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HPAI PRO-POOR HPAI RISK REDUCTION
HIGHLY PATHOGENIC AVIAN INFLUENZA

Quantitative Risk Assessment of HPAI Virus H5N1 Release via Cock Fighting Activities into the 1-km Buffer Zones Surrounding Compartmentalised Broiler Chicken Farms in Thailand

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Preface

Outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 in Eurasia and Africa since 2003 have had a devastating impact on the poultry production sectors of affected countries, as well as national economies and affected farmers' livelihoods. Although the virus can produce fatal disease in susceptible poultry and humans, it is currently not able to transmit effectively between humans. However, it is possible that mutation of the virus could result in sustained human to human transmission. Affected countries have implemented control measures adapted to their resources, infrastructure and characteristics of the poultry production sector.

In order to obtain maximum effectiveness of HPAI control strategies and risk reduction measures, decision making should be based on the best available scientific evidence. The UK Department for International Development (DFID) has funded a collaborative, multi-disciplinary HPAI research project for Southeast Asia and Africa. The specific purpose of the project is to assist decision makers in the development of evidence-based, pro-poor HPAI control measures at both 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 comprise the majority of livestock producers in these countries.

With the above in mind, this document describes a quantitative risk assessment conducted for Thailand in relation to the release of HPAI virus (HPAIV) subtype H5N1 via cock fighting activities into the 1-km buffer zone surrounding compartmentalised broiler chicken farms.

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Disclaimer

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Keywords

Avian Flu, Risk Assessment, Highly Pathogenic Avian Influenza (HPAI), Cock fighting Activities, Buffer zones, Thailand, Fighting Cocks, Compartmentalised Farms.

More information

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

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Executive Summary

After the first outbreak in Thailand caused by highly pathogenic avian influenza virus (HPAIV) subtype H5N1, the Department of Livestock Development (DLD) implemented a range of control measures, including culling (with compensation), movement restriction, improvements in hygiene and biosecurity, and an active surveillance programme (the "X-ray programme"). The DLD has also encouraged broiler chicken farmers to establish compartmentalised production systems, where biosecurity and surveillance measures are implemented within both the boundaries of the farm itself and within the surrounding 1km area around the farm (the 'buffer zone') to protect the health status of the farm's chicken population. The use of compartmentalisation is not only a tool for prevention of HPAIV infection, but also creates disease free areas, allowing a continuation of trade activities. A qualitative risk assessment conducted as part of a larger project funded by the UK Department for International Development (DFID) through the Food and Agriculture Organisation of the United Nations assessed the risk of introduction and transmission of highly pathogenic avian influenza virus (HPAIV) H5N1 into the 1-km buffer zones surrounding compartmentalised poultry farms in Thailand. The results indicated that a more detailed and quantitative risk assessment should be conducted focussing on risk pathways associated with backyard chickens and fighting cocks. Following discussions with the DLD, it was agreed to evaluate the risk of introducing HPAIV subtype H5N1 via cock fighting activities into the buffer zones surrounding compartmentalised broiler farms.

Risk question and methodology

"What is the risk per year for a province in Thailand of viable HPAI virus (HPAIV) subtype H5N1 being released at least once into the buffer zone surrounding a compartmentalised broiler chicken farm via activities associated with cock fighting?"

It needs to be emphasized that the above risk question does only address the likelihood of virus entering a buffer zone, but not its potential subsequent spread.

Risk pathway diagrams were developed for each factor of interest (fighting cocks, human, vehicles and equipment). Quantitative risk assessment models were then developed to estimate the probability of virus introduction for each of these pathways separately, before combining these to produce an overall estimate of the annual risk for a province

(assuming an average of 20 compartments per province). It is important to note that this risk assessment only considers mechanical introduction of HPAIV via contaminated objects and surfaces, incl. externally contaminated humans and fighting cocks, into the buffer zones, although infected birds were considered as a pathway of release of virus into cock fighting establishments.

