around the very large assumption that Kampong chickens will show similar ranging behaviour to semi-domesticated jungle fowl (*A22*). Moreover, we assume that free-ranging poultry do not show specific territorial behaviour, i.e. that the ranges of birds from different households may overlap and therefore birds from more than one household may include a broiler farm within their home-range (*A23*). Whilst this assumption would seem reasonable given the high density of free-range birds in this part of Indonesia, territoriality in domestic fowl has been demonstrated in some studies (McBride and Foenander 1962; Craig and Guhl 1969), but not others (Hughes et al. 1974; Preston and Murphy 1989). The estimate is based on the additional assumption that there are not fences surrounding the perimeter of the broiler farm that would be adequate to prevent the entry of free-ranging chickens (*A24*). This is reportedly common in Bogor, but additional

survey based studies are required to fully assess the degree to which free-ranging poultry can enter the boundary of a broiler farm.

Release via live poultry: summary

Having estimated N_{hh}, the predicted contact rate can be combined with predictions of the probability of infection given contact in order to estimate the number of backyard flocks that might be expected to become infected through direct contact during an HPAI H5N1 outbreak on a small-scale broiler farm.

6 Exposure assessment: Dose-response models

Dose-response modelling involves the development of a mathematical relationship between an exposure (i.e. the dose) and a measure of effect (i.e. the response), and is a key component in quantitative microbial risk assessments (Haas et al. 1999).

Estimation of the dose-response relationship relies on the results of experiments in which animals are inoculated with a defined dose of infectious agent and a response, such as death, recorded as evidence of infection at that dose. By applying such experiments to samples of animals, the probability of becoming infected as a result of exposure to a particular dose can be ascertained. To our knowledge, a dose-response relationship has not yet been determined for HPAIV H5N1 in chickens, although several authors report a presumed 'minimum' infectious dose (as a BID_{50}, i.e. the dose that results in infection of 50% of birds) (Table 13). Such estimates rely on the traditionally held assumption that a 'threshold' of infectious particles is required in order for infection to occur, and therefore that exposure to doses below this threshold will not result in infection. The use of this hard classification has been challenged by other authors, who suggest that 'single-hit' models provide a better explanation of the data (Sutmoller and Vose 1997; Buchanan et al. 2000; French et al. 2002; Teunis et al. 2004). Single hit models describe a scenario in which each infectious particle has an explicit probability of causing infection. Two non-threshold models, the exponential and beta-Poisson, are considered to be particularly suitable for dose-response modelling of infectious agents (Haas et al. 1999). The exponential dose-response equation is derived by assuming the dose is Poisson distributed and that the probability of each organism actually ingested causing illness/infection is binomial. It is given by the equation:

where *r* is the probability of infection by a single virus and D is dose, so that *Pi* is the probability of infection of an individual that was exposed to the average dose *D*.

The beta-Poisson model is approximated by:

Where N_{50} is the median dose to give the response and α is the exponential fitting parameter.

Table 13: Estimated minimum infectious doses (BID50) for a variety of HPAIV H5N1 viruses

Virus	Host	BID_{50} (EID_{50} log_{10})	Source
A/chicken/Scotland/1959 H5N1	Chicken	2.6	(Swayne and Slemons 2008)
A/turkey/England/50–92/1991 H5N1	Chicken	3.9	(Swayne and Slemons 2008)
A/chicken/Korea/ES/2003 H5N1	Chicken	2.5 – 3.1	(Swayne and Beck 2005)
A/chicken/Yamaguchi/7/2004 H5N1	Chicken	2.5	(Tsukamoto et al. 2007)
A/Whooper Swan/Mongolia/7/2005 H5N1	Chicken	2.8	(Brown et al. 2007)
A/chicken/Miyazaki/K11/2007 H5N1	Chicken	2.5	((Yamaguch, 2007) cited in Swayne and Slemons 2008)
A/Hong Kong/486/1997 H5N1	Chicken	2.4	(Swayne and Slemons 2008)
A/Whooper Swan/Mongolia/7/2005 H5N1	Wood duck	0.95	(Brown et al. 2007)

6.1 Identification of data for dose-response modelling

In general, susceptibility, infectivity, and transmissibility data for poultry are minimal for most of the HPAIV (Swayne and Slemons 2008). Several studies have investigated the response of birds to a fixed dose of HPAIV H5N1 in order to develop vaccines or to study pathology in infected birds (Swayne and Beck 2005, Swayne and Slemons 2008). Relatively few of these studies have investigated the effect of variable doses on the likelihood of disease in inoculated birds, and of those that have the majority only report the minimum infectious dose (e.g. Table 13).

In order to be suitable for dose-response modelling, data should demonstrate a general monotonic relationship (i.e. increasing response with increasing dose) and give a response other than 0 and 100% at intermediate dose groups (Haas et al. 1999). Based on these criteria, two studies involving chickens were identified (Brown et al. 2007; Bublot et al. 2007b) (Table 14).

Table 14. Data used to parameterize the dose-response model (Both studies involved chickens inoculated intra-nasally with HPAIV H5N1).

Study	Strain	Dose (log10 EID_{50})	Number tested	Number died
Bublot et al. 2007b	A/chicken/SouthKorea/ES/03	0.5	10	0
		2.0	10	0
		3.5	10	8
		5.0	10	10
		6.5	10	10
		8.0	10	10
Brown et al. 2007	A/WhooperSwan/Mongolia/244/05	1.0	5	0
		3.0	5	3
		5.0	5	5

6.2 Fitting the dose-response model

The data from Brown et al. (2007) and Bublot et al. (2007b) were fit with the one-parameter exponential model and two-parameter Beta-Poisson model using the open-source statistical package, R. The dose-response model parameters were estimated using a maximum likelihood estimation (MLE) procedure, with the BFGS algorithm used for nonlinear minimization (Bartrand et al. 2008). Models were considered to exhibit adequate fit if the minimized deviance was less than the 95% confidence value for the χ^2 distribution with degrees of freedom equal to the number of doses tested minus the number of parameters (Haas et al. 1999) (Table 15).

Table 15. Dose-response parameter estimations and model fit

Source	Model	Parameters	Minimized Deviance	Model fit (*p*-value)
Brown et al. 2007	Exponential	r = 0.0009	0.09	0.96
	Beta- Poisson	α = 72.6, N_{50} = 767.2	0.092	0.76
Bublot et al. 2007b	Exponential	r = 0.0005	1.01	0.96
	Beta- Poisson	α = 663, N_{50} = 1470.9	1.011	0.91

In both cases, the exponential model gave the best fit. In order to determine whether the two data sets could be pooled, the summed minimized deviances from the two individual models were compared with those from the pooled model. Pooling was considered acceptable since the difference between the models was less than the 95% confidence value for the χ^2 distribution at degrees of freedom equal to the sum of the number of parameters used in fitting individual data sets minus the number of parameters used in fitting the pooled data set (Table 16) (Haas et al. 1999).

Table 16. Best fit model for pooled data

Source	Model	Parameters	Minimized Deviance	Model fit (*p*-value)
Pooled (Brown et al. 2007; Bublot et al. 2007b)	Exponential	r = 0.00051	0.23	0.63

6.3 Estimating uncertainty around dose-response estimate

Confidence intervals for the best fitting model were determined using the bootstrapping method described by Haas et al. (1999). For this, bootstrap replicates were generated by random sampling from a binomial distribution at each dose within the pooled dose response data set. The exponential model was then fitted to every re-sampled set to generate a distribution of maximum likelihood parameters. The 2.5th and 97.5th percentiles were extracted from the resultant distribution following 5000 model iterations. The resultant dose response curve is shown in Figure 177.

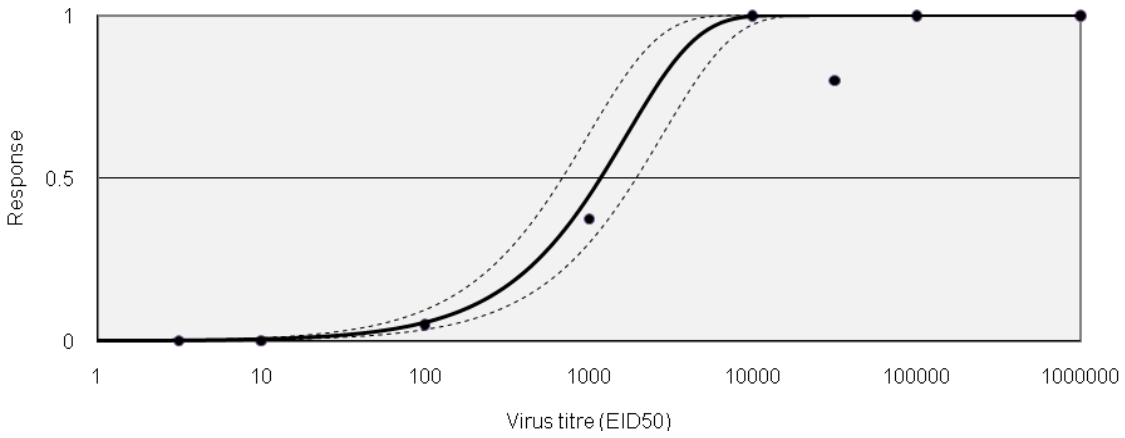

Figure 17: Dose response curve for the pooled data of Brown et al. (2007) and Bublot et al. (2007b).

7 Exposure assessment: Exposure following contact with fomites

Clearly, infectious virus particles must detach from a contaminated fomite in order to pose a transmission risk. An estimation of the amount of virus transferred from contaminated fomites arriving on a susceptible farm, and the resultant 'dose' in exposed birds, can be combined with the estimate of *r* from the dose-response model to predict the probability of infection following exposure.

The rate of virus transfer depends on the adherence properties of the organism, and on environmental conditions (Haas et al. 1999). Estimates of transfer rates for viruses in general are somewhat lacking (Rheinbaben et al. 2000), and, to our knowledge, are not available for AIV. Moreover, those estimates that are available for other viruses relate to transfer from fomite to hand, hand to fomite, or more rarely, fomite to fomite. It is therefore necessary to extrapolate from such studies to estimate a 'fomite to chicken' transfer rate for HPAIV H5N1. Examples of the expected efficiency of transfer for a variety of viruses are presented in Table 17. The range of transfer rates is wide and there appears to be substantial differences between viruses, although some of this variability may be associated with differences in experimental conditions. It is anticipated that the effectiveness of virus transfer will be highly variable, however, and in the absence of specific data on the efficiency of transfer of AIV, the true extent of this variability is uncertain. In an attempt to incorporate the range of possible values for virus transfer, and in particular to model the transfer of HPAIV H5N1 from contaminated hands, we assume transmission efficiencies may range anywhere between 0 and 22% (i.e. the maximum from the Ansari et al. (1988) study, and model transfer efficiency (*TE*) as a uniform distribution.

Table 17: Efficiency of transfer of virus from fomites

Virus	Experimental conditions[1]	Transfer	Proportion recovered (s.d.)	n	Source
Rhinovirus	Wet mucous	Hand → Hand	9.73 (± 9.2)	6	(Pancic et al. 1980)
		Hand → Surface → Hand	5.6 (± 3.3)	8	
Parainfluenza virus 3	Dry mucous	Hand → Surface	0.92 (± 0.3)	3	(Ansari et al. 1991)
		Surface → Hand	0.67 (± 0.1)	3	
		Hand → Hand	0.71 (± 0.2)	3	
Rotavirus	Air dried inoculum	Hand → Surface	16.1 (± 5.4)	5	(Ansari et al. 1988)
		Surface → Hand	16.8 (± 5.2)	5	
		Hand → Hand	6.6 (± 2.1)	5	
Calicivirus	Air dried inoculum	Hand → Surface	13.0 (± 3.6)		(Bidawid et al. 2004)

[1] Describing the state of the contaminating virus on the fomite

7.1 Risks associated with contaminated hands

Hands that have been contaminated with HPAIV H5N1 may transfer the virus to the feathers of poultry when these are handled. Broilers of a wide range of ages have been observed to spend approximately 10% of their day engaged in preening (Kubikova et al. 2001; Weeks et al. 2000). Such behaviour is intended to maintain feather condition, and to remove contaminating material. Indeed, Caldwell et al. (2001) showed that day old chicks sprayed with a fluroscein dye ingested between 0.005 and 0.022% of the applied fluroscein dye after only 1 minute preening activity, with chicks preening themselves between 1 and 15 times during that time. Hence, if virus is transferred to feathers, it is very likely that birds may ingest a proportion of the contaminating virus through preening.

The average surface area of a 1 kg bird is 1000 cm^2 (Mitchell 1930; Leighton et al. 1966). The average palm surface area, and therefore the area that may come into contact with a broiler, is estimated to be 224 cm^2 (Lee et al. 2007). Hence, a person handling a 1 kg bird may contact at least 450 cm^2, or around 45% of the bird's surface during the handling process. Persons handling birds by the legs (e.g. collectors) will contact a considerably smaller surface area.

7.1.1 Probability of infection following handling

In order to predict the probability of infection following HPAIV H5N1 contamination, we developed a simple stochastic model of exposure. The model describes the situation in which an individual bird is handled by a animal health worker who had previously handled infected birds on a broiler farm on which an outbreak is occurring, received viral contamination on their hands and travelled to the susceptible farm without performing sanitary measures. We assume that handling the susceptible bird results in contamination over a fixed area of 450 cm^2 of the bird's 1000 cm^2 area, with the degree of contamination of each cm^2 defined by a random value drawn from the second-order distribution describing viral contamination of hands (V_h) and *TE* (i.e. the distribution representing the efficiency of transfer). For this, we modelled the surface area of a chicken as a matrix of 1000 cells, 45% of which receive variable levels of virus contamination (i.e. combining random values of V_h and *TE*), and 65% receive zero contamination. This process is repeated over 3000 matrices, the result being a variability distribution describing 3000 contamination scenarios, representing 3000 contaminated 'birds' following handling.

Virus exposure was modelled by simulating intermittent preening activities for each of the 3000 contamination scenarios. We assumed that a bird may preen itself at least once every 10 minutes (*A25*), and incorporated the variability in the interval between preening as a uniform distribution between 0 and 10 minutes. For each preening 'event', the simulated bird preens over a randomly selected 1 cm^2 area, and may ingest between 0 and 100% of any virus present, with the proportion ingested (*Pi*) defined by a random value selected from a uniform distribution for each preening event. The virus remaining in the specific cell of the matrix that has been 'preened' is therefore (1 - *Pi*). This process is repeated over multiple iterations for each of the 3000 contamination scenarios.

In order to account for the decay of virus on the surface of the bird (and therefore decreasing risk over time), the concentration of virus remaining within each cell of the matrix following each preening event (V_t) is defined as:

=

where the value of *t* is defined by the time between preening, and *k* is a fixed value of virus decay derived from the uncertainty distribution.

We consider each 'preening' exposure as independent, and the probability of infection per exposure as:

Where a fixed value for r is drawn from its uncertainty distribution and D relates to the dose the bird ingests for each random preening event. The simulation is run over multiple iterations, and the probability of infection for each contamination scenario after each step is given as:

Each simulation was run until the mean probability of infection for all scenarios stabilized (i.e. at the point at which contaminating virus on the surface of the chicken had been entirely diminished through the combined effects of consumption and virus decay; this was typically after 2 simulated days).

The process was repeated over 25 simulations, with a random value derived from the uncertainty distributions representing virus transformation (*k*), and the dose response parameter (*r*) at the start of each simulation and incorporated into each iteration as a fixed value for each of the 3000 contamination scenarios.

The model was implemented using Visual Basic for Applications (VBA, Microsoft Corp.) in Microsoft Excel. A summary of the parameters used in the model is presented in Table 18 and the model structure is presented in the appendix (Figure 1A).

Table 18: Summary of model parameters used to predict the risk of infection for a broiler chicken handled by someone with HPAIV contaminated hands

Parameter	Description	Distribution/value	Classification[1]
V_h	Virus contamination per cm^2 hand	Second-order distribution (see text)	V + U
P_{area}	Palm surface area (cm^2)	224	F
C_{area}	Chicken surface area (cm^2)	1000	F
TE	Transfer efficiency through handling	Uniform(0,0.22)	V(+U)
t	Time between preening 'events'	Uniform(0, 10)	V
Pi	Proportion of virus consumed per preening events	Uniform(0, 1)	V
k	Virus inactivation rate	Uniform(0.1604, 0.1972)	U
r	Exponential dose response parameter	Bootstrap replicates (see text)	U

[1]V = variability, U = uncertainty, F= Fixed values

Model outputs

The model predictions for a 1 kg broiler, handled by someone who has previously handled an infected bird on a farm on which an outbreak is occurring and who has not applied sanitary measures, are presented in Table 19. The cumulative second-order distribution for the probability of infection is presented in Figure 18 18. Given the uncertainty in both the rate of virus decay and the dose-response values, the summaries provided in Table 19 and presented in Figure 18 are the range of values derived from 25 simulations, each composed of 3000 contamination and exposure scenarios.

Table 19: Probability of infection of a single chicken following handling by someone with HPAIV H5N1 contaminated hands

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Handling	$2.9x10^{-2} – 9.5x10^{-2}$	$2.0x10^{-3} – 7.6x10^{-3}$	$1.5x10^{-2} – 5.3x10^{-2}$	$1.0x10^{-1} – 3.4x10^{-1}$

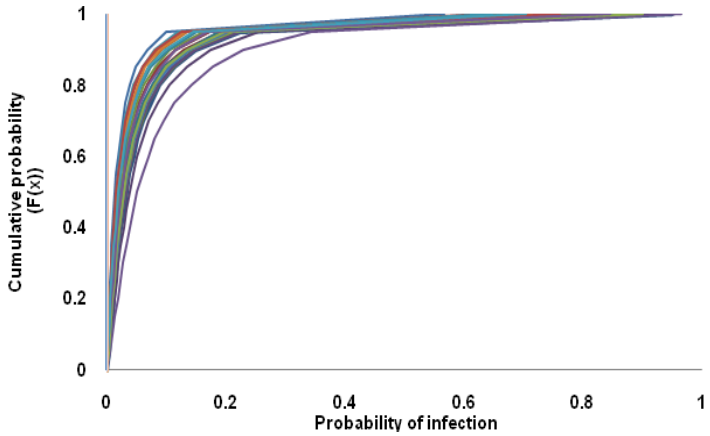

Figure 18: Cumulative second-order distribution for the risk of infection of a 1 kg chicken following handling by someone with HPAIV H5N1 contaminated hands.

The model output indicates that on 95 times out of 100, the probability of infection of a bird handled by someone with contaminated hands will be less than 10 to 34%, and that that median estimate is between 2 and 5%. The mean value for all 25 simulations, each comprising 3000 different contamination scenarios is 5%. That is, on average, 5 out of every 100 birds that are handled by someone who has previously handled an infected bird and travelled to a susceptible farm might be expected to become infected, but this probability ranges from 2.9–9.5%. It should be noted that these estimates relate to the first bird handled; the risks associated with handling will decrease as more birds are handled and the virus concentration on contaminated hands decreases. Broilers are highly stress sensitive, and therefore handling of these birds is typically kept to a minimum until the end of the production cycle. Hence, whilst the risks associated with handling are unlikely to be negligible, the estimates do not take into account the frequency of handling.

Moreover, in the absence of estimates regarding viral contamination of hands, the estimation of risk relies on a large assumption regarding the concentration of virus that could be expected on hands as a result of handling infected birds. Furthermore, whilst the estimates incorporate the predicted range of travelling times between farms in Bogor, and the effect of travelling time on virus decay, it does not incorporate the impact of stop-overs between farm visits.

7.2 Effectiveness of hand sanitation in reducing risk

Clearly, measures that prevent the contamination of hands will remove the risk associated with the transfer of HPAIV H5N1 through handling. Hence, the use of disposal gloves when handling birds should be recommended for all visitors to broiler farms who handle birds, and these should be changed between farm visits.

Where such measures are not possible, simple hand washing or hand sanitation following the handling of birds is likely to substantially reduce the risk of disease transmission associated with handling. Hand washing with soap and water alone would generally be expected to remove between 1 to 2 logs of contaminating micro-organisms. A disinfectant hand sanitizer (i.e. a alcohol based gel used on hands without gross contamination), might be expected to remove between 2 and 3 logs, although there may substantial differences between viruses (Mbithi et al. 1993; Sattar et al. 2000; Lages et al. 2008). In one of the few studies to investigate hand-washing and the use of hand

sanitizers on Influenza A survival on hands, Grayson et al. (2009) showed that H1N1 could be reduced to non-detectable levels via hand-washing with soap or through the use of alcohol based sanitizers.

The effect of removal of 90, 99% and 99.9% (i.e. 1, 2 and 3 log reduction) of infectious virus on hands on the probability of infection as a result of handling is presented in

Table 20. Based on the model described previously, with 25 simulations, each with 3000 iterations, a 1 log reduction in the virus titre per cm^2 of contaminated hands would be expected to reduce the probability of infection following handling from 0.05 to 0.008, i.e. 8 out of every 1000 handling events (of the next bird handled following the handling of an infected bird) with contaminated hands would be expected to result in infection. More effective hand washing, particularly when combined with hand sanitizers and an associated 2 or 3 log reduction in contaminating virus, would be expected to reduce the risk to near negligible levels of 0.00084 and 0.00010 respectively (i.e. around 8 and 1 out of every 10,000 handling occasions).

Table 20: Effect of hand sanitation on the probability of infection of a single chicken with HPAIV H5N1 following handling

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Handling – 1 log reduction	$6.7x10^{-3}$– $9.7x10^{-3}$	$4.3x10^{-4}$– $6.4x10^{-4}$	$3.3x10^{-3}$– $4.8x10^{-3}$	$2.4x10^{-2}$– $3.5x10^{-2}$
Handling – 2 log reduction	$6.9x10^{-4}$– $9.9x10^{-4}$	$4.3x10^{-5}$– $6.6x10^{-5}$	$3.2x10^{-4}$– $4.7x10^{-4}$	$2.5x10^{-3}$– $3.5x10^{-3}$
Handling – 3 log reduction	$4.9x10^{-5}$– $2.0x10^{-4}$	$3.2x10^{-6}$– $1.3x10^{-5}$	$2.3x10^{-5}$– $9.6x10^{-5}$	$1.7x10^{-4}$– $7.0x10^{-4}$

8 Exposure assessment: Litter contamination by collectors

Contamination of the broiler environment, and subsequent ingestion by birds through coprophagia or litter consumption, is an important means of spread of infectious disease within broiler flocks (Montrose et al. 1985). If any of the faecal contamination present on collectors' shoes, or on collecting crates, is able to contaminate litter in the house of a susceptible broiler flock, there is the potential for infection of birds engaging in litter consumption or coprophagia.

Consumption of litter by broilers

Malone et al. (1983) recorded the proportion of litter consumed by broilers of different ages, and revealed that litter may constitute up to 6.35% of a bird's daily intake of feed, but that this varied by litter type and age (Table 21). The majority of broiler producers in Bogor use rice hulls for litter, which are expected to be consumed at a similar rate to wood fibres; consumption of rice hulls is reportedly common (Anisuzzaman and Chowdhury 1996). Hence, we used a Betapert distribution to represent the presumed variability in the rate of litter consumption (C_l), where the most likely value was taken as the consumption rates of wood fibres for birds aged 22–49 days (since this is the age at which collectors visit), but the distribution was defined by the maximum and minimum values for all litter types and all ages of bird presented in Table 21 (i.e. Betapert [0, 0.0128, 0.0635]).

Table 21. Litter consumption rates in broilers at different ages

Litter type	1–7 days	8–21 days	22–49 days
Wood chips	0.32%	0.14%	0
Saw dust	2.31%	3.08%	0.95%
Wood fibres	6.35%	4.82%	1.28%
Newspaper	0.39%	0.49%	0.1%
Newspaper fibres	0.15%	0.29%	0.04%

Source: Malone et al. (1983)

Daily feed consumption by broilers aged 25 days and older (i.e. the age at which collectors visit the farm) was estimated from a variety of sources. Feed consumption can be highly variable, particularly in tropical climates, and may be influenced by stocking density and environmental conditions (Daghir 2008). In order to capture the likely variability in this parameter, we used the minimum and maximum values presented in Table 22, and assume that these represent the true range of feed consumption rates of broilers between 25 and 40 days. An estimate of the daily feed intake was then multiplied by the proportion of the intake that is litter in order to estimate the amount (in grams) of litter consumed by broilers in the course of a single day (C_L).

Contamination of susceptible broiler house

Collectors entering the broiler house on a susceptible farm may contaminate litter as a result of transfer of virus from shoes and or from carrying crates.

Table 22: Estimates of broiler feed consumption per day

Age (days)	Feed consumption per day (g)	Source
25	89	(Brake et al. 1992)
30	107	
35	125	
40	142	
28	75 – 115	(RCI 2002)
35	86 – 150	
28 – 49	98 – 125	(Perry 1981)

The virus concentration present on shoes and crates upon arrival to the farm was defined by the distributions for $\overline{\mu}_{\text{shoes}}$ and $\overline{\mu}_{\text{crates}}$, described previously. The amount of faeces, and therefore virus, transferred from shoes or from crates is unknown, but theoretically could range anywhere between 0 and 100% of the amount present. However, we use an estimate of between 1 and 10% to describe the amount of faeces (TE_{shoes}) that is transferred from the amount contaminating shoes, and an estimate of between 0.1 and 1% to describe the amount of faecal transfer from each of the carrying crates brought onto the farm ($TE_{creates}$). Based on these parameters, we estimated the amount of virus that may be transferred into the house, C_v.

We consider two contamination scenarios; 1) collectors using a truck, bringing a maximum of 49 crates into the broiler house and, 2) collectors using a pick-up truck, bringing a maximum of 8 crates into the broiler house.

We make the simplifying assumption that transferred faeces, and the virus within, becomes completely mixed with litter already present in the house through the action of broiler movements (A26). Hence the concentration of virus within the litter of the susceptible broiler house following release of faeces from shoes/crates can be described as a Poisson process.

Estimating viral contamination of litter

Based on consultation with poultry experts in Indonesia, an average small-scale broiler flock would be expected to use between 125 to 250 bags of rice hulls, each bag weighing between 20 to 30 kg. These variables were modelled as uniform distributions and were combined with an estimate of the faecal contamination present in the susceptible broiler house. For this we assumed that collectors would only visit the susceptible broiler flock after the 25th day. We selected random values from the distribution for broiler flock size (*n*) and defined the expected cumulative faecal weight at 25 days and 40 days given this flock size. A random value from a uniform distribution defined using these values as minimum and maximum was then used to predict the weight of faeces already present in

the susceptible broiler house at the time of the collector's visit (W_{fc}). The overall weight of litter in the house (W_{litter}) was then the sum of these two parameters.

By assuming complete mixing within the broiler house, the virus concentration (per g) within the litter of the receiving farm was estimated as:

litter

The probability that a bird within the flock will become infected was then given by the exponential dose-response model as:

Where the dose, D, is:

The probability that at least one bird within the flock will become infected is then,

The parameters used in the model are summarized in Table 23 and the model structure is given in the appendix (Figures 3A to 7A). The model was run as a set of 50 simulations where random values for the rate of virus decay (*k*) and the dose-response parameter (*r*) are drawn from their respective uncertainty distributions, and each individual simulation was composed of 3000 iterations whereby random values are drawn from the variability distribution describing the amount of virus present in the susceptible broiler house following contamination and the exposure (i.e. dose) broiler chickens may receive ( ).

8.1 Risks associated with collectors and their equipment

The summarized outputs for the probability that at least one bird within the broiler house becomes infected as a result of collectors bringing HPAIV H5N1 contaminated shoes and equipment into the broiler house are presented in Table 24.

Table 23: Summary of model parameters used to predict the risk of transmission of HPAIV H5N1 to a broiler flock as a result of contamination of litter by collectors and their equipment

Parameter	Description	Distribution/value	Classification*
n	Flock size (on susceptible farm)	Empirical distribution (see 'Modelling an outbreak' section)	V
$\bar{\mu}_{shoes}$	Virus concentration on shoes after arrival at broiler farm	Second-order non-parametric distribution (see text)	V
$\bar{\mu}_{crates}$	Virus concentration on collecting crates after arrival	Second-order non-parametric distribution (see text)	V

	at broiler farm		
TE_{shoes}	Proportion of faecal material (and virus) transferred from shoes	Uniform(0.01, 0.1)	V(+U)
TE_{crate}	Proportion of faecal material (and virus) transferred from crates	Uniform(0.001, 0.01)	V(+U)
S_{litter}	Number of sacks of litter used per broiler house	Uniform(125, 250)	V(+U)
W_{sack}	Weight of litter per sack	Uniform(20, 30)	V(+U)
W_{fc}	Weight of faeces in the house	See text	V(+U)
W_{litter}	Weight of litter and faeces in the house	$(S_{litter} \times W_{sack}) + W_{fc}$	V(+U)
k	Virus inactivation rate	Uniform(0.1604, 0.1972)	U
r	Exponential dose response parameter	Bootstrap replicates	U
C_l	Litter consumption	Betapert(0, 0.0128, 0.0635)	V(+U)
N_c	Number of collectors per truck	Uniform(1,3)	V

*V = variability, U = uncertainty, F= Fixed values

Table 24. Summarized results for the probability of infection on the *next* farm after collectors have visited a small-scale broiler farm on which an HPAI H5N1 outbreak is occurring

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Collectors using a truck	3.2×10^{-2} – 1.3×10^{-1}	4.2×10^{-4} – 2.3×10^{-3}	1.2×10^{-2} – 5.9×10^{-2}	1.2×10^{-1} – 5.2×10^{-1}
Collectors using a pick-up	2.1×10^{-2} – 8.6×10^{-2}	1.9×10^{-4} – 9.9×10^{-4}	5.8×10^{-3} – 2.8×10^{-2}	8.4×10^{-2} – 3.8×10^{-1}

Given that collectors using a truck may bring more collection crates into the broiler house than those collectors using a pick-up truck (i.e. 49 vs. 8), the risk of transmission for the former group is higher than that for the latter (Figure 9 and 20).

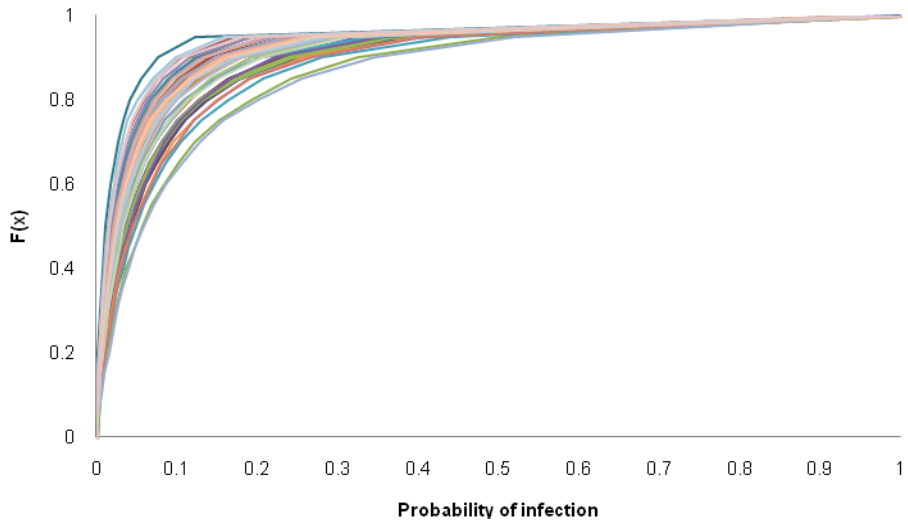

Figure 19: Cumulative second-order distribution of the probability of infection following contamination by the shoes and collecting crates of collectors using a truck and who have previously visited a broiler farm on which an outbreak of HPAI H5N1 is occurring.

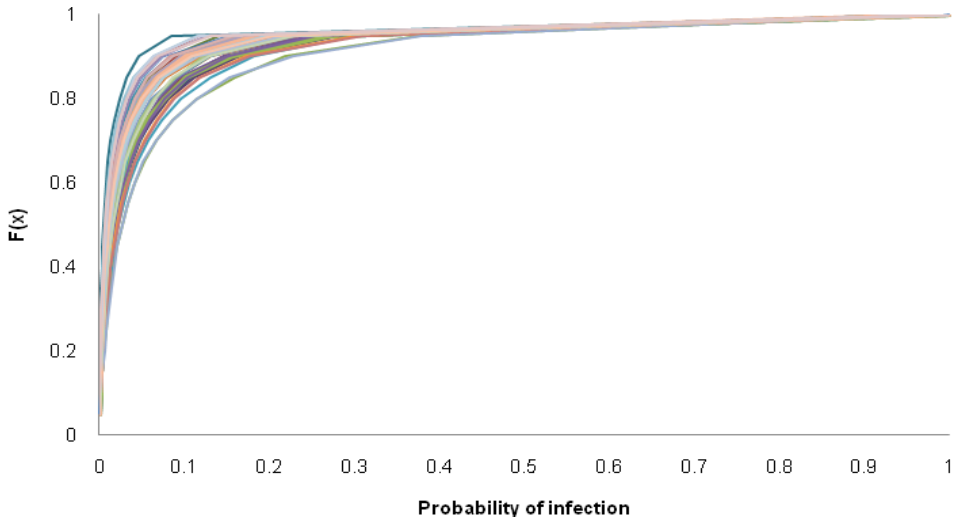

Figure 20: Cumulative second-order distribution of the probability of infection following contamination by the shoes and collecting crates of collectors using a pick-up truck and who have previously visited a broiler farm on which an outbreak of HPAI H5N1 is occurring.

The mean value for the probability that at least one bird will become infected in a flock of broilers following contamination of litter by collectors using a truck, over all simulations, is 0.0714. Hence, we would expect that, on average, on around 7 out of every 100 occasions on which a farm is visited

by collectors who have previously visited a farm on which an outbreak is occurring, at least one bird would become infected with HPAIV H5N1. Given the presumed high basic reproduction number (R_0) of HPAIV H5N1 in a flock of ground-dwelling birds, the infection of a single bird is assumed to be equivalent to infection of the entire flock. The estimate for collectors using a pick-up truck is 0.0474. Each estimate is associated with an uncertainty distribution of between 0.032 to 0.129 and 0.021 to 0.086, respectively. It should be noted that these estimates are specific to the *next* farm visited following the initial visit to the index farm (i.e. the farm on which the outbreak is occurring); subsequent visits would be associated with lower risks as the quantity of contaminating virus decreases through the actions of virus decay and transfer.

8.2 Impact of altering model parameters

In order to assess the impact of altering model parameters on the risk estimates derived, we selected those parameters in which there was considerable uncertainty in the true extent of variability, and used extreme values in order to represent a wider range of possible values for each factor (Henderson-Sellers and Henderson-Sellers 1996). The parameters selected were:

– Amount of faecal material transferred from shoes (TE_{shoes})
– Amount of faecal material transferred from crates (TE_{crate})

We also considered the impact of altering the transmission parameters in the stochastic transmission model (i.e. from the release assessment), and in particular the impact of altering R_0 and the number of birds initially infected on the risk of onward transmission via litter contamination.

1) Faecal transfer from shoes (TE_{shoes})

The impact of altering the amount of faeces, and therefore virus that can enter the broiler house from shoes is presented in

Table 25. Clearly, as the amount of HPAIV H5N1 contaminating the litter increases, the risk to broiler chickens within the house increases. For example, if 50% of faecal material (and therefore virus) is transferred from the shoes of collectors using a truck when they enter the broiler house, the mean risk estimate over 50 simulations, each with 3000 iterations, increases to 0.23, or on average, 23 out of every 100 visits would be expected to result in infection. If all contaminating material is transferred, the probability is increases to 0.37 for those collectors using a truck, or 0.35 for collectors using a pick-up truck. Whilst the transfer of such a large amount of material seems unlikely, these estimates can serve as an indication of the *potential* risk associated with the viral contamination of shoes.

Table 25: The impact of altering the proportion of faecal material that is transferred from shoes (TE_{shoes}) on the predicted probability of transmission of HPAIV H5N1 to a susceptible flock of broilers

	Transfer	*Mean*	*5th percentile*	*50th percentile*	*95th percentile*
Truck	***0.001***	3.2×10^{-2}	2.5×10^{-4}	1.1×10^{-2}	1.3×10^{-1}
	0.01	4.1×10^{-2}	4.6×10^{-4}	1.6×10^{-2}	1.6×10^{-1}
	0.1	9.7×10^{-2}	1.4×10^{-3}	4.1×10^{-2}	4.1×10^{-1}
	0.25	1.6×10^{-1}	2.4×10^{-3}	7.0×10^{-2}	6.7×10^{-1}
	0.5	2.3×10^{-1}	3.9×10^{-3}	1.1×10^{-1}	8.8×10^{-1}
	1	3.7×10^{-1}	6.2×10^{-3}	1.9×10^{-1}	9.8×10^{-1}
Pick–up truck	***0.001***	6.7×10^{-3}	6.0×10^{-5}	2.3×10^{-3}	2.6×10^{-2}
	0.01	1.5×10^{-2}	1.7×10^{-4}	5.2×10^{-3}	5.9×10^{-2}
	0.1	7.4×10^{-2}	7.0×10^{-4}	2.3×10^{-2}	3.4×10^{-1}
	0.25	1.4×10^{-1}	1.4×10^{-3}	4.8×10^{-2}	6.4×10^{-1}
	0.5	2.1×10^{-1}	2.4×10^{-3}	8.8×10^{-2}	8.7×10^{-1}
	1	3.5×10^{-1}	4.1×10^{-3}	1.6×10^{-1}	9.8×10^{-1}

2) Faecal transfer from crates (TE_{crate})

The impact of altering the amount of faeces, and therefore virus, entering the house from collecting crates is presented in

Table 26. The baseline model uses values between 0.1 and 1%, and increasing these estimates is expected to result in potentially large increases in risk. For example, if 50% of all faeces predicted to be present on un-sanitized collection crates is transferred to the litter of the broiler house, the average risk for the infection of at least one bird associated with a visit by those collectors using a truck is 0.61 and for those collectors using a pick-up truck is 0.29.