Data used in the assessment were obtained from the scientific literature, DLD records and a field study. This field study consisted of a face-to-face interview with individuals involved in cock fighting activities as well as direct observation of the practices associated with cock fighting. It was conducted in 2 provinces, Province A in Northern Thailand and Province B in Central Thailand, between May and July 2009. These provinces were selected as they had a high density of compartmentalised poultry farms and frequent cock fighting activities. The detailed findings from the cock-fighting activity field study are presented in a separate report (Appendix C).

The model was used to evaluate three disease and risk management scenarios: (1) absence of evidence of infection, but HPAI H5N1 possibly present at a lower-than-detectable level of prevalence (current situation), (2) epidemic occurrence of HPAI H5N1 outbreaks without risk management procedures being applied at cock fighting establishments based on DLD- hygiene and biosecurity recommendations, and (3) epidemic occurrence of HPAI H5N1 outbreaks with such risk management measures being applied.

Results

Table I. Results of the quantitative risk assessment investigating the annual probability of releasing HPAIV H5N1 into at least one of a province's buffer zones surrounding compartmentalised broiler chicken farms through various cock-fighting associated risk pathways under three different disease and risk management scenarios.

RISK PATHWAY	Absence of evidence of infection, HPAI H5N1 may be present at lower-than-detectable prevalence (current situation)			Epidemic occurrence of HPAI H5N1 disease outbreaks in province without risk management specific to cock fighting *			Epidemic occurrence of HPAI H5N1 disease outbreaks in province with risk management specific to cock fighting*		
	5%	Mode	95%	5%	Mode	95%	5%	Mode	95%
Cock fighting establishments - Open once a week									
Fighting cock	*1.19E-04*	*1.98E-04*	*0.01*	*7.74E-04*	*2.86E-03*	*0.03*	*8.75E-07*	*1.62E-06*	*2.29E-04*
Human	*3.47E-05*	*4.23E-05*	*3.47E-03*	*2.24E-04*	*1.04E-03*	*0.012*	*1.95E-06*	*6.31E-06*	*3.17E-04*
Vehicles	*3.58E-06*	*2.05E-05*	*1.23E-03*	*4.54E.-5*	*9.10E-05*	*7.85E-03*	*8.22E-08*	*2.70E-07*	*3.87E-05*
Equipment	*1.29E-05*	*1.71E-05*	*4.4E-03*	*8.18E-05*	*8.18E-05*	*0.016*	*1.06E-06*	*1.21E-06*	*5.78E-04*
Overall	**2.12E-04**	**6.55E-04**	**0.017**	***1.42E-03***	***4.59E-03***	***0.06***	**8.02E-06**	**6.86E-05**	**1.07E-03**
Cock fighting establishments - Open every day									
Fighting cock	*2.38E-04*	*3.97E-04*	*0.02*	*1.55E-03*	*5.72E-03*	*0.06*	*1.75E-06*	*3.24E-06*	*4.52E-04*
Human	*2.43E-04*	*8.96E-04*	*0.024*	*1.57-E-03*	*7.29E-03*	*0.079*	*1.37E-05*	*4.43E-05*	*2.12E-03*
Vehicles	*2.51E-05*	*1.44E-04*	*8.63E-03*	*3.18E-04*	*6.39E-04*	*0.054*	*5.77E-07*	*1.90E-06*	*2.72E-04*
Equipment	*2.58E-05*	*3.42E-05*	*8.77E-03*	*1.54E-04*	*1.64E-04*	*0.031*	*2.12E-06*	*2.23E-06*	*1.55E-03*
Overall	**6.70E-04**	**1.78E-03**	**0.056**	***4.56E-03***	***0.014***	***0.20***	**2.93E-05**	**4.85E-05**	**3.76E-03**

** hygienic and biosecurity management at cock fighting establishments recommended by the DLD*

Table II. Expected number of years between releases of HPAIV H5N1 via cock fighting associated activities