Table 26: The impact of altering the proportion of faecal material that is transferred from crates (TE_{crates}) on the predicted probability of transmission of HPAIV H5N1 to a susceptible flock of broilers

	Transfer	*Mean*	*5th percentile*	*50th percentile*	*95th percentile*
Truck	***0.001***	4.8×10^{-2}	4.8×10^{-4}	1.4×10^{-2}	2.1×10^{-1}
	0.01	9.3×10^{-2}	1.5×10^{-3}	4.3×10^{-2}	3.7×10^{-1}
	0.1	3.2×10^{-1}	6.7×10^{-3}	2.2×10^{-1}	9.3×10^{-1}
	0.25	4.8×10^{-1}	1.4×10^{-2}	4.4×10^{-1}	1.0
	0.5	6.1×10^{-1}	2.6×10^{-2}	6.7×10^{-1}	1.0
	1	7.3×10^{-1}	5.2×10^{-2}	9.0×10^{-1}	1.0
Pick-up truck	***0.001***	4.3×10^{-2}	2.5×10^{-4}	9.2×10^{-3}	2.0×10^{-1}
	0.01	5.2×10^{-2}	5.9×10^{-4}	1.7×10^{-2}	2.2×10^{-1}
	0.1	1.2×10^{-1}	1.9×10^{-3}	5.8×10^{-2}	4.6×10^{-1}
	0.25	2.0×10^{-1}	3.3×10^{-3}	1.1×10^{-1}	7.0×10^{-1}
	0.5	2.9×10^{-1}	5.4×10^{-3}	1.9×10^{-1}	8.9×10^{-1}
	1	4.0×10^{-1}	9.5×10^{-3}	3.4×10^{-1}	9.9×10^{-1}

3) Basic reproduction number

The impact of altering the basic reproduction number on the risk of onward transmission is presented in Table 27. These results suggest that reductions in the transmissibility of infection on the index farm are associated with reductions in the risk of onward transmission (when compared to the model in which R_0 is allowed to range between 20 to 30), but that these reductions are relatively small. For example, the average risk of infection on the receiving farm when the value of R_o on the outbreak farm is 10 is 0.041 for collectors using a truck and 0.026 for those collectors using a pick-up truck (compared to the baseline results of 0.0714 and 0.0474, respectively). The average risk is further reduced when the R_0 is 5 to 0.020 and 0.0089, respectively.

Table 27: The impact of altering the basic reproduction number on the index farm (i.e. the farm on which the outbreak is occurring) on the probability of transmission of HPAIV to a susceptible flock of broilers

	R_o	*Mean*	*5th percentile*	*50th percentile*	*95th percentile*
Truck	10	4.1×10^{-2}	2.0×10^{-4}	1.5×10^{-2}	1.7×10^{-1}
	5	2.0×10^{-2}	2.5×10^{-5}	5.8×10^{-3}	8.3×10^{-2}
Pick-up	10	2.3×10^{-2}	1.2×10^{-4}	6.3×10^{-3}	9.4×10^{-2}
truck	5	8.9×10^{-3}	1.5×10^{-5}	2.3×10^{-3}	3.5×10^{-2}

4) Number of birds initially infected

When the source of contamination on the index farm is contaminated feed or water, it might be expected that a large proportion of the flock will become infected at or around the same time. The impact of this effect is presented in Table 28. Compared to the baseline model, in which a single bird is infected, the probability of onward transmission increases as the number of birds initially infected within the flock on which the outbreak is occurring increases, so that an outbreak in which 1% of the flock are initially infected is associated with an average risk of onward transmission of 0.086 via collectors using trucks, whilst an outbreak on which 50% of the flock are initially infected is associated with an average risk of onward transmission of 0.11.

Table 28: The impact of the number of birds initially infected on the index farm on the probability of infection as result of the contamination of litter on a susceptible farm by collectors.

	Proportion of flock initially infected	*Mean*	*5th percentile*	*50th percentile*	*95th percentile*
Truck	1%	8.6×10^{-2}	2.5×10^{-3}	3.6×10^{-2}	3.6×10^{-2}
	10%	1.0×10^{-1}	3.0×10^{-3}	4.3×10^{-2}	4.1×10^{-1}
	50%	1.1×10^{-1}	3.2×10^{-3}	4.6×10^{-2}	4.6×10^{-1}
Pick-up truck	1%	6.2×10^{-2}	9.7×10^{-4}	1.8×10^{-2}	2.9×10^{-1}
	10%	7.4×10^{-2}	1.1×10^{-3}	2.3×10^{-2}	3.4×10^{-1}
	50%	8.4×10^{-2}	1.2×10^{-3}	2.4×10^{-2}	3.9×10^{-1}

5) Time at which the virus is introduced into the house

The time at which the HPAIV H5N1 was introduced into the flock was altered to reflect the anecdotal evidence that HPAI H5N1 outbreaks typically occur only after the second week in broiler flocks. For this, the uniform distribution describing time of virus introduction was altered to range only

between 14 and 40 days. The resulting model outputs (in which all other model parameters were held at their baseline, i.e. R0 between 20 and 40, introduction of a single infected bird etc) are presented in Table 29. These results suggest that the time of virus introduction into the flock has a minimal impact on the risk of onward transmission via collectors, with the average risk estimate and the range of values derived being roughly equivalent for the baseline and adjusted model.

Table 29: Summarized results for the probability of infection on the *next* farm after collectors have visited a small-scale broiler farm on which an outbreak is occurring and received HPAIV H5N1 contamination of shoes and carry crates. The model is adjusted so that HPAIV H5N1 on the outbreak farm is only introduced after the 14th day of production

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Truck	7.2×10^{-2}	9.0×10^{-4}	2.9×10^{-2}	2.9×10^{-1}
Pick-up	4.8×10^{-2}	4.3×10^{-4}	1.4×10^{-2}	2.1×10^{-1}

6) Mixing within the house

For the baseline scenario we make the important assumption that any introduced virus mixes completely with all litter present within the house. It would be reasonable to assume, however, that the movement of chickens within the broiler house will result in mixing with only a proportion of litter, for example the top layer only. The impacts of reducing the amount of litter with which virus is able to mix are presented in Table 30. In the first case, we consider mixing with 50% of the litter in the house (i.e. the top 50% of litter), and the second scenario considers mixing with 10% of litter in the house. Clearly, the less mixing that takes place, the greater the concentration of virus per gram of litter, and the probability of infection within the receiving broiler house increases. Based on a model with 50 simulations, each with 3000 iterations, the mean estimate of risk based on the 50% mixing assumption would be 0.123 for collectors using trucks, whilst the estimate for collectors using a pick-up truck would be 0.082. Similarly, the estimate for 10% mixing within the litter of the broiler house is 0.347 for collectors using a truck and 0.241 for collectors using a pick-up truck. Clearly, then, this assumption is extremely important and can alter the output from the model from a low to medium risk (i.e. 7 out of every 100 visits resulting in infection) to a high to very high risk, i.e. 35 out of every 100 visits resulting in infection). Unfortunately, this uncertainty is unlikely to be resolved and the impact of this large assumption should therefore be considered when interpreting the risks associated with contamination of litter on the receiving broiler farm.

Table 30: Summarized results for the probability of infection associated with the movement of collectors and their equipment under the assumption that virus mixes with 50% or 10% of litter in the receiving broiler house

Mixing assumption	Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
50%	*Truck*	1.2×10^{-1}	1.9×10^{-3}	5.6×10^{-2}	5.0×10^{-1}
	Pick-up	8.2×10^{-2}	8.5×10^{-4}	2.6×10^{-2}	3.7×10^{-1}
10%	*Truck*	3.5×10^{-1}	9.1×10^{-3}	2.5×10^{-1}	9.7×10^{-1}
	Pick-up	2.4×10^{-1}	4.2×10^{-3}	1.2×10^{-1}	9.1×10^{-1}

Summary: risks associated with collectors and their equipment

Although the baseline model considers the scenario in which all of the introduced virus becomes mixed with the very large amount of litter present within the house of the susceptible broiler farm, and therefore that individual birds are exposed to extremely small doses, the fact that such a large number of birds are exposed to HPAIV H5N1 results in a relatively high predictions of risk. Hence, the assumption of complete mixing of virus within the house and hence the exposure of all birds is an extremely important one.

Altering model parameters such as the proportion of virus entering the house from shoes and collecting crates will clearly influence the risk of disease spread; as more virus is introduced, the risk that at least one bird becomes infected increases.

8.3 Impact of mitigation strategies: litter contamination by collectors

We considered the impact of a range of mitigation strategies that could be implemented to reduce the risk associated with transmission from collectors. These were:

- A mandatory delay period between farm visits.
- The prevention of collectors visiting farms on which outbreaks are occurring, either through incentivizing reports of HPAI H5N1 or penalization of the sale of live broilers in the event of a disease outbreak.
- Sanitation of collector's equipment between farm visits

The impact of all interventions was evaluated using the baseline model (i.e. a single infected bird, R_0 between 20 and 40, complete mixing within the house, etc.).

8.3.1 Mandatory 24 hr delay period between farm visits by collectors

The establishment of regulations to ensure a delay of 24 hours between visits to farms by collectors is expected to result in a substantial decrease in the risk of disease spread from an infected farm to a susceptible farm (Table 31). Based on a model with 50 simulations, each with 3000 iterations, the average estimate of risk associated with transmission from collectors using a truck would be reduced from 0.071 to 0.0023, or approximately two occasions out of 1000 in which collectors transmit infection to a susceptible flock, if collectors were forced to wait for 24 hours between farm visits. The mean risk associated with collectors using a pick-up truck would be reduced from 0.047 to 0.00064. Similarly, the probability of transmission of infection following a 48 hour delay is extremely small, with the average risk for those collectors using a truck being 0.0000349, or infection on 3 or 4

occasions out of every 100,000 visits to a susceptible farm following a visit to a farm on which an outbreak is occurring.

Table 31: Predicted probability of infection following the establishment of a mandatory 24 hr delay period for movement between farms for collectors using trucks and pick-up trucks

	Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
24 hr delay	Truck	9.5×10^{-4} – 4.5×10^{-3}	8.0×10^{-6} – 5.4×10^{-5}	2.8×10^{-4} – 1.5×10^{-3}	3.2×10^{-3} – 1.8×10^{-2}
	Pick-up	6.4×10^{-4} – 3.0×10^{-3}	3.5×10^{-6} – 2.4×10^{-5}	1.4×10^{-4} – 7.0×10^{-4}	2.2×10^{-3} – 1.2×10^{-2}
48 hr delay	Truck	7.7×10^{-6} – 9.1×10^{-5}	8.6×10^{-8} – 9.0×10^{-7}	2.6×10^{-6} – 2.9×10^{-5}	2.7×10^{-5} – 3.1×10^{-4}
	Pick-up	5.0×10^{-6} – 6.2×10^{-5}	3.9×10^{-8} – 4.3×10^{-7}	1.2×10^{-6} – 1.3×10^{-5}	1.7×10^{-5} – 2.1×10^{-4}

Whilst we acknowledge that mandatory delay periods between farm visits would be difficult to enforce, a similar impact could be achieved if collectors were encouraged to change footwear and carry crates between farm visits, ensuring that these items had a 24 or 48 hour rest period between farm visits.

8.3.2 Prevention of collectors visiting farms on which an HPAI outbreak has been detected or is suspected

The litter contamination model has considered the situation in which producers detecting disease attempt to sell broilers as early as possible. However, if it is possible to incentivize producers to report an outbreak of HPAI within their flock upon detection, and therefore to reduce the likelihood that they will use collectors, the risk of onward transmission via collectors is likely to be reduced, but not prevented, given that collectors may visit a farm on which an HPAI outbreak is occurring, but on which disease has not yet been detected.

In order to estimate the probability of onward transmission associated with collectors who *only* visit a farm before an outbreak is detected, we repeated each step of the release and exposure assessment, but selecting only those data from the stochastic transmission model that relate to collector visits before the disease has been detected. The summarized outputs from 50 simulations, each with 3000 iterations are presented in expected to be reduced by at least one order of magnitude by ensuring that collectors do not visit a farm on which HPAI H5N1 is suspected.

Table 32 and the cumulative second-order distributions for collectors using trucks and those using pick-up trucks are presented in Figure 21 and 22. Whilst the mean value for trucks, over all simulations was reduced to 0.0076, and for pick-up trucks 0.0025, the distribution of observed values is extremely wide. This is due to the fact that the within flock prevalence at the time the collector visits is highly variable, with the potential for collectors to visit when only a few birds are infected, to situations in which over 90% of birds within the flock are infected and shedding virus (e.g. at extreme values of the range of R_0 together with short latent periods). However, on average,

the risks of onward transmission via litter contamination might be expected to be reduced by at least one order of magnitude by ensuring that collectors do not visit a farm on which HPAI H5N1 is suspected.

Table 32: Summarized results for the probability of infection on the *next* farm after collectors have visited a small-scale broiler farm on which an HPAI outbreak is occurring but has not yet been detected

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Collectors using truck	2.8×10^{-3}-1.6×10^{-2}	5.2×10^{-7}-2.3×10^{-6}	1.5×10^{-4}-7.1×10^{-4}	1.4×10^{-2}-7.8×10^{-2}
Collectors using pick-up	7.7×10^{-4}-4.5×10^{-3}	4.4×10^{-8}-1.9×10^{-7}	4.2×10^{-5}-2.0×10^{-4}	3.7×10^{-3}-2.2×10^{-2}

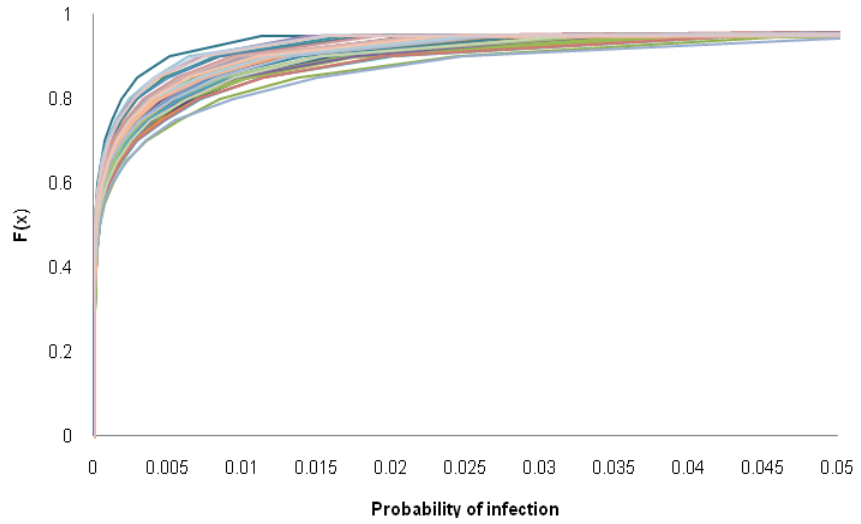

Figure 21: Cumulative second-order distribution for the predicted probability of infection following the entry into the broiler house of a group of collectors using a truck who has previously visited a farm on which an HPAI outbreak is occurring, but has not yet been detected.

8.3.3 Sanitation of footwear and crates

If collectors can be encouraged to sanitize their footwear and equipment following each farm visit, it is likely that the risk of onward transmission will be reduced. The expected impact of cleaning or disinfection of shoes or carry crates, or both, on the risk of transmission associated with collectors is presented in Table 33.

A 2 to 3 log reduction in virus titre on footwear is a theoretically achievable through the use of foot dips, however this requires very regular maintenance of chemical concentrations and is unlikely to be achieved in the field (Dwyer 2004). Moreover, the disinfectants of choice for broiler producers in Indonesia tend to be quaternary ammonium compounds, and whilst these have been shown to be effective against AIV (De Benedictis et al. 2007; Patnayak et al. 2008), the presence of organic

material (as would be expected on contaminated footwear) may severely reduce their effectiveness. Reduction of virus titres on footwear will reduce the risk associated with collectors somewhat (i.e. to a minimum average risk of 0.032 for a 3 log reduction (99.9%) in virus titre on shoes of collectors using a truck), but given that collectors can also contaminate the broiler house environment through collecting crates, the risk remains reasonably high if these remain un-sanitized. Similarly, a 2 to 3 log reduction in the expected H5N1 virus titre on crates will substantially reduce the risk of virus transmission via the crates, however the predicted probability of transmission remains at 0.04 if collectors do not also sanitize their footwear.

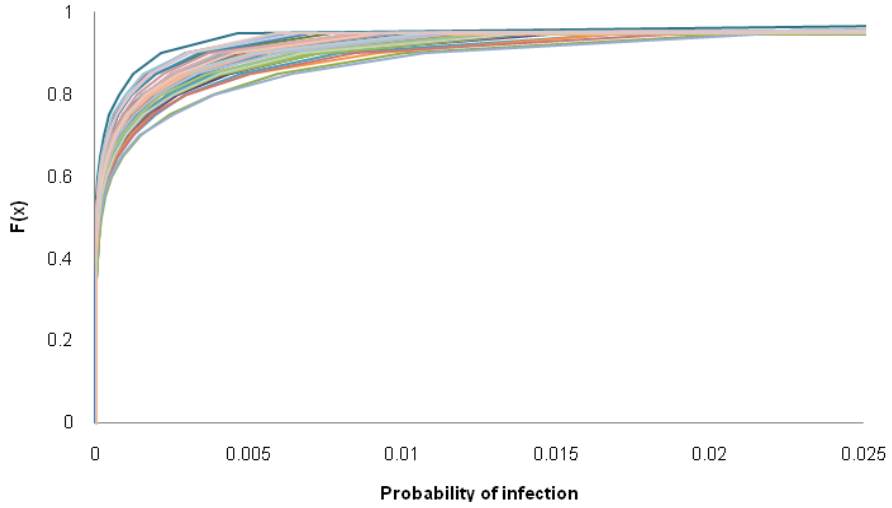

Figure 22: Cumulative second-order distribution for the predicted probability of infection following the entry into the broiler house of a group of collectors using a pick-up truck who have previously visited a farm on which an HPAI outbreak is occurring, but has not yet been detect.

Table 33: Summarized results for the probability of infection through the entrance of collectors following the sanitation and 1, 2 or 3 log removal of virus contaminating shoes and/or collection crates

	Log reduction	Mean	5th Percentile	50th Percentile	95th Percentile
Shoes					
Collectors	1	3.6×10^{-2}	3.5×10^{-4}	1.4×10^{-2}	1.5×10^{-1}
using	2	3.2×10^{-2}	2.3×10^{-4}	1.1×10^{-2}	1.3×10^{-1}
trucks	3	3.2×10^{-2}	2.0×10^{-4}	1.0×10^{-2}	1.3×10^{-1}
Collectors	1	1.1×10^{-2}	1.2×10^{-4}	3.8×10^{-3}	4.3×10^{-2}
using	2	6.3×10^{-3}	4.9×10^{-5}	2.0×10^{-3}	2.5×10^{-2}
pick-up	3	5.8×10^{-3}	3.3×10^{-5}	1.7×10^{-3}	2.3×10^{-2}
Crates					
Collectors	1	4.3×10^{-2}	3.6×10^{-4}	1.1×10^{-2}	2.0×10^{-1}
using	2	4.3×10^{-2}	1.9×10^{-4}	8.5×10^{-3}	2.0×10^{-1}
trucks	3	4.2×10^{-2}	1.4×10^{-4}	8.2×10^{-3}	2.0×10^{-1}
Collectors	1	4.3×10^{-2}	2.1×10^{-4}	8.7×10^{-3}	2.0×10^{-1}
using	2	4.3×10^{-2}	1.4×10^{-4}	8.1×10^{-3}	2.0×10^{-1}
pick-up	3	4.2×10^{-2}	1.4×10^{-4}	8.1×10^{-3}	2.0×10^{-1}
Shoes and Crates					
Collectors	1	9.1×10^{-3}	9.2×10^{-5}	2.9×10^{-3}	3.5×10^{-2}
using	2	9.5×10^{-4}	9.3×10^{-6}	2.9×10^{-4}	3.5×10^{-3}
trucks	3	9.6×10^{-5}	9.3×10^{-7}	2.9×10^{-5}	3.5×10^{-4}
Collectors	1	6.1×10^{-3}	4.2×10^{-5}	1.3×10^{-3}	2.4×10^{-2}
using	2	6.5×10^{-4}	4.3×10^{-6}	1.3×10^{-4}	2.4×10^{-3}
pick-up	3	6.6×10^{-5}	4.2×10^{-7}	1.3×10^{-5}	2.4×10^{-4}

Hence, in order to effectively reduce the risks of transmission associated with collectors through sanitation, the cleaning of all potentially contaminated equipment (i.e. footwear *and* collecting crates) must be conducted. If this can be achieved, a 2 to 3 log reduction in contaminating virus titres can be considered to result in reduction of risk of transmission to very low levels.

9 Exposure assessment: Litter contamination by animal health workers

We applied the same litter contamination model to predict the risks associated with the contamination of litter as a result of transfer of faecal material to the broiler house from the shoes of animal health workers (i.e. veterinarians or pharmaceutical officers). Additional risks would be associated with the handling of the birds on the susceptible broiler farm, and these are described above.

The model outputs are given in Table 34, and the cumulative second-order distribution is presented in Figure 223. The mean probability of onward transmission, over all simulations, is 0.087, or the model predicts that approximately 9 out of every 100 small-scale broiler farms would become infected as a result of the entry of animal health workers who have previously visited a small-scale broiler farm on which an outbreak is occurring, and who have not applied sanitary measures. The uncertainty range for this mean estimate is between 0.039 and 0.15. This assessment considers the specific example of animal health workers called to the farm at the first suspicion of infectious disease within the flock.

Table 34:. Summarized results for the probability of infection on the next farm after veterinarians or animal health workers have visited a small-scale broiler farm on which an HPAI outbreak has just been detected

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Veterinarian/animal health worker	3.9×10^{-2}- 1.5×10^{-1}	8.5×10^{-4} -4.8×10^{-3}	1.4×10^{-2}- 7.0×10^{-2}	1.6×10^{-1}- 6.3×10^{-1}

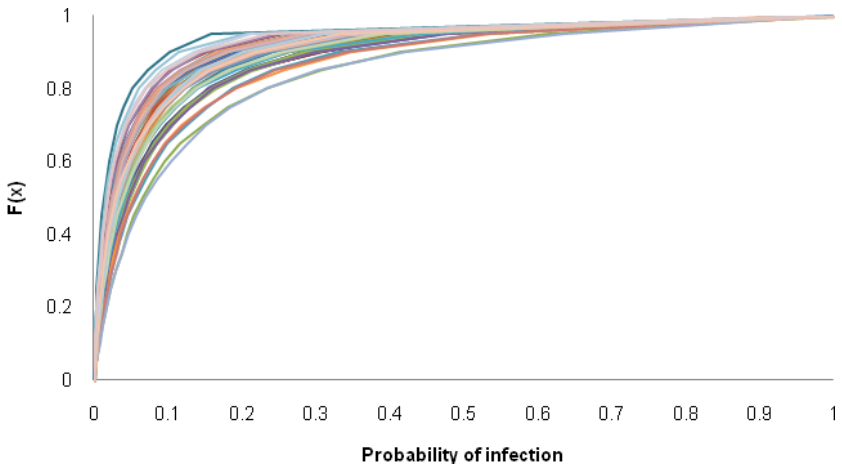

Figure 23: Cumulative second-order distribution for the probability of infection following the entry of a veterinarian or animal health worker who has previously visited a farm on which an HPAI H5N1 outbreak has recently been detected.

9.1 Impact of mitigation strategies: litter contamination by animal health workers

We considered the impact of two mitigation strategies that could be implemented to reduce the risk associated with transmission from animal health workers. These were:

- A mandatory delay period between farm visits by animal health workers.
- Provision of disinfectant footbaths prior to entry to the house

9.1.1 Mandatory 24 hr delay period between farm visits by veterinarians

As is the case with collectors, the establishment of regulations to ensure a delay of 24 hours between visits to farms by veterinarians and animal health workers can result in a potentially large decrease in the risk of spread of infection from a farm on which an outbreak is occurring to a susceptible farm (Table 35). Based on 50 simulations, each comprising 3000 iterations, the mean estimate is reduced to very low levels of 0.0016 (with an associated uncertainty range of 0.00064 - 0.0034).

Table 35: Predicted probability of HPAIV H5N1 infection following the establishment of a mandatory 24 hr delay period for movement between farms for veterinarians and animal health workers.

Probability of infection	Mean	5th Percentile	50th Percentile	95th Percentile
Veterinarian/animal health worker: 24 hour delay	6.4×10^{-4}- 3.4×10^{-3}	4.1×10^{-5}- 2.1×10^{-4}	4.0×10^{-4}- 2.0×10^{-3}	2.2×10^{-3}- 1.1×10^{-2}

9.1.2 Impact of the provision of disinfectant footbaths prior to entry of the broiler house

The expected impact of disinfectant foot dips on the risks associated with faecal contamination on the feet of veterinarians and animals health workers is presented in Table 36. Whilst difficult to achieve, if a 2 to 3 log reduction in virus titres on footwear can be achieved, the risk of spread via the footwear of such visitors could be expected to be reduced to low levels.

Table 36: Predicted impact of disinfectant footbaths on the probability of disease spread via veterinarians and animal health workers following a visit to a farm on which an HPAI outbreak is ongoing

Probability of infection		5th Percentile	50th Percentile	95th Percentile
Veterinarians and animal health worker: foot dips	*1 log reduction*	2.8×10^{-4}- 9.1×10^{-4}	2.9×10^{-3}- 9.4×10^{-3}	1.8×10^{-2}- 5.8×10^{-2}
	2 log reduction	3.0×10^{-5}- 9.3×10^{-5}	2.8×10^{-4}- 9.3×10^{-4}	1.7×10^{-2}- 5.5×10^{-3}
	3 log reduction	2.9×10^{-6}- 9.4×10^{-6}	2.8×10^{-5} -9.2×10^{-5}	1.8×10^{-4}- 5.9×10^{-4}

10 Exposure assessment: Water-borne transmission

Based on estimates of the concentration of virus in surface water (C_r) under three dilution scenarios, the exposure dose (*D*) received by chickens consuming water from rivers contaminated by carcasses from birds that have died from HPAI H5N1 was estimated (Schijven et al. 2005a):

Where values of C_r are defined by the second order-distribution representing the concentration of virus in water and *Fc* is the drinking rate of domestic chickens per day. We estimated *Fc* by defining the distribution of daily water consumption rates from a single study investigating water consumption on broiler farms at 5 sites and over 6 production cycles (Manning et al. 2007) (Table 37). Water consumption rates were approximately normally distributed, with best fitting values of $\mu = 0.17$, $\sigma = 0.02$. The distribution was truncated at 0 and 1 litre.

Table 37: Average daily water consumption by broiler chickens (l/bird/day) at 5 sites

	Water consumption (l/bird/day)				
Crop	**1**	**2**	**3**	**4**	**5**
1	0.159	0.167	0.168	0.156	0.184
2	0.159	0.139	0.137	0.151	0.177
3	0.156	0.228	0.221	0.153	0.170
4	0.155	0.172	0.177	0.158	0.175
5	0.186	0.183	0.183	0.162	0.189
6	0.188	0.184	0.187	0.158	0.176
Average	0.167	0.179	0.177	0.156	0.178

Source: Manning et al. (2007)

We considered exposure over the course of a single day (i.e. $t = 1$); after one day, we expect that virus concentrations at a specific location will be diminished through the combined actions of dispersion and advection (*A27*) (Schijven et al. 2005a).

10.1 Probability of infection in a broiler flock

The probability that a single bird exposed to dose *D* will become infected can be estimated based on the exponential dose response model as,

Given that all birds within a flock of broilers will be exposed to the same source of drinking water, we estimated the probability that at least one bird within the flock would become infected as:

Where is the number of birds in the susceptible broiler flock. The full list of parameters used in both the release and exposure assessment for the transmission of HPAIV H5N1 as a result of the consumption of contaminated river water is given in Table 38.

Table 38: Parameters used in the assessment of the risks of transmission associated with the consumption of H5N1 contaminated river water by broilers

Parameter	Description	Distribution/value	Classification[1]
F_{day}	Faecal output per day (g)	Betapert(20, 40, 50)	V (+ U)
R_{day}	Respiratory secretion per day (ml)	Betapert(0,5,20)	V (+ U)
$\bar{\tau}$	Virus titre per gram faeces (EID_{50})	Parametric bootstrap from observed data	V (+ U)
$\bar{\rho}$	Virus titre per ml respiratory secretion (EID_{50})	Parametric bootstrap from observed data	V (+ U)
$T_{disposal}$	Time between death and disposal into river (hrs)	Uniform (1,12)	V
k	Virus inactivation co-efficient (in faeces)	Uniform(0.1604, 0.1972)	U
π	Virus inactivation co-efficient (in water)	Lognormal(0.043, 0.064)	U
$F_{r\,(small)}$	Flow rate - small river	8.6×10^4 m^3/day	F
$F_{r\,(medium)}$	Flow rate - medium river	2.2×10^6 m^3/day	F
$F_{r\,(large)}$	Flow rate - large river	2.3×10^7 m^3/day	F
d	Drinking rate	Normal(0.172, 0.02)	V (+U)
r	Exponential dose response parameter	Bootstrap replicates	U
n_{flock}	Number of birds in the flock	Empirical distribution	V
ε	Mortality rate (detection threshold)	Uniform(0.01, 0.02)	V (+U)

[1]V = variability, U = uncertainty, F = fixed values

The model was run as a set of 50 simulations, each comprising 5000 iterations. For each simulation, a random value was drawn from the uncertainty distributions representing the dose response parameter, *r*, virus inactivation co-efficient in faeces, *k*, and the virus inactivation co-efficient in water, π. The ranges of distributional outputs are presented in Table 39. The risk associated with the

contamination of small rivers is relatively high, with a mean risk estimate, over all simulations, of 0.18 (and associated uncertainty distribution between 0.12 and 0.31). Hence, we would predict that at least one bird within a flock will become infected 18 times out of 100 when a random flock uses untreated water from a small river within a one-day flow from a farm on which an HPAI outbreak is occurring, and on which contaminated carcasses amounting to between 1 and 2% of the flock size are thrown into the small river.

Table 39: Estimates of the probability of infection of at least one bird within a broiler flock (Pi_{flock}) following exposure to water from small, medium and large rivers contaminated by carcasses comprising between 1 and 2% of a broiler flock on which an outbreak is occurring

Dilution scenario	Mean Pi_{flock}	5th percentile	50th percentile	95th percentile
Small	1.2×10^{-1} – 3.1×10^{-1}	2.8×10^{-3} – 1.4×10^{-2}	4.3×10^{-2} – 1.9×10^{-1}	5.4×10^{-1} – 9.8×10^{-1}
Medium	7.6×10^{-3} – 3.3×10^{-2}	1.1×10^{-4} – 5.6×10^{-4}	1.7×10^{-3} – 8.1×10^{-3}	3.0×10^{-2} – 1.5×10^{-1}
Large	7.8×10^{-4} – 3.7×10^{-3}	1.0×10^{-5} - 5.4×10^{-5}	1.6×10^{-4} – 7.8×10^{-4}	2.9×10^{-3} – 1.5×10^{-2}

Clearly, as the size of the river increases, and the value of Cr decreases through dilution, the risks to broiler flocks decrease. Hence, a broiler farm using water from a 'medium' sized river has a average risk of 0.014 (uncertainty range 0.0076 - 0.033), and a broiler flock using water from a 'large' sized river would be expected to have a risk of infection of 0.0016 (uncertainty range 0.00078 - 0.0037). The cumulative second-order distributions for each of these scenarios are presented in

Figure 24, 25 and 26.

Given that so many birds within a single flock are exposed to potentially infectious doses of HPAIV H5N1 within drinking water, the risk that at least one bird within the flock becomes infected is relatively high, even though the specific risk for an individual within the flock is extremely small.

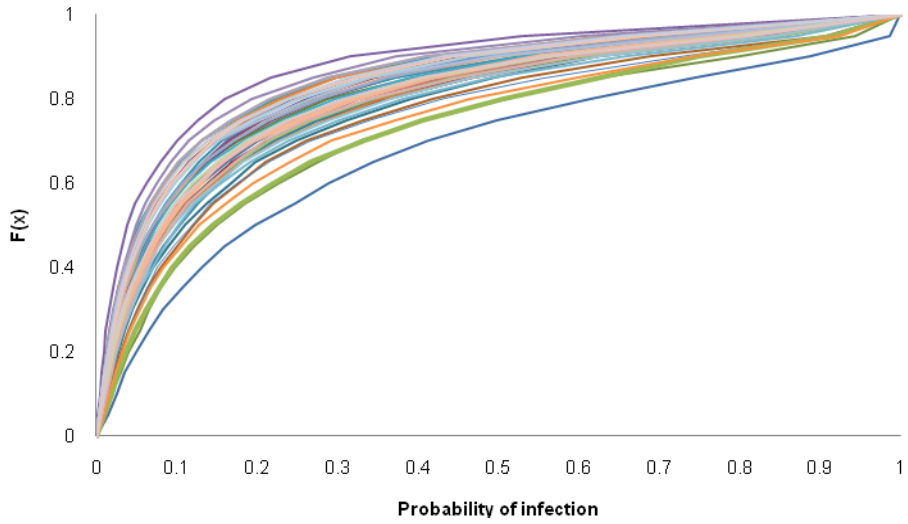

Figure 24: The cumulative second order distribution for the risk of HPAIV H5N1 infection of *at least one* bird in a small-scale broiler flock following consumption of untreated water from a 'small' river contaminated by carcasses.

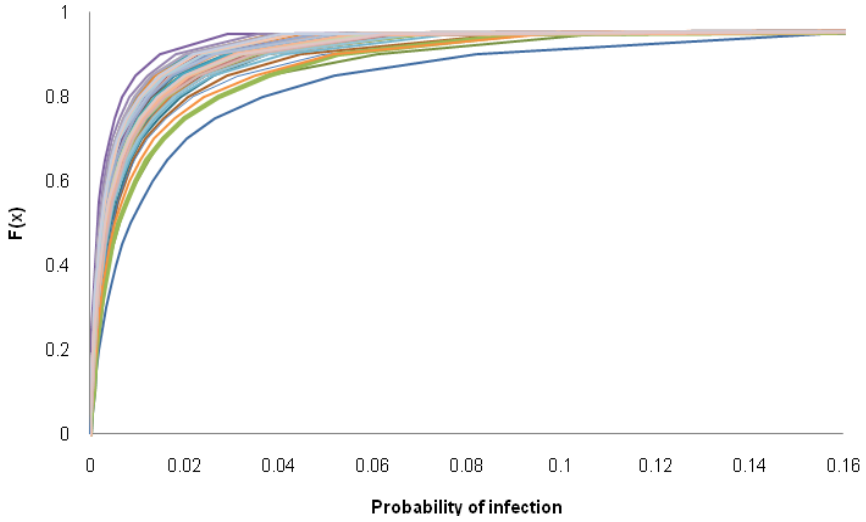

Figure 25: The cumulative second order distribution for the risk of HPAIV H5N1 infection of *at least one* bird in a small-scale broiler flock following consumption of untreated water from a 'medium' sized river contaminated by carcasses.

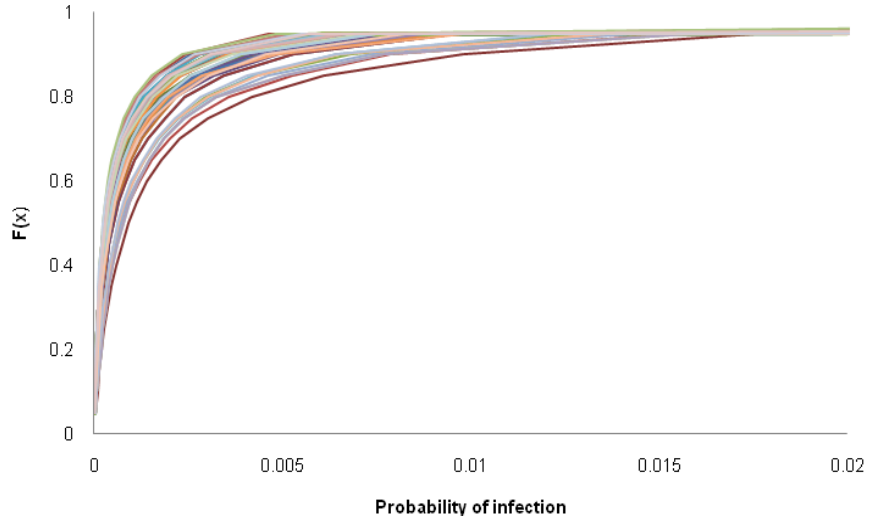

Figure 26: The cumulative second order distribution for the risk of HPAIV H5N1 infection of *at least one* bird in a small-scale broiler flock following consumption of untreated water from a 'large' sized river contaminated by carcasses.

It is important to note that this model makes the large assumption that the distribution of virus within river water is homogeneous (i.e. that there is complete mixing) (*A28*). This is a model simplification, and it is very likely that there is additional variability in the dose with which birds are exposed which is not accounted for in this model, as discussed by Schijven et al. (2005a).

Moreover, these estimates are only relevant for those broiler producers making use of river water, and for those broiler producers that are downstream of another broiler flock. The risks posed by piped or ground water have not been assessed.