SCENARIO	Frequency of opening cock fighting	5th Percentile	Mode	95th Percentile
Absence of evidence of infection, HPAI H5N1 may be present at lower-than-detectable prevalence (current situation)	*Once a week*	59	1,527	4,673
	Every day	18	562	1,493
HPAI H5N1 outbreaks in province without risk management specific to cock fighting *	*Once a week*	16	218	876
	Every day	5.1	72	220
HPAI H5N1 outbreaks in province with risk management specific to cock fighting*	*Once a week*	936	14,577	124,660
	Every day	267	20,618	34,167

** hygienic and biosecurity management at cock fighting establishments recommended by the DLD*

Conclusions and Recommendations

Note that these conclusions are only about the risk pathways considered in the model and the outcome is the risk per province per year of viable HPAI virus H5N1 being released into at least one compartmentalised broiler chicken farm's buffer zone. It does not express the risk of exposure of susceptible poultry within the buffer zones or the associated compartmentalised farm.

At province level, given the current HPAI H5N1 risk (not outbreaks or infection reported, but a with the available methods undetectable level of prevalence possible) and current risk management practices at cock fighting establishments and assuming cock fighting establishments are open only once a week, the model estimates that the modal risk (i.e. 50% of the simulation iterations resulted in less than this value) of HPAIV H5N1 being released into the buffer zone of at least one compartmentalised broiler chicken farm within a province per year, through activities associated with cock fighting, to be about once in 1,500 years. The uncertainty around that estimate is considerable in that there is a 5% chance that it occurs more often than once in every 59 years. If the fighting cock arenas are opened every day of the week, the modal risk becomes at least one virus release every 562 years, but there is a 5% chance that it occurs more often than once every 18 years. In the presence of epidemic HPAI within the province without implementation of risk management procedures specific to cock fighting and assuming cock fighting establishments being open

only once a week, the modal risk of HPAIV being released into the buffer zone of at least one compartmentalised broiler chicken farm was at least one virus release every 218 years, and a 5% chance of more than one virus release every 16 years. With the same epidemic HPAI background risk, strict implementation of DLD-recommended hygiene and biosecurity measures at the cock fighting arenas reduced the modal risk to at least one virus release every 14,577 years with there being a 5% chance of it one such event more often than every 936 years. Given the same risk scenario, but assuming daily opening of cock fighting establishments strongly increased the risk estimates, in that the modal risk became one virus release every 20,618 years with a 5% chance of more than 1 such release every 267 years.

The model emphasizes the importance of the background outbreak risk in the province as a key factor influencing the release of HPAIV H5N1 into the buffer zone of compartmentalised broiler chicken farms. In the presence of epidemic disease (i.e. occurrence of outbreaks within the province throughout the year), fighting cocks can play a role in releasing the virus into a buffer zone, and there is a 95% chance that virus is released into at least one farm's buffer zone less than once every 5 years (assuming fighting places being open daily). While full compliance with recommended risk management procedures at cock fighting establishments would reduce that risk substantially, it has to be recognised that it is unlikely that full compliance can be achieved across the whole province. Risk managers might therefore wish to take precautionary action during periods of epidemic outbreak occurrence and prohibit all cock fighting activity.

Although the continuation of currently used risk management procedures at cock fighting establishments in the absence of HPAI H5N1 outbreaks is advised, implementation of additional measures will further reduce the risk of HPAIV release into buffer zones surrounding compartmentalised broiler chicken farms. In particular, the risk assessment indicates that current measures aimed at reducing contamination upon exit from cock fighting establishments may be insufficient to prevent the spread of HPAIV via risk pathways such as contamination of fighting cocks, human clothing and equipment. Therefore, further research on risk mitigation measures targeted at this step in the release pathway is advised, as are measures aimed at encouraging compliance with footbath use amongst visitors to cock fighting establishments and fighting cock passport use by fighting cock owners.