10.1.1 The impact of water treatment

The risks posed to broiler chickens drinking contaminated river water appear to be high, particularly for flocks using a small river for as a water source. In reality, however, it is likely that the majority of broiler producers in Indonesia chlorinate water before making this available for birds; this precaution is typically undertaken in order to reduce the risk associated with enteric pathogens (such as *E. coli*) rather than being specifically directed against HPAIV H5N1. Although chlorination of water has long been expected to be effective in the elimination of AIV from drinking water, Rice et al. (2007) were the first to quantify the effectiveness of chlorination in the elimination of HPAIV H5N1 by demonstrating that the maintenance of a free chlorine residual between 0.52-1.08 mg/L was sufficient to inactivate the virus by >3 orders of magnitude within an exposure time of 1 minute. It is generally recommended that commercial poultry producers using chlorination for water sources maintain a free chlorine concentration of between 1 and 5 mg/L, which would be expected to rapidly remove, or diminish the infectivity of HPAIV H5N1, and therefore, if properly applied, reduce the risks associated with river water to low levels. The predicted impact of 1 to 6 log reductions in virus titre on the probability of infection following exposure to water from a small river are presented in Table 40. Whilst a 3 log reduction (Rice et al. 2007) would be expected to reduce the risks to low levels (0.00018 to 0.0011), if broiler producers are required to use river water due to a lack of

alternatives, they should be encouraged to maintain free chlorine concentrations as high as possible to ensure the risk of transmission to their flocks remains at very low levels (as is likely to be achieved at 5 to 6 log reductions in virus concentration).

Table 40: The impact of chlorination on the probability of infection in a small-scale broiler flock exposed to H5N1 contaminated river water from a 'small' river

Log reduction	Mean	5th Percentile	50th Percentile	95th Percentile
1	1.8×10^{-2} – 6.6×10^{-2}	2.8×10^{-4} – 1.4×10^{-3}	4.2×10^{-3} – 2.2×10^{-2}	7.3×10^{-2} – 2.9×10^{-1}
2	2.0×10^{-3} – 8.5×10^{-3}	2.7×10^{-5} – 1.5×10^{-4}	4.2×10^{-4} – 2.2×10^{-3}	8.1×10^{-3} – 3.7×10^{-2}
3	1.8×10^{-4} – 1.1×10^{-3}	2.9×10^{-6} – 1.4×10^{-5}	4.5×10^{-5} – 2.1×10^{-4}	7.3×10^{-4} – 3.9×10^{-3}
4	2.1×10^{-5} – 1.3×10^{-4}	2.7×10^{-7} – 1.3×10^{-6}	4.4×10^{-6} – 2.1×10^{-5}	8.5×10^{-5} – 3.5×10^{-4}
5	1.9×10^{-6} – 1.0×10^{-5}	3.1×10^{-8} – 1.3×10^{-7}	4.3×10^{-6} – 2.3×10^{-6}	7.2×10^{-6} – 3.9×10^{-5}
6	1.9×10^{-7} – 9.3×10^{-7}	2.8×10^{-9} – 1.4×10^{-8}	4.5×10^{-8} – 2.1×10^{-7}	7.5×10^{-7} – 3.5×10^{-6}

10.2 Probability of infection in a household flock

The exposure assessment was repeated by considering the exposure of backyard poultry within a single household to contaminated river water. The same variability and uncertainty distributions were used, but an empirical distribution for the number of poultry owned by a household replaced the number of broilers in the risk model. A specific estimation of the number of poultry owned per household was derived from DGLS surveillance data from Bogor District and Municipality. The mean value based on this distribution was 8, with a range between 1 and 311 birds per household.

Given that the number of susceptible birds owned by a household is considerably smaller than the number present within a small-scale broiler flock, the risk of infection as a result of exposure to contaminated surface water is comparatively small per household (Table 41). The mean estimate for those households using water from a small river is 9.6×10^{-4} (with an uncertainty range of 4.60×10^{-4}-2.32×10^{-3}). Hence, it might be expected that of 10,000 households using river water that has been contaminated by carcasses representing between 1 and 2% of a broiler flock size, on average, around 10 households would be expected to become infected. The average estimates for household flocks using surface water from a medium and large-sized river are considerably smaller at 3.1×10^{-5} and 3.7×10^{-6}.

Table 41: Estimates of the probability of infection of at least one bird within a household owned (sector 4) flock (Pi_{hh}) following exposure to water from small, medium and large rivers contaminated by carcasses comprising between 1 and 2% of the broiler flock on an infected farm

Dilution scenario	Mean Pi_{hh}	5th percentile	50th percentile	95th percentile
Small	4.6×10^{-4} – 2.3×10^{-3}	6.1×10^{-6} – 3.2×10^{-5}	8.6×10^{-5} – 4.0×10^{-4}	1.7×10^{-3} – 8.8×10^{-3}
Medium	1.8×10^{-5} – 9.5×10^{-5}	2.4×10^{-7} – 1.2×10^{-6}	3.3×10^{-6} – 1.6×10^{-5}	6.6×10^{-5} – 3.4×10^{-4}
Large	1.7×10^{-6} – 9.1×10^{-6}	2.3×10^{-8} – 1.2×10^{-7}	3.2×10^{-7} – 1.5×10^{-6}	6.3×10^{-6} – 3.3×10^{-5}

It should be noted that these estimates are based on the assumption that domestic ducks (and other poultry) owned by the household are subject to the same dose-response relationship as domestic chickens. Given that values of r were defined based on chicken-only data, this assumption is not necessarily appropriate, but is applied given the absence of dose and response data for domestic ducks.

10.3 Probability of infection within a *desa*

The process was repeated by considering the exposure of all backyard chickens within a single *desa*, assuming that all households within the *desa* use river water to water their poultry. The number of backyard poultry within a *desa* was derived from DGLS surveillance data, and these were used to derive an empirical distribution with a mean of 5606, and a range of 15 to 23,936. These estimates relate to backyard poultry only. The range of uncertainty outputs from the model with 50 simulations, each with 5000 iterations, is presented in Table 42. Given that the mean estimate of the number of poultry per *desa* is greater than the number per broiler farm, on average, the risk from consumption of contaminated river water would appear to be greater per *desa* compared to a broiler farm. It is also considerably less likely that backyard poultry producers would chlorinate water given to backyard poultry than is the case for broiler producers, and therefore these risk estimates may be more realistic. It is important to note, however that these estimates are based on the large assumption that *all* households in the *desa* make use of river water for the watering needs of their poultry and should therefore be interpreted in this light. Whilst this may be the case for rural communities in close proximity to rivers, it is likely that, in many *desa*s, poultry in households with access to piped or groundwater may make use of these sources for the needs of their poultry and therefore the *desa* poultry population over represent the exposed population.

Table 42: Estimates of the probability of infection of at least one backyard chicken within a *desa* following exposure to water from small, medium and large rivers contaminated by carcasses comprising between 1 and 2% of the broiler flock

Dilution scenario	Mean Pi_{desa}	5th percentile	50th percentile	95th percentile
Small	1.3×10^{-1} – 3.3×10^{-1}	1.5×10^{-3} – 6.8×10^{-3}	4.3×10^{-2} – 1.9×10^{-1}	6.9×10^{-1} – 9.9×10^{-1}
Medium	1.1×10^{-2} – 4.4×10^{-2}	6.0×10^{-5} – 2.7×10^{-4}	1.7×10^{-3} – 8.4×10^{-3}	4.4×10^{-2} – 2.2×10^{-1}
Large	1.3×10^{-3} – 5.8×10^{-3}	5.7×10^{-6} – 2.5×10^{-5}	1.7×10^{-4} – 8.0×10^{-4}	4.3×10^{-3} – 2.3×10^{-2}

11 Exposure assessment: Transmission via contact between live birds

The release assessment provided the basis for estimation of the degree of contact expected between small-scale broiler farms and free-ranging poultry flocks in the District and Municipality of Bogor. The unit of 'contact' in this context is between a broiler farm (i.e. including contact with live birds or, more commonly, with the farm environment) and a backyard flock. The likelihood that a free-ranging flock will become infected following such contact (i.e. whether the contact is 'effective'), *p,* was estimated. We modelled a range of possible values for *p* from 0.01 to 1, and these estimates were multiplied by values drawn at random from the distribution representing the contact rate (i.e. *cp*). Values of *cp* were in turn multiplied by values drawn at random from an empirical distribution for the duration of an outbreak (*d*) derived from the SEIR transmission model developed as part of the release assessment. The output was then an estimate of the number of free-ranging poultry flocks that would be expected to become infected following an outbreak on a small-scale broiler farm and resulting from contact between the infected 'farm' and free-ranging flocks (i.e. excluding indirect routes of transmission). The model was run over 10,000 iterations, and for each iteration a single outbreak scenario was simulated, with the length of the outbreak (in days) determined by a random value from the empirical distribution for *d*, a fixed value for the probability of transmission, *p*, and a random value for the number of household flocks visiting the farm per day. The output for each iteration was then the total number of household flocks becoming infected, given that the number of susceptible birds decreases each time a specific household flock becomes infected.

The parameters used in the model are presented in Table 43.

Table 43: Parameters used in the model predicting the number of household flocks that would become infected through free-ranging following an outbreak on a small-scale broiler farm

Parameter	*Description*	*Distribution/calculation*
c	Contact rate sector 4 flock with small-scale broiler farms (per day)	Empirical distribution (*see text*)
p	Probability of transmission to free-range flock	Uniform(0,1)
cp	Rate of 'effective' contacts	$c \times p$
d	Duration of infection on broiler flock	Empirical distribution (*see text*)

The range of predicted number of household flocks becoming infected at a variety of values of *p* is presented in Table 44. Whilst the value of *p* is likely to be highly variable, depending on the type of broiler farm and its population, the model predicts that there will be relatively little variation in the number of household flocks that become infected. This is perhaps unsurprising given that the mean

outbreak duration is 11 days (5th percentile = 2 days, 95th percentile = 24 days), hence the distribution of number of household flocks becoming infected largely reflects the distribution of flocks visiting a farm, even at relatively low values of *p*.

Table 44: Distributional values for the number of backyard chicken (sector 4) flocks that are expected to become infected as a result of ranging into the environment of a broiler farm on which an H5N1 outbreak is occurring at a range of values for p, the probability of infection in a backyard chicken flock given contact with an infected broiler farm

p	Mean	5th Percentile	50th Percentile	95th Percentile
0.01	0.19	0.004	0.072	0.79
0.05	0.71	0.019	0.30	2.89
0.1	1.09	0.032	0.51	4.25
0.2	1.42	0.058	0.75	5.10
0.3	1.65	0.079	0.89	5.81
0.4	1.68	0.080	0.92	5.72
0.5	1.81	0.087	0.97	6.03
0.6	1.90	0.093	1.01	6.44
0.7	1.94	0.098	1.08	6.50
0.8	1.98	0.10	1.09	6.50
0.9	2.05	0.10	1.10	6.90
1	2.06	0.11	1.12	6.89

This relatively simple model makes the assumption that the probability of transmission to free-ranging flocks is the same for each day of an outbreak, which is not necessarily the case; as demonstrated in Figure 12, on average, the amount of virus entering the environment increases very rapidly until the 4th or 5th day, after which the number of infectious birds in the flock declines (as the number of susceptible birds declines), and hence it might be expected that the risk of transmission to free-ranging chicken flocks would decrease.

11.1 Impact of mitigation strategies: reducing free-ranging during outbreaks

In Bogor, it is generally recommended that all free-ranging poultry within a *desa* are confined for the duration of an HPAI outbreak involving either commercial, small-scale commercial or household flocks. However, given that an outbreak on a small-scale broiler farm can occur for up to 3 days before detection, there may be considerable opportunity for contact between free-ranging flocks and the infected broiler farm before such a policy can even begin to be implemented. Table 45 presents the distributional estimates for the predicted number of backyard flocks that may become infected as a result of contact with an HPAIV H5N1 infected broiler farm before the disease has been detected, but in which contact ceases following detection. These results indicate that the enforcement of measures to prevent contact between backyard flocks and the broiler farm following disease detection are likely to be effective in reducing the average number of backyard flocks that become infected, but this effect is relatively small at high values of *p* (i.e. if the probability of infection following contact is high, then relatively few contacts are necessary for at least one flock to become infected). Hence, on average, contact between free-ranging household flocks and a farm on

which the value of *p* is 0.4 or greater is expected to result in infection of at least one backyard flock within the *desa*, even before the disease has been detected.

Table 45: Distributional estimates of the number of backyard chicken (sector 4) flocks that are expected to become infected as a result of ranging into the environment of a broiler farm on which an H5N1 outbreak is occurring but has not been detected

p[a]	Mean	5th Percentile	95th Percentile
0.01	0.03	0.00	0.03
0.05	0.14	0.00	0.14
0.1	0.28	0.00	0.28
0.2	0.53	0.01	0.53
0.3	0.78	0.01	0.78
0.4	1.00	0.02	1.00
0.5	1.26	0.02	1.26
0.6	1.34	0.02	1.34
0.7	1.58	0.03	1.58
0.8	1.69	0.03	1.69
0.9	1.90	0.03	1.90
1	1.96	0.03	1.96

[a] *p* = the probability of infection in a backyard chicken flock given contact with an infected broiler farm

It is likely that the value of *p* will be influenced to a large extent by the cleanliness of the environment surrounding the broiler farm; those farms that dispose of carcasses, manure and litter into the environment of the farm are more likely to have higher values of *p* than those that dispose of waste products adequately (i.e. using incineration and burying). Hence, as well as recommending that household poultry producers continue to confine poultry upon detection of disease within the *desa*, additional recommendations to improve the cleanliness of the broiler environment (and therefore reduce *p*) can be expected to reduce the number of backyard flocks that become infected following an outbreak in a small-scale broiler flock.

12 Discussion: summary of risk mitigation approaches

12.1 Biosecurity

Biosecurity can be defined as any practice or system that prevents the spread of infectious agents from infected to susceptible animals, or that prevents the introduction of infection into a herd or flock (Radostits 2001). There are a variety of practical control policies that broiler producers can adopt to reduce the risk of disease entry, and these have been described by a number of authors (Otte et al. 2006; Permin and Detmer 2007; FAO 2008a).

The specific disease prevention measures that have been addressed in this report, several of which are already promoted in Indonesia, include:

a) Appropriate disinfection of shoes and equipment brought onto the farm

The use of effective disinfectant foot dips will reduce the risk of virus introduction into a broiler flock. We estimate that the probability of HPAIV H5N1 spread via the footwear of collectors and animal health workers can be reduced to very low levels if at least 2 to 3 log reductions in the contaminating virus titre can be achieved. However, the effective use of foot dips relies on maintenance of disinfectant concentrations at the recommended levels. Moreover, the activity of these agents is generally reduced by the presence of organic material, such as faecal contamination (Dwyer 2004). Hence, poultry producers should be encouraged to make use of foot baths as much as possible but in order to effectively reduce the risks of HPAIV H5N1 transmission between farms, it may also be necessary to conduct producer education on the appropriate preparation and use of disinfectant foot baths. Protective equipment that is available for visitor's use on the farm (i.e. gum boots, protective shoes covers etc.) is also likely to reduce the risk of disease transmission.

Visitors who enter a broiler house may introduce virus on contaminated equipment, with collection crates used by collectors expected to represent a particular risk. In such circumstances, the dipping of feet alone cannot be expected to adequately reduce the risks of HPAIV H5N1 introduction. Producers and visitors should therefore be encouraged to ensure equipment is adequately cleaned and disinfected before it is allowed to enter the broiler house. Those items that are heavily contaminated with faecal material are likely to pose the greatest risk since faeces can contain large amounts of virus and such material can easily be dislodged into the environment of the broiler house. Hence, even if disinfectants are not readily available, the removal of gross faecal contamination (e.g. via spray washing) is likely to reduce risk of onward transmission to low levels and should be encouraged as a matter of course, particularly for collectors.

b) Encourage single depopulation of broilers

This risk assessment has suggested that the movement of poultry collectors between farms may represent a potential route for the spread of HPAIV H5N1 in Bogor District and Municipality. Clearly, the establishment of infection within a flock following the introduction of virus relies on the continued presence of birds within the flock. Hence, if an 'all-out' policy could be adopted for broiler flocks, and all birds removed from the farm as part of a single depopulation, the risks associated with virus introduction by collectors could be effectively eliminated. There is a theoretical risk that a farm

contaminated by collectors could remain infectious for the next batch of birds, although this is likely to be very small (but has not been explicitly assessed in this risk assessment).

Single depopulation of small-scale broiler farms on a large scale would require substantial changes in the way in which broilers are marketed and sold.

c) Treatment of water and/or reduced reliance on rivers as a water source for poultry

This study has demonstrated that the risk of introduction of HPAIV H5N1 into a flock of broilers through consumption of untreated river water is potentially high. Chlorination is likely to be effective against HPAIV H5N1, and all sources of water for broilers should be treated prior to use: this is reportedly already common practice for broiler producers in Bogor District and Municipality. If it is necessary to use river water for the needs of broiler chickens, water treatment should always be employed and free chlorine levels should be held as high as is possible and for as long as possible. Although such an approach will not completely eliminate the risk associated with the use of river water, this risk can be reduced to very low levels.

Whilst the risks posed to individual household flocks from contaminated river water are very low, the risk that at least one bird within a *desa* becomes infected is relatively high if all birds are exposed to contaminated (and untreated) water sources. Clearly, the greater the number of birds within a *desa* that are exposed, the greater the likelihood that at least one will become infected. Hence, although the treatment of river water could be encouraged for all backyard poultry producers, this is likely to be particularly important in those *desa* with large populations of backyard poultry in which river water is used for the drinking needs of a large proportion of poultry.

d) Sanitation of hands

Although broilers are highly stress sensitive and generally handled infrequently, the handling of birds with contaminated hands may result in infection of the birds following transfer of virus to feathers. Any person handling broilers, and particularly those that move from farm to farm, should be encouraged to employ simple hand washing measures with soap and water, and/or the use of a hand sanitizer between farms. These measures are likely to be particularly important for animal health workers, who may handle sick birds in the course of a disease investigation on several farms.

e) Mandatory delays between farm visits

Time is likely to be an effective disinfectant for HPAIV H5N1, which is readily inactivated in the environment. Hence, for all scenarios in which transmission occurs via the environment, measures that allow a delay of 24, or better still, 48, hours for the movement of people between farms are expected to reduce the risk of transmission to low or very low levels.

f) Containment of free-ranging poultry

Given the high density of both sector 4 poultry and small-scale broiler farms in the District and Municipality of Bogor, there is great potential for contact if backyard poultry are allowed to range during the day. Whilst complete confinement of birds in this sector would be the lowest risk strategy, such a strategy is likely to be unrealistic for such a low input system. *Desa*-wide actions to confine free-ranging poultry in the event of an outbreak on a small-scale broiler farm would be expected to reduce the risk of transmission between sectors. Dialogue could be encouraged between broiler farmers and the households that surround their farm; both parties should be aware

of the potential for the transmission of HPAIV H5N1 through the movement of free-ranging backyard flocks and the need for confinement of these in the event of an outbreak on either a broiler farm or in the backyard flock itself.

12.2 Biocontainment

Biocontainment describes the set of practices that can be adopted to reduce the risk of transmission between animals on a farm or from an infected to susceptible farm (Radostits 2001). Hence, whilst biosecurity may reduce the risks associated with HPAIV H5N1 'exposure' on a susceptible small-scale broiler farm or in a susceptible backyard flock, bio-containment can reduce the likelihood that HPAIV H5N1 is 'released' from a farm undergoing an outbreak.

a) Incentivising reporting

If outbreaks of HPAI H5N1 on a broiler farm are reported at the earliest possible opportunity (i.e. following detection or suspicion of disease) we would expect that the risk associated with transmission, particularly via collectors, will be significantly reduced, although not removed entirely. In the model described there may still be substantial risks of onward transmission via water, the movement of animal health workers and contact with free-ranging poultry, even before the disease has been detected on the farm undergoing the outbreak. Incentivising the reporting of infectious disease is a complex issue, but the potential impacts on the onward transmission of virus via collectors are a potential benefit.

b) Maintaining a clean environment around the broiler house

This study has predicted that the rate of contact between free-ranging backyard flocks and small-scale broiler farms can potentially be high. This high rate of contact may allow the infection of at least one free-ranging backyard flock following an outbreak on a small-scale broiler farm. Broiler farmers should be encouraged to maintain as clean an environment as possible around the broiler house, for example by burning or burying waste products and carcasses. The erection of appropriate fences, which completely prevent the entry of free-ranging flocks, would also be expected to reduce the risk of spread of disease between sector 3 and 4 farms within a *desa*.

c) Adequate disposal of poultry carcasses

The disposal of HPAIV H5N1 contaminated poultry carcasses into surface water bodies is likely to present a risk to those broiler chickens and backyard poultry that are within a one-day flow downstream. It is feasible that such practices also pose a risk to public health if untreated river water is consumed by people. These risks could be reduced if broiler producers are educated about the most appropriate ways to dispose of poultry carcasses, and the risks posed to their neighbours downstream could be used as a platform for this. In those areas in which broiler producers have been profiled, such activities can be targeted to those producers located within a certain distance of a river.

13 Conclusion

This risk assessment has used available data and current knowledge in order to predict the risk of spread of HPAIV H5N1 along a variety of transmission routes. The estimates derived should not be considered definitive, but rather a 'best guess', which is as close to the true estimate of risk as it is possible to achieve at this time (Hartnett 2001). In order to reflect uncertainty in a number of key parameters, such as the rate of virus decay in the environment and the virus dose-response relationship, we have employed a second-order modelling approach, and the summary statistics derived from this process have been presented as ranges of values to reflect the uncertainty in these estimates. It should be noted that the risk assessment model is based on a wide variety of assumptions, necessitated by the lack of available data for many of the pathways. Where such assumptions are likely to have a large impact on the risk estimates derived or where there is considerable uncertainty in the assumption, we have attempted to demonstrate the potential impact of alterations in the basic assumption. The assumptions made in this risk assessment are presented in the appendix.

This risk assessment was developed in order to assess the risks of transmission, in quantitative terms, along a selection of high risk pathways. The development of this model has also allowed the testing of a variety of potential risk mitigation strategies that could be established, or should continue to be promoted, in order to reduce the risk of transmission. It is intuitive that the mitigation strategies tested would reduce the risk of transmission, either by reducing contact between susceptible birds and virus in the environment or direct contact between infected and susceptible birds. However, through an estimation of the magnitude of the risk reduction, and potentially, consideration of the associated economic cost of strategy and its likely up-take, these outputs could assist in policy decisions.

Clearly, additional information that would reduce the degree of variability or uncertainty present in distributional estimates would be of use for many of the risk pathways described in this study, however we would like to highlight a selection of key areas in which additional information would be particularly important.

Additional work is required to fully characterize virus survival at the range of environmental conditions observed in Indonesia (and other areas in which HPAI H5N1 is endemic), and, critically, estimates of titre should be taken at more regular intervals in order to fully parameterize the virus decay curve.

The movement of HPAIV H5N1 through the soil profile, and therefore the potential for viruses to contaminate groundwater, is unknown but may present a potential route for the transmission of HPAIV H5N1 (that has not been assessed in this risk assessment). Sampling from groundwater (as well as surface water) at sites from those areas in which outbreaks are ongoing would provide a useful indication of the potential importance of surface and groundwater in the epidemiology of HPAI H5N1.

The ability for the H5N1 virus to detach from carcasses contaminating water bodies is unknown, but is a critical component of a risk assessment considering transfer from carcasses.

Experimental estimates of the dose-response relationship are available from a few studies in domestic chickens, however the sample sizes used and the range of titres tested are typically small. Additional work is required in order to fully characterize this vital parameter, and such studies should use a wider range of infectious doses for multiple virus strains, with a particular emphasis on the low dose estimates.

Additional data are required on the behaviour of collectors in Indonesia, for example real-life estimates for travelling time between farms and collecting yards, and risk behaviour on the farm (in terms of the potential for contamination of the broiler house). Moreover, and given that collectors may play an important part in the transmission of HPAIV H5N1 between farms, additional data are required regarding the disease prevention strategies they adopt.

Observational studies into the distances over which flocks of free-ranging backyard poultry may travel and the degree to which territories may overlap are required to fully assess the importance of contact between sector 3 and 4 flocks in the transmission of HPAIV H5N1.

Out of necessity, and in the absence of adequate data describing the prevalence of infection in the semi-commercial broiler population in Bogor District and Municipality, this risk assessment has considered the risks associated with transmission from an infected farm rather than the current risks associated with transmission of HPAIV H5N1 for all broiler farms or backyard producers in Bogor. In order to consider these specific risks, an estimation of the prevalence of infection within small-scale broiler flocks would need to be incorporated. These estimates are currently unavailable.

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Appendix 1

A lack of data, or the need to simplify complex processes, means most quantitative risk assessments incorporate assumptions, which may have a large influence on the model outcomes. Hence, the assumptions made should be viewed critically when the outputs from a risk assessment model are interpreted. The range of general assumptions used in the model is listed below, and the distributional assumptions and sources of data for the parameters used in the model are presented in Table 1A.

A1: A flock of ground dwelling broiler chickens undergoes random mixing.

A2: Each bird within a flock of ground dwelling broiler chickens makes a fixed number of contacts per unit time, regardless of population size.

A3: Infectious period and latent period are log-normally distributed.

A4: Small-scale broiler farmers in the District and Municipality of Bogor do not vaccinate against H5N1.

A5: Broiler farmers will include H5N1 as a differential diagnosis if the flock mortality exceeds the background level.

A6: All broiler producers will alter their behaviour in the face of an HPAI H5N1 outbreak in their flock.

A7: An average day old chick in Indonesia is 20 g, reaches a weight of 1000 g by 25 days and a weight of 1250g by 45 days.

A8: Virus in faeces and respiratory secretions is Poisson distributed.

A9: Faecal and respiratory HPAIV H5N1 secretions enter the litter where the virus becomes entirely mixed, and therefore viral concentration within the litter can be modelled as a Poisson distribution.

A10: Faecal adherence is equivalent to soil adherence and the distribution described by Finley et al. (1994) (describing soil adherence to hands) can be used as a proxy for the amount of faecal contamination remaining on hands once gross contamination is removed, which, in turn, can be used to predict the amount of virus present.

A11: Any person handling a single infected broiler chicken will be subject to the same degree of viral contamination as someone handling a number of infected broilers.

A12: The rate of decay of HPAIV H5N1 on fomites is the same as the rate of decay of HPAIV H5N1 in faeces.

A13: Poultry collectors will only visit a small-scale broiler farm after the 25th day of production.

A14: The broiler grow-out period finishes by the 40th day of production in Bogor.

A15: Collectors travel between farms via collection yards, and all collection yards used by collectors are located in Bogor city.

A16: All carry crates used by collectors contain up to 25 birds.

A17: Following detection of HPAI, animal health workers visit the farm 6 hours following detection (i.e. mid-way through the day of detection).

A18: The amount of faecal and respiratory secretions available for contamination of a river is equivalent to one day's faecal and respiratory output per bird, and this is leached over the course of a single day.

A19: The rate of HPAIV H5N1 decay in carcasses is equivalent to decay in faeces.

A20: There is complete mixing of leached faecal and respiratory secretions, and complete detachment of virus allowing a relatively homogeneous suspension of virus contaminated river water.

A21: The Euclidean distance between a household with a backyard poultry flock and a small-scale broiler farm is linearly associated with the probability that the backyard flock will enter the confines of the broiler farm.

A22: Kampong chickens show similar ranging behaviour to semi-domesticated jungle fowl.

A23: Free-ranging poultry do not show specific territorial behaviour and the ranges of birds from different households may overlap.

A24: There are not fences surrounding the perimeter of small-scale broiler farms in the District and Municipality of Bogor that would be adequate to prevent the entry of free-ranging chickens.

A25: A broiler chicken will undergo preening *at least* once every 10 minutes.

A26: The concentration of virus within the litter of the susceptible broiler house, following release of faeces from shoes/crates, can be modelled as a Poisson process.

A27: After one day, the virus concentrations at a specific location of a river will be diminished through the combined actions of dispersion and advection (Schijven et al. 2005a).

A28: The distribution of virus within river water following leaching from contaminated carcasses is homogeneous (i.e. there is complete mixing).

Input	*Value/distribution*	*Source*	*Assumptions/ comments*
Transmission model			
Flock size (*n*)	Empirical distribution	(FAO, 2008b)	The cut-off for a 'small-scale' farm was set at 5000 birds or less
Basic reproduction number (R_0)	Uniform(20, 40)	*Assumption*	Assume transmission parameters are higher in a population of ground dwelling birds than predictions based on experimental data (Bouma et al. 2009, Poetri et al. 2009).
Latent period (δ)	Lognormal (0.24, 0.043)	(Bouma et al. 2009)	Latent period is lognormally distributed
Infectious period (γ)	Lognormal (2.1, 0.33)	(Bouma et al. 2009)	Infectious period is lognormally distributed
Number of birds initially infected (N_{inf})	1	-	Baseline scenario. Range of values tested in a sensitivity analysis.
Within-flock prevalence	*Derived from transmission model*		
Time of introduction (T_{inf})	Uniform(1,40)	*Assumption*	Assume virus can be introduced at any point during broiler production with equal probability. The impact of this assumption is estimated.
Visitor contamination			
Detection threshold (mortality rate) (ε)	Uniform(0.01, 0.02)	*Assumption*	Mortality within a single day.
Mean H5N1 respiratory titre (per ml) ($\bar{P}$)	Parametric (Poisson) bootstrap	(Lee et al. 2005; Subbarao et al. 2003; Imai et al. 2007; Li et al. 2008; Bublot et al. 2007a; Bublot et al. 2007b; Swayne et al. 2006)	Virus titre in respiratory secretions is Poisson distributed
Mean H5N1 faecal titre (per g) ($\bar{\tau}$)	Parametric (Poisson) bootstrap	(Lee et al. 2005; Subbarao et al. 2003; Imai et al. 2007; Li	Virus titre in faeces is Poisson distributed

		et al. 2008; Bublot et al. 2007a; Bublot et al. 2007b; Swayne et al. 2006)	
Bird growth rate	DOC =20g, 25 day bird = 1 kg, 45 day bird = 1.25 kg	Assumption	The expected production targets for Broilers in Bogor. Based on these estimates, the daily bird weight was predicted using polynomial regression.
Faecal production per day per gram (F_{day})	Betapert(30, 40,50)/1250	(EPA, 2001)	The variability around this estimate (minimum, most likely, maximum) is assumed, based on available data.
Respiratory production per day (R_{day})	Betapert(0, 5,20)/1250	*Assumption*	The variability around this estimate (minimum, most likely and maximum) are assumed, based on report author's opinion
Virus decay constant (k)	Uniform(0.1604, 0.1972)	(EPA, 2009; Shortridge et al. 1998)	Virus decay on fomites is equivalent to virus decay in faeces. Estimates are based on studies predicting virus survival in the environmental conditions expected in a broiler house.
Day collector visits	Betapert(25, 35, 40)	Assumption	If the disease is detected earlier than this estimate, collectors visit 24 hours following disease detection, but not earlier than 25 days following the start of production. The last day a collector will visit is on the 40th day
Faecal contamination of footwear *(g)* (C_{shoes})	Uniform(30,100)	Assumption	Considers total contamination of shoes for a single visitor entering the broiler house. These data are assumed, however the estimates could be estimated based on a simple experimental study.
Faecal adherence to hands (mg/cm^2) (H_f)	Lognormal(0.52, 0.9)	(Finley et al. 1994)	Assume faecal adherence is equivalent to soil adherence. This assumption is untested. We also assume that the concentration of virus in faeces adhering to hands can be predicted based on estimates of $\overline{\tau}$.
Hand surface area (cm^2)	448	(Lee et al. 2007)	This estimate was incorporated into the model as a fixed value

Speed of travel (Km/hr)	Uniform(10, 50)	*Assumption*	Estimates of speed of travel were combined with distances between farms and distance between farms and Bogor Municipality in order to estimate travelling time between farms (t_f) and travelling time to Bogor (t_b).
Distance between small-scale broiler farms	Empirical distribution	(FAO 2008b)	Prediction based on Euclidean distance (i.e. the straight line) distance between farms.
Distance between small-scale broiler farms and Bogor city	Empirical distribution	(FAO 2008b)	Prediction based on Euclidean distance only (i.e. the straight line distance between farms and Bogor city). Assumes that all collecting yards are in Bogor Municipality, and therefore that all broiler producers in the District and Municipality of Bogor use collectors that are based in the Municipality of Bogor.
Stop-over time for collectors in collecting yards (hrs)	Uniform(0.5, 2)	*Assumption*	These estimates are assumed; additional data collection is required to describe the true range of 'stop-over' values
Minimum number of birds present in flock for collector to visit	Uniform(30, 50)	*Assumption*	These estimates are assumed; additional data collection is required to fully describe the true range of minimum bird numbers
Number of birds present with-in a collection crate	25	Expert opinion (EO)	
Number of crates carried by truck	49	EO	
Number of crates carried by pick-up truck	8	EO	
Faecal production by birds in collection crates per hr	$((F_{day})*2)/24$	(EPA 2001)	We assume that the stress of transport will result in doubling of the faecal output per bird. This is combined with travelling time to Bogor (t_b) to predict the total faecal production per journey
Proportion of faeces remaining in a collection crate	Uniform(0.01, 0.05)	*Assumption*	This is a large assumption, and one that is likely to be difficult to test.

Water contamination			
Flow rate F_r (m^3/day) [1]	8.6×10^4 2.2×10^6 2.3×10^7	Schijven et al. 2005a; Schijven et al. 2005b	Describes 3 hypothesized 'rivers' (small, medium and large). These are considered to be representative of a range of rivers, however these estimates do not reflect the true variability in dilution in surface water
Virus inactivation rate constant in water (μ)	Lognormal(0.043, 0.064)	Brown et al. 2007; Stallknecht et al. 1990a; Stallknecht et al. 1990b	Experimental estimates between 25 and 29°C used (reflecting estimated river water temperature in Bogor, derived from http://www.gemstat.org).
Faeces leached per carcass per day	Betapert(30, 40,50)	Assumption	Based on estimates of F_{day}. Used to predict the contamination of river water by carcasses. The estimate does not include virus that may be present in tissues or organs of the bird, and may therefore be an underestimate.
Respiratory secretions leached per carcass per day	Betapert(0, 5,20)	Assumption	Based on estimates of R_{day}. Used to predict the contamination of river water by carcasses. The estimate does not include virus that may be present in tissues or organs of the bird, and may therefore be an underestimate.

EXPOSURE ASSESSMENT			
Dose-response parameter, (r)	0.00051 (95% C.I. 0.00033 - 0.0013)	Brown et al. 2007; Bublot et al. 2007b	Derived using exponential model. Distributional estimates derived using bootstrapping (see text)
Handling model			
Transfer efficiency (from handling) (TE)	Uniform(0, 0.22)	Pancic et al. 1980, Ansari et al. 1988, Ansari et al. 1991, Bidawad et al. 2004	Estimates describing the efficiency of transfer form hands to the surface of a chicken as a result of handling. The range of estimates used is based on variety of viruses (not including AIV)
Surface Area of 1 kg broiler chicken (cm^2)	1000	Mitchell 1930, Leighton et al. 1966	The surface area of a broiler chicken was incorporated as a fixed value
Time between successive preening activities in broiler chickens (mins)	Uniform(0,10)	*Assumption*	Estimates based on observations from Weeks et al. (2000) and Kuikova et al. (2001)
Proportion of contaminating virus consumed as a result of a single preening activity	Uniform(0,1)	*Assumption*	There is substantial uncertainty in this estimate; therefore the full range of possible values was modelled.
Litter contamination model			
Weight of litter per *sack,* W_{sack}	Uniform(20, 30)	*Assumption,* based on EO	
Number of sacks of litter used per broiler house, S_{litter}	Uniform(125, 250)	*Assumption,* based on EO	
Virus concentration contaminating shoes after arrival at broiler farm, $\bar{\mu}_{shoes}$ (EID_{50})	-	Second order non-parametric distribution	Estimates derived from the visitor contamination module, based on outputs from the SEIR transmission model
Virus concentration contaminating collecting	-	Second order non-	Estimates derived from the visitor contamination module, based

crates after arrival at broiler farm, $\bar{\mu}_{crates}$ (EID_{50})		parametric distribution (see text)	on outputs from the SEIR transmission model
Amount of faecal material transferred from shoes, TE_{shoes}	Uniform(0.01, 0.1)	*Assumption*	This is a large assumption that has an important influence on the risk estimates derived.
Amount of faecal material transferred from crates, TE_{crate}	Uniform(0.001, 0.01)	*Assumption*	This is a large assumption that has an important influence on the risk estimates derived.
Number of collectors per truck, N_c	Uniform(1,3)	*Assumption*	
Broiler feed consumption per day (g)	Uniform(75, 150)	Perry 1981; Brake et al. 1992, RCI 1999,	Feed consumption may vary depending on environmental conditions and bird age.
Litter consumption per day (proportion of total feed consumption), C_l	Betapert(0,0.0128, 0.0635)	Malone et al. 1983	Literature based estimate for a variety of litter substrates, not including rice hulls (the most commonly used litter in Bogor)

Water consumption model			
Drinking rate of domestic chickens per day, *Fc*	Normal(Normal(0.17,0.02)	Manning et al. 2007	Distribution truncated at 0 and 1
Time between bird death and carcass disposal into river (hrs)	Uniform (1,12)	*Assumption*	It is assumed that producers will check for their flocks at least once per day, and discard poultry carcasses at this time
Broiler flock size ($n_{broiler}$)	Empirical distribution	FAO data	The cut-off for a 'small-scale' farm was set at 5000 birds or less
Household flock size (n_{hh})	Empirical distribution	DGLS data	Includes all poultry species owned by a household

Number of poultry within a *desa*	Empirical distribution	DGLS data	Considers only non-commercial poultry within the *desa*
Direct bird-bird transmission model			
Probability of transmission to free-range flock, *p*	Range of values (0.01 to 1)	Assumption	There is a large degree of uncertainty in these estimates, which are likely to vary from farm to farm
Duration of infection on broiler flock, *d*	*Derived from transmission model*		
Proportion of households owning chickens	Betapert(0.01, 0.477, 1)	IFPRI, DGLS, FAO data	The range of values is wide, as indicated by results from the DGLS surveillance data
Proportion of households in which birds are free-ranging	Parametric bootstrap based on observed data	IFLS data (see text)	
Contact rate sector 4 flock with small-scale broiler farms (per day), *c*	-	Empirical distribution (*see text*)	

Appendix 2 Transfer via handling model

Figure 1A shows the simplified spreadsheet model that was used to estimate the probability that a broiler chicken will become infected following handling by someone who has previously handled an infected bird on a farm on which an outbreak is ongoing.

The model considers 3000 separate contamination scenarios (cells C2 to DKM2), where the degree of contamination of each of the cells representing the surface area of a chicken (i.e. cells C27 to C1026 for the first contamination scenario, cells D27 to D1026 for second, and so on) is defined by the concentration of virus per cm^2 of palm surface (C9 to DKM9) and the transfer rate (C11 to DKM11). For each contamination scenario, the parameters describing the contamination of hands (i.e. concentration of virus per cm of faeces, τ (cells c4 to DKM4) and faecal adherence to hands, H_f (cells C6 to DKM6) were drawn at random from their respective probability distributions. In order to incorporate the effect of travel time on virus concentration per cm^2 of contaminated hand, a random estimate from the empirical distribution describing travel times between farms (t_f) was combined with a fixed estimate for the virus decay co-efficient, k.

Following the contamination of its surface area, the modelled 'bird' preens itself at intervals defined by values in cells C15 to DKM15. Each model iteration describes a distinct preening event, with the cm^2 of bird preened defined by a random value between 1 and 1000 (cells C14 to DKM14). The bird is subsequently exposed to a proportion of the virus present in the randomly selected cell (if any), defined by values in cells C17 to DKM17. The exposure dose (cell C18 to DKM18) is combined with a fixed estimate from the uncertainty distribution describing the dose response parameter in order to define the probability of infection given exposure to that dose.

The concentration of virus contaminating each cm^2 of bird declines over time at a rate defined by the virus decay co-efficient (k) and the time between preening events. Moreover, each time a cell is 'preened' the proportion of virus that is ingested by the bird is removed from the amount present (this is automated so that the virus concentration is reduced only after the bird has 'preened'). The simulation continues until the concentration of virus per cell is negligible and therefore the probability of infection (cells C21 to DKM21) remains constant.

The model was repeated over 25 simulations with a randomly selected value from the uncertainty distributions describing *k* and *r* used to populate cells C8 to DKM8 and C19 to DKM19 (respectively) for each simulation. This process, and all model iterations, was automated using VBA in Microsoft Excel with distributions defined in @risk (Palisade Corporation 2009).

	B	C	D	DKM
2	Contamination scenario	1	.....	3000
3	*Virus concentration on hands*			
4	Virus titre	="VIRUS_TITRE_FAECES"	.....	="VIRUS_TITRE_FAECES"
5	Travel time	="Tf"	.....	="Tf"
6	mg/cm	="Hf"	.....	="Hf"
7	g/cm	=C6/1000	.....	=DKM6/1000
8	decay	="K"	.....	="K"
9	Virus per cm hand	=((C4*C7)*EXP(-C8*C5))	.....	=((DKM4*DKM7)*EXP(-DKM8*DKM5))
10	*Virus transfer to chickens*			
11	Transfer rate	="Te"	.....	="Te"
12	Virus per cm3 bird contacted at time zero	=C9*C11	.....	=DKM9*DKM11
13	*Chicken exposure*			
14	Cm^2 preened	=RANDBETWEEN(1,1000)	.....	=RANDBETWEEN(1,1000)
15	Interval between preening	=RANDBETWEEN(0,10)	.....	=RANDBETWEEN(0,10)
16	Cumulative time between preening (t)	=(C16+C15)*$B$24	.....	=(DKM16+DKM15)*$B$24
17	virus consumed in preening	=RANDBETWEEN(0, 100)/100		=RANDBETWEEN(0, 100)/100
18	Dose per per 'preen'	=OFFSET(C26, C14,0)*C17	.....	=OFFSET(DKM26, DKM14,0)*DKM17
19	dose response	="r"	.....	="r"
20	Probability of no infection	=IF($B$25=1,1*(1-(1-EXP(-C19*C18))),C20*(1-(1-EXP(-C19*C18)))*$B$24)	.....	=IF($B$25=1,1*(1-(1-EXP(-DKM19*DKM18))),DKM20*(1-(1-EXP(-DKM19*DKM18)))*$B$24)
21	Probability of infection	=(1-C20)*$B$24	.....	=(1-DKM20)*$B$24
22				
23	Random value	=C14	.....	=DKM14
24	Model "key" (set at 0 or 1)			
25	"Counter"=(B25+1)*B24			
26				
27	1	"see below"	.....	"see below"
476	450	"see below"	.....	"see below"
1026	1000	0	.....	0
1028		C27=		DKM27=
1029		IF(C27=0,(C$9*C$11),IF($B27= C$23,C27*EXP(-C$8*(C$15/60))*(1-C$17),C27*EXP(-C$8*(C$15/60)))))*$B$24		IF(DKM27=0,(DKM$9*DKM$11),IF($B27=DKM$23,DKM27*EXP(-DKM$8*(DKM$15/60)))*(1-DKM$17),DKM27*EXP(-DKM$8*(DKM$15/60))))*$B$24
1030				
1031		C476 =		DKM476=
1032		IF(C476=0,(CC$9*C$11),IF($B476=C$23,C476*EXP(-C$8*(C$15/60))*(1-C$17),C476*EXP(-C$8*(C$15/60))))*$B$24		IF(DKM476=0,(DKM$9*DKM$11),IF($B476=DKM$23,DKM476*EXP(-DKM$8*(DKM$15/60))*(1-DKM$17),DKM476*EXP(-DKM$8*(DKM$15/60))))*$B$24
1033				
1034				
1035				

Figure 1A: Spreadsheet model describing the probability of infection following contamination of the surface of a broiler chicken through handling with contaminated hands

Appendix 3 Transfer via water model

Figure 2A shows the simplified spreadsheet based model used to predict the probability of infection following exposure to river water contaminated through leaching from HPAIV H5N1 contaminated carcasses. The fixed values in the model were estimates of flow rate for a small, medium and large rivers (as described by Schijven et al. 2005a and Schijven et al. 2005b) (Cells C15, C16 and C17, respectively). The model was run over 5000 iterations, with random values drawn from the probability distribution describing mortality rate on the outbreak broiler farm (C3), the population at risk on the outbreak farm (C4), the amount of faeces and respiratory secretion expected to leach from the contaminated carcass (C6 and C7), virus titre per gram of faeces (C8) and respiratory secretion (C9), the chicken drinking rate (litres/day) (C19), the number of broilers on the susceptible broiler farm (C22) or the number of birds within a sector 4 flock (C23) or within a whole *desa* (C24). For each iteration, these estimates were combined with a fixed estimate drawn at random from the distribution describing the rate of virus decay in the carcass (k) (C10), the rate of virus inactivation in water (π) (C18) and the dose response parameter, r (C20) in order to predict the probability of infection of *at least one* bird within a broiler flock, a household flock, or within a *desa* for each of the dilution scenarios described. The process was repeated over 50 simulations.

The model was run in Microsoft Excel with the @Risk extension (Palisade Corporation, 2009)

	B	C	D	E
2			DOSE	
3	Mortality rate on outbreak broiler farm	=RiskUniform(0.01, 0.02)	SMALL	=(C15*$C$19*EXP(-$C$18*$C$21))
4	Number dead birds	=ROUNDUP(C3*"BROILER_DISTRIBUTION_outbreakfarm",0)	MEDIUM	=(C16*$C$19*EXP(-$C$18*$C$21))
5	Delay between death and entering river (hrs)	=RiskUniform(1, 12)	LARGE	=(C17*$C$19*EXP(-$C$18*$C$21))
6	Faecal leachate per dead bird (g)	=RiskPert(30,40,50)	RESPONSE	
7	Respiratory leachate per dead bird (ml)	=RiskPert(1,5,20)	SMALL	=1-EXP(-C20*E3)
8	Virus titre faeces (EID50 per g)	=10^"Virus_Titre_faeces"	MEDIUM	=1-EXP(-C20*E4)
9	Virus titre respiratory secretion (EID50 per ml)	=10^"Virus_titre_respiratory"	LARGE	=1-EXP(-C20*E5)
10	Virus inactivation rate in faeces (hrs)	=RiskSimtable("K")		
11	Contamination from dead bird	=(((C8*C6)+(C9*C7))*C4)*EXP(-C10*C5)	At least one bird in any broiler unit in Bogor	
12	Small river flow rate (l/day))	=8.6*10^7	*SMALL*	=RiskOutput()+1-(1-E7)^C22
13	Medium flow rate (l/day))	=2.2*10^9	*MEDIUM*	=RiskOutput()+1-(1-E8)^C22
14	Large flow rate (l/day))	=2.3*10^10	*LARGE*	=RiskOutput()+1-(1-E9)^C22
15	Conc. of virus (per L) small river (Cr_small)	=$C$11/C12		
16	Conc. of virus (per L) medium river (Cr_medium)	=$C$11/C13	At least one bird IN HOUSEHOLD	
17	Conc. of virus (per L) large river (Cr_large)	=$C$11/C14	*SMALL*	=RiskOutput()+1-(1-E7)^C23
18	Virus inactivation in water (π) (days)	=RiskSimtable("π")	*MEDIUM*	=RiskOutput()+1-(1-E8)^C23
19	Chicken water consumption rate (L/day)	=RiskNormal(0.17,0.02, RiskTruncate(0,1))	*LARGE*	=RiskOutput()+1-(1-E9)^C23
20	Dose response parameter (r)	=RiskSimtable("r")		
21	Time (t) (days)	1	At least one bird in the VILLAGE	
22	Number of broilers on a farm	=ROUNDUP("BROILER_DISTRIBUTION_susceptiblefarm",0)	*SMALL*	=RiskOutput()+1-(1-E7)^C24
23	Number of chickens within a HH	="HOUSEHOLD_CHICKEN_DISTRIBUTION"	*MEDIUM*	=RiskOutput()+1-(1-E8)^C24
24	Number of chickens within a desa	="POULTRY_PER_DESA"	*LARGE*	=RiskOutput()+1-(1-E9)^C24

Figure 2A. Spreadsheet model to predict the probability of infection of at least one bird in a small-scale broiler unit, a backyard flock, or a *Desa* [village] following exposure to river water contaminated by H5N1 contaminated carcasses.

Appendix 4 Transfer via contaminated litter model

Figures 3A to 7A show the structure of the simplified spreadsheet model used to predict the contamination of litter in a susceptible broiler house following the transfer of HPAIV H5N1 contaminated faecal material from shoes and collection crates. The outbreak farm characteristics in Figure 3A were derived from the SEIR disease transmission model. As the within flock prevalence (cell B13, defined by cell E5029) and the weight at the time the birds are collected (B14, defined by values from F5029) were highly correlated ($\rho = 0.79$, $p=<0.01$), these values were incorporated into the model as linked values. The estimate of bird weight at the time of collection was combined with the prediction of faecal output per gram per hour (B17) and the travelling time to Bogor (B20) to predict a single bird's faecal output over the course of the journey (B21). Given that birds may increase their faecal production as a result of the stress of transport, these estimates were doubled. The result was multiplied by 25 to reflect the total faecal output per crate per journey (B22). In order to predict the average amount of HPAIV H5N1 contamination present in collecting crates, matrices were developed approximating the arrangement of collecting crates in a truck (Figure 4A) and pick-up truck (Figure 5A). The number of infectious birds present in each collection crate of the truck or pick-up truck were estimated using separate binomial processes (J5 to P11 and K36 to N37, respectively) where the probability of success (i.e. infection) was defined by the within-flock prevalence at the time of collection (B13). In order to estimate the virus titre per gram of faeces in the crate for the top row of crates on a truck or pick-up truck, the total faecal virus output en route to Bogor was added to the total respiratory virus output and multiplied by the number of infectious birds in the crate (S5 to Y5 and T36 to W36). These estimates were then divided by the total faecal output for all birds in the crate. Hence, we made the simplifying assumption that all virus and faeces produced within a crate are completely mixed (and therefore that faecal output by non infected birds contributes to the denominator). We assumed that between 99 and 95% of faeces produced within a crate would exit via the slats and openings, and this was defined by a separate uniform distribution for each crate in the model (i.e., cells J15 to P21 and K42 to N43)). We therefore assumed that faecal material (and any virus contained within) produced within an upper crate could mix with the faecal material produced by birds within the lower crates. Hence, for example, for the bottom row of crates (S11 to Y11), the estimation of the virus titre per gram of faeces incorporates that produced in the crate itself, as well as virus production in each of the crates above it. As only between 1 to 5% of the faecal material that is produced in the crate (and mixed with faecal material from the crates above) remains, an estimation of the total virus titre per crate was derived by multiplying the estimate of virus concentration per gram of faeces with the amount of faeces remaining (AB5 to AH11 and AB36 to AE37). The average virus titre per crate was then the average of these estimates (AA16 and AA43).

Cells AQ43 to AQ53 in Figure 6A describe total amount of litter and faeces present in the susceptible broiler house at the time of the collector's visit. This relies on the total weight of added litter (AQ47) as well as an estimate of the total amount of faeces present in the house (AQ52). The value in AQ52 is derived by defining a uniform distribution between the expected amount of faeces produced by all birds in the house after 25 days of production and 40 days of production, given the variability in the number of broilers in the house (AQ44), faecal output per gram of broiler chicken per day (AQ51) and fixed polynomial co-efficients describing the growth rate of broilers (AQ48 to AQ50). The second module in the model, predicts litter contamination as a proportion (AQ63 and AQ58) of virus

contamination that is present on carry crates (AQ59 and AQ60) and shoes (AQ57) given a variable number of collectors (AQ56) and a fixed number of crates (AQ61 and AQ62). The degree of virus contamination that collectors could be expected to receive on their footwear was estimated from the SEIR transmission model, and since this parameter was correlated with both the within flock prevalence and the weight of birds at the time of the collectors visit to the outbreak farm, outputs from the SEIR model were incorporated as linked values. Based on the ratio between the amount of virus that is transferred from shoes and crates and the total amount of litter in the house an estimate of HPAIV H5N1 in litter was derived, under the assumption of complete mixing (AS58 and AT58). Given an expected litter consumption rate for broilers per day, a total exposure dose per bird was estimated (AT63 and AT65) and introduced into the dose response model (AU70 and AU71).

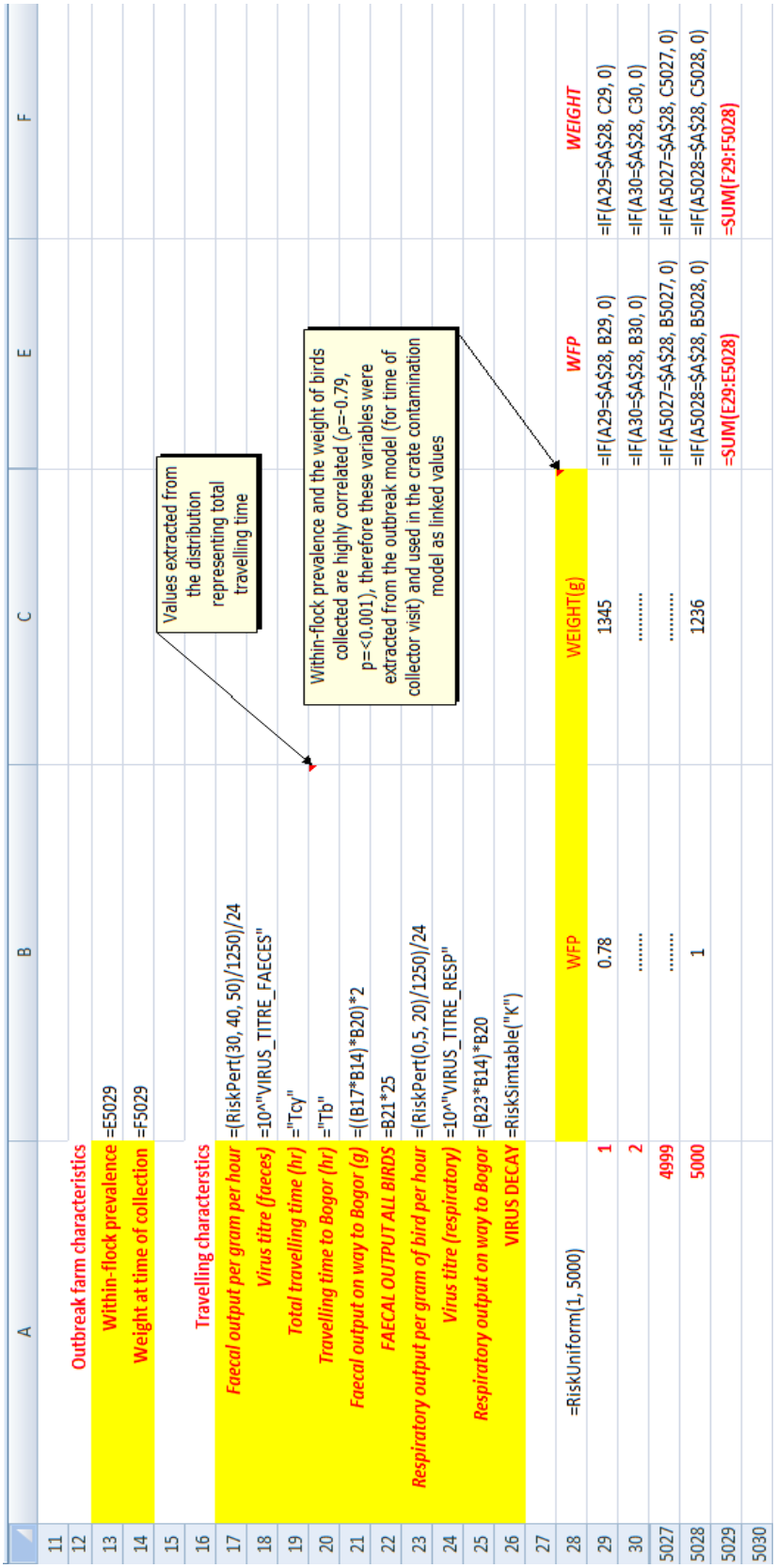
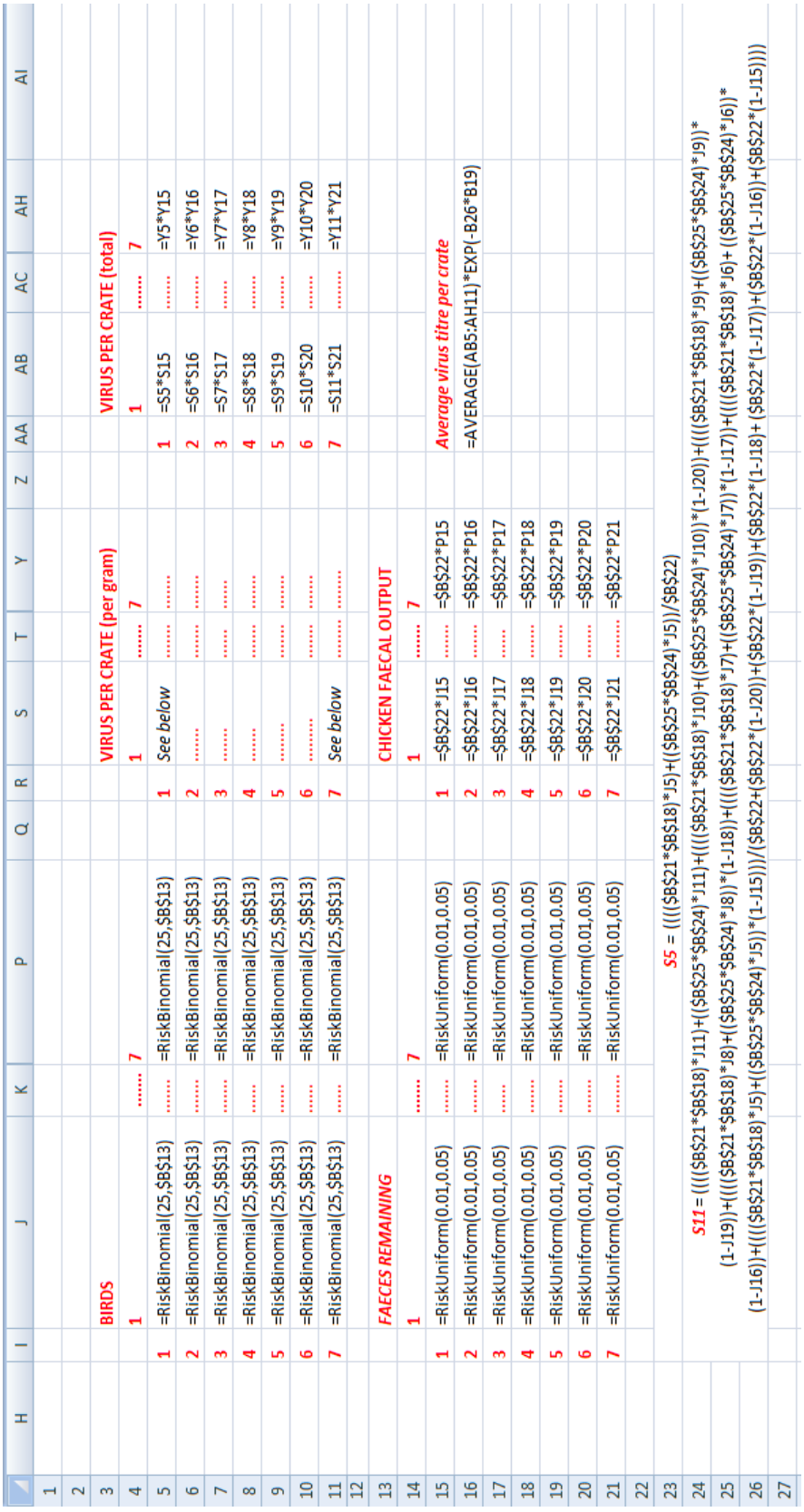
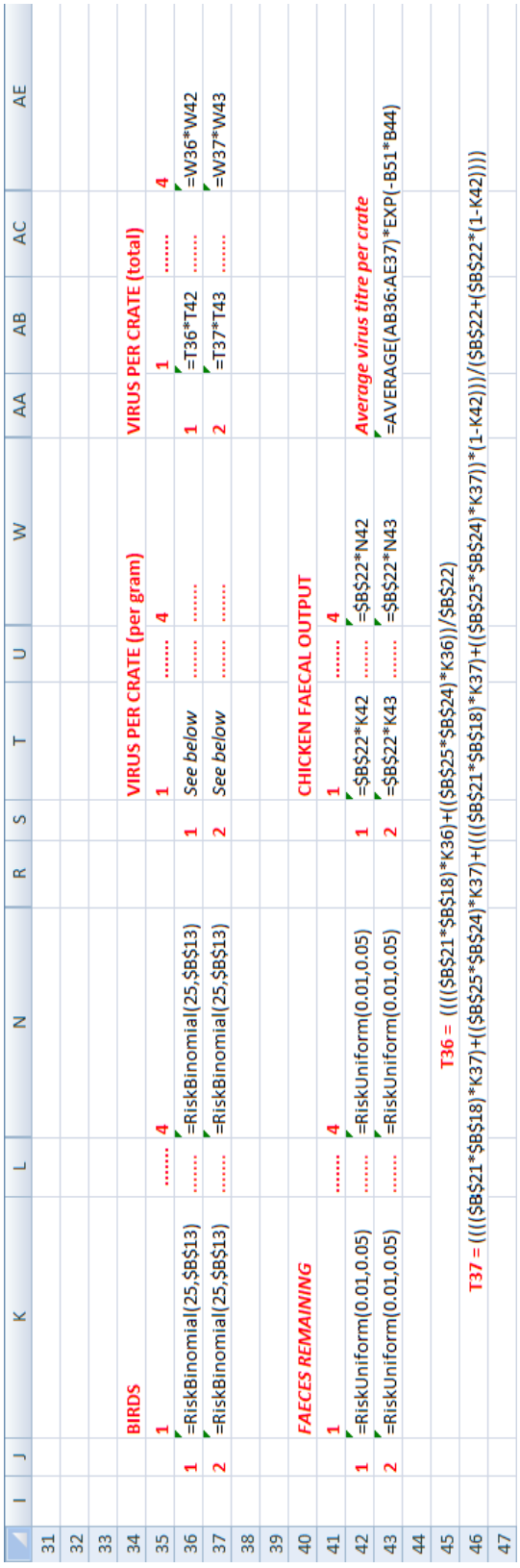


Figure 3A. The spreadsheet model describing the parameters used to estimate the degree of viral contamination expected for collection crates



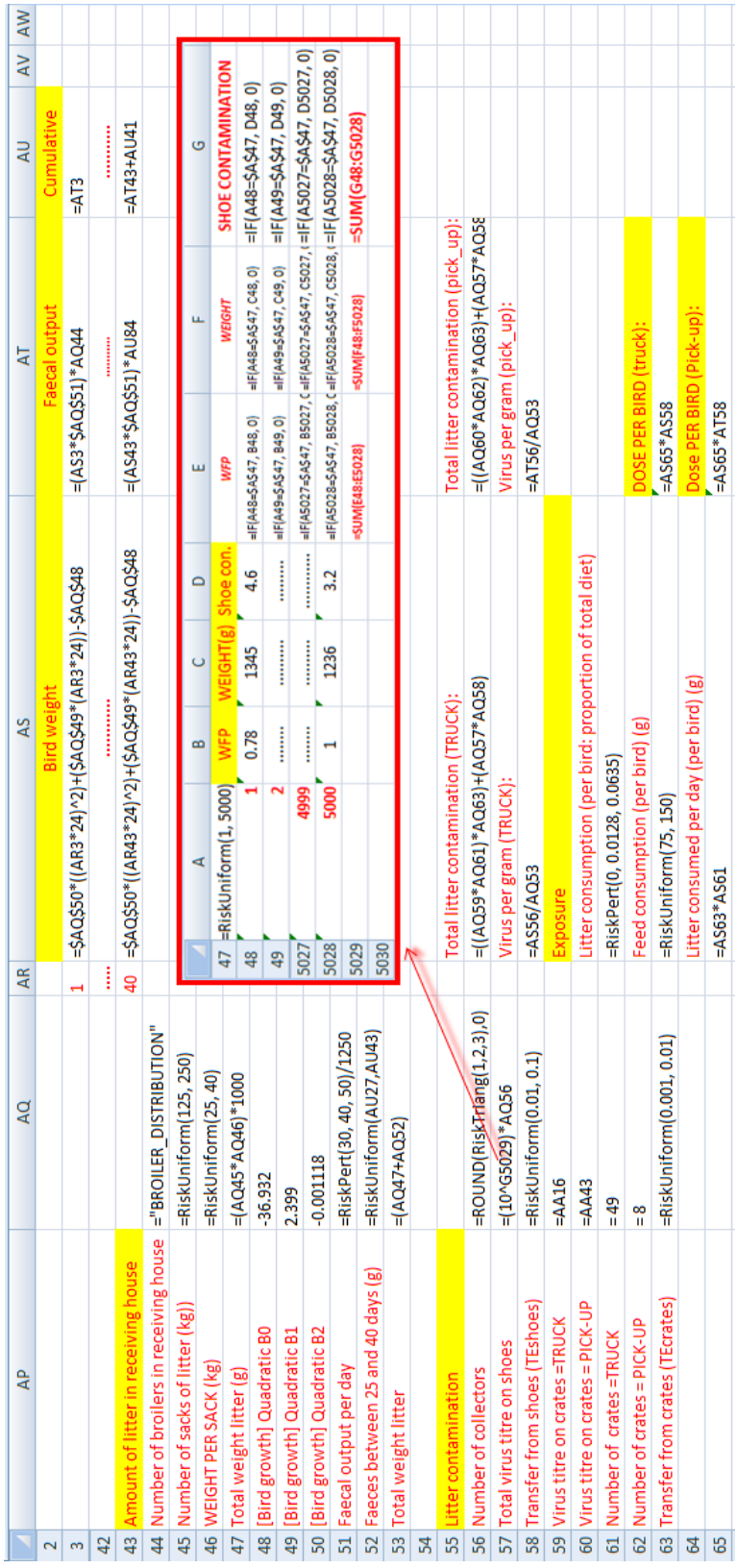
	H	I	J	K	P	Q	R	S	T	Y	Z	AA	AB	AC	AH	AI
1																
2																
3			BIRDS					VIRUS PER CRATE (per gram)					VIRUS PER CRATE (total)			
4			1	.....	7			1	.....	7			1	.....	7	
5		1	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		1	*See below*	.....	.....		1	=S5*S15	.....	=Y5*Y15	
6		2	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		2	.....	.....	.....		2	=S6*S16	.....	=Y6*Y16	
7		3	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		3	.....	.....	.....		3	=S7*S17	.....	=Y7*Y17	
8		4	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		4	.....	.....	.....		4	=S8*S18	.....	=Y8*Y18	
9		5	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		5	.....	.....	.....		5	=S9*S19	.....	=Y9*Y19	
10		6	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		6	.....	.....	.....		6	=S10*S20	.....	=Y10*Y20	
11		7	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		7	*See below*	.....	.....		7	=S11*S21	.....	=Y11*Y21	
12																
13			*FAECES REMAINING*					CHICKEN FAECAL OUTPUT								
14			1	.....	7			1	.....	7						
15		1	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		1	=$B$22*J15	.....	=$B$22*P15			*Average virus titre per crate*			
16		2	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		2	=$B$22*J16	.....	=$B$22*P16			=AVERAGE(AB5:AH11)*EXP(-B26*B19)			
17		3	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		3	=$B$22*J17	.....	=$B$22*P17						
18		4	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		4	=$B$22*J18	.....	=$B$22*P18						
19		5	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		5	=$B$22*J19	.....	=$B$22*P19						
20		6	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		6	=$B$22*J20	.....	=$B$22*P20						
21		7	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		7	=$B$22*J21	.....	=$B$22*P21						
22																
23					*S5* = ((((($B$21*$B$18)*J5)+(($B$25*$B$24)*J5))/$B$22)											
24					*S11* = ((((($B$21*$B$18)*J11)+(($B$25*$B$24)*J11)+((((($B$21*$B$18)*J10)+(($B$25*$B$24)*J10))*(1-J20))+((((($B$21*$B$18)*J9)+(($B$25*$B$24)*J9))*											
25					(1-J19))+((((($B$21*$B$18)*J8)+(($B$25*$B$24)*J8))*(1-J18))+((((($B$21*$B$18)*J7)+(($B$25*$B$24)*J7))*(1-J17))+((((($B$21*$B$18)*J6)+ (($B$25*$B$24)*J6))*											
26					(1-J16))+((((($B$21*$B$18)*J5)+(($B$25*$B$24)*J5))*(1-J15)))/($B$22+($B$22*(1-J20))+($B$22*(1-J19))+($B$22*(1-J18)+ ($B$22*(1-J17))+($B$22*(1-J16))+($B$22*(1-J15))))											
27																

Figure 4A. Spreadsheet based model predicting the HPAIV H5N1 contamination of the 49 collecting crates present within a collection truck, if all crates are filled with birds.



	I	J	K	L	N	R	S	T	U	W	AA	AB	AC	AE
31														
32														
33														
34			BIRDS					VIRUS PER CRATE (per gram)		VIRUS PER CRATE (total)				
35			1	 4				1	 4			1	.....	4
36		1	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		1	*See below*	.....	.....	1	=T36*T42	.....	=W36*W42
37		2	=RiskBinomial(25,$B$13)	.....	=RiskBinomial(25,$B$13)		2	*See below*	.....	.....	2	=T37*T43	.....	=W37*W43
38														
39														
40			*FAECES REMAINING*					CHICKEN FAECAL OUTPUT						
41			1	 4				1	 4					
42		1	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		1	=$B$22*K42	.....	=$B$22*N42		*Average virus titre per crate*		
43		2	=RiskUniform(0.01,0.05)	.....	=RiskUniform(0.01,0.05)		2	=$B$22*K43	.....	=$B$22*N43		=AVERAGE(AB36:AE37)*EXP(-B51*B44)		
44														
45					T36 = ((((($B$21*$B$18)*K36)+((($B$25*$B$24)*K36))/$B$22)									
46					T37 = ((((($B$21*$B$18)*K37)+((($B$25*$B$24)*K37)+((((($B$21*$B$18)*K37)+((($B$25*$B$24)*K37))*(1-K42))))/($B$22+($B$22*(1-K42))))									
47														

Figure 5A. Spreadsheet based model predicting HPAIV H5N1 contamination of the 8 collecting crates present in a pick-up truck, if all are filled with birds



	AP	AQ	AR	AS	AT	AU	AV	AW
2				Bird weight	Faecal output	Cumulative		
3			1	=$AQ$50*((AR3*24)^2)+($AQ$49*(AR3*24))-$AQ$48	=(AS3*$AQ$51)*AQ44	=AT3		
42			.....	.....	.....	.....		
43	Amount of litter in receiving house		40	=$AQ$50*((AR43*24)^2)+($AQ$49*(AR43*24))-$AQ$48	=(AS43*$AQ$51)*AU84	=AT43+AU41		
44	Number of broilers in receiving house	="BROILER_DISTRIBUTION"						
45	Number of sacks of litter (kg))	=RiskUniform(125, 250)						
46	WEIGHT PER SACK (kg)	=RiskUniform(25, 40)						
47	Total weight litter (g)	=(AQ45*AQ46)*1000						
48	[Bird growth] Quadratic B0	-36.932						
49	[Bird growth] Quadratic B1	2.399						
50	[Bird growth] Quadratic B2	-0.001118						
51	Faecal output per day	=RiskPert(30, 40, 50)/1250						
52	Faeces between 25 and 40 days (g)	=RiskUniform(AU27,AU43)						
53	Total weight litter	=(AQ47+AQ52)						
54								
55	Litter contamination			Total litter contamination (TRUCK):	Total litter contamination (pick_up):			
56	Number of collectors	=ROUND(RiskTriang(1,2,3),0)		=((AQ59*AQ61)*AQ63)+(AQ57*AQ58)	=((AQ60*AQ62)*AQ63)+(AQ57*AQ58			
57	Total virus titre on shoes	=(10^G5029)*AQ56		Virus per gram (TRUCK):	Virus per gram (pick_up):			
58	Transfer from shoes (TEshoes)	=RiskUniform(0.01, 0.1)		=AS56/AQ53	=AT56/AQ53			
59	Virus titre on crates =TRUCK	=AA16		Exposure				
60	Virus titre on crates = PICK-UP	=AA43		Litter consumption (per bird: proportion of total diet)				
61	Number of crates =TRUCK	= 49		=RiskPert(0, 0.0128, 0.0635)				
62	Number of crates = PICK-UP	= 8		Feed consumption (per bird) (g)	DOSE PER BIRD (truck):			
63	Transfer from crates (TEcrates)	=RiskUniform(0.001, 0.01)		=RiskUniform(75, 150)	=AS65*AS58			
64				Litter consumed per day (per bird) (g)	Dose PER BIRD (Pick-up):			
65				=AS63*AS61	=AS65*AT58			

	A	B	C	D	E	F	G
47	=RiskUniform(1, 5000)	WFP	WEIGHT(g)	Shoe con.	WFP	WEIGHT	SHOE CONTAMINATION
48		1	0.78	1345	4.6	=IF(A48=$A$47, B48, 0)	=IF(A48=$A$47, C48, 0)	=IF(A48=$A$47, D48, 0)
49		2	.....	.....	.....	=IF(A49=$A$47, B49, 0)	=IF(A49=$A$47, C49, 0)	=IF(A49=$A$47, D49, 0)
5027		4999	.....	.....	.....	=IF(A5027=$A$47, B5027, 0)	=IF(A5027=$A$47, C5027, 0)	=IF(A5027=$A$47, D5027, 0)
5028		5000	1	1236	3.2	=IF(A5028=$A$47, B5028, 0)	=IF(A5028=$A$47, C5028, 0)	=IF(A5028=$A$47, D5028, 0)
5029						=SUM(E48:E5028)	=SUM(F48:F5028)	=SUM(G48:G5028)
5030								

Figure 6A. Spreadsheet based model predicting contamination of the litter of a broiler house with HPAIV H5N1 by poultry collectors

	AH	AT	AU
62		DOSE PER BIRD (truck):	
63		=AS65*AS58	
64		Dose PER BIRD (Pick-up):	
65		=AS65*AT58	
66		Dose reponse parameter	=RiskSimtable("r")
67		Response_Truck	=1-EXP(-AU66*AT63)
68		Response_pick-up	=1-EXP(-AU66*AT65)
69			
70		Probability of infection_TRUCKS	=RiskOutput("Trucks")+1-(1-AU67)^AQ44
71		Probability of infection_PICK_UP	=RiskOutput("Pick_up")+1-(1-AU68)^AQ44
72			

Figure 7A. Spreadsheet based model predicting probability of infection of broilers given exposure to HPAIV H5N1 contaminated litter