Limitations of the study

Due to limited data availability for some parameters, this risk assessment model had to be based on a number of assumptions. In relation to the HPAIV H5N1 disease prevalence in poultry within a province for the hypothetical epidemic outbreak scenario, the background HPAIV prevalence associated with each risk release pathway was assumed to be equal to the reported HPAI disease outbreak prevalence in the province's poultry population in previous years. It was also assumed that no control measures such as movement restrictions were applied in the province in response to outbreaks, other than the normal DLD recommended biosecurity and hygiene measures for cock fighting activities. In reality, any detected outbreak would result in implementation of control measures that would reduce the disease risk. The model outputs are therefore likely to be an overestimate of true risk per year. It was also assumed that disinfectant foot- and wheel- baths, cleaning procedures for vehicles and equipment, and human showering and changing the clothes were 100% effective at removing HPAIV contamination. These assumptions may lead to underestimation of the true risk.

As pointed out above, the results of this study relate to the release assessment only and do not incorporate exposure and consequence assessment. This means that the model did not estimate the risk of poultry itself becoming exposed to infection or in fact becoming infected in the buffer zone or the compartmentalised broiler chicken farm. Risk management procedures are likely to be in place, particularly in relation to the compartmentalised broiler chicken farms, which would result in some degree of risk mitigation, should HPAIV H5N1 be released into the buffer zone.

Introduction

A UK Department for International Development (DFID) funded qualitative risk assessment of the risk of introduction into and transmission of highly pathogenic avian influenza virus (HPAIV) H5N1 within the 1-km buffer zones surrounding compartmentalised poultry farms in Thailand was conducted (as part of the same project) prior to this study. The study identified that movements of live poultry (including backyard chickens and fighting cocks) presented a very low yet non-negligible risk (with high uncertainty) of introducing the virus into the buffer zones, indicating that a more detailed and quantitative risk assessment should be conducted focussing on backyard chickens and fighting cocks. A workshop was conducted with local veterinary officers to define the risk question for the quantitative risk assessment. It was decided to restrict the study to a quantitative risk assessment of HPAIV H5N1 introduction via cock fighting activities into the buffer zones surrounding compartmentalised broiler chicken farms. In this study, we conducted a release assessment based on the Office International des Epizooties (OIE) methodology for risk assessments (OIE 2005) to evaluate the likelihood of release of HPAIV H5N1 via cock fighting activities into the buffer zone surrounding at least one of a province's compartmentalised commercial broiler chicken farms. Three preventive risk management scenarios were considered: (1) absence of evidence of infection, but HPAI H5N1 may present at a lower-than-detectable level of prevalence (current situation), (2) epidemic occurrence of HPAI H5N1 disease outbreaks without risk management measures at cock fighting establishments based on the Department of Livestock Development's (DLD) hygiene and biosecurity recommendations, and (3) epidemic occurrence of HPAI H5N1 disease outbreaks with such risk management measures.

Glossary and Definitions

Buffer zone: An area of 1-kilometre radius around a compartmentalised broiler chicken farm in which targeted disease surveillance (e.g. routine clinical surveillance and sampling of cloacal swabs) has been implemented. Abattoirs and live bird markets are not permitted within a buffer zone.

Cock fighting activities: These relate to either cock fighting competitions or training matches. In a cock fighting competition the winner receives a reward agreed to prior to the

fight by the owners of a pair of fighting cock combatants, whereas training matches are aimed at cocks improving their fighting experience and stamina. Involvement in these activities influences the risk of transmission into and out of the buffer zone of compartmentalised broiler farms, and the associated risk pathways are investigated in this study.

Cock fighting establishments: The stadiums or training arenas where cock fighting activities take place:

- Permanent fighting cock stadium (PFCS): A permanent stadium with a seating capacity of more than 100 persons and at least 2 permanent arenas for fighting. These are also referred to in this report as 'stadiums'.
- Temporary fighting cock training arena (TFCTP): A temporary arena for training of fighting cocks. This type of arena can be packed up and stored.
- Permanent fighting cock training arena (PTP): A permanent arena for training fighting cocks with a seating capacity of less than 50 persons.

Comparison of fighting cocks: In the context of this study, this relates to the first stage of the cock fighting competition or training. Fighting cocks are brought into the arena/ring of the cock fighting establishment by their owners in order to compare their size, height and weight and select a suitable competitor. If no suitable competitor is found, the fighting cock will not engage in a fight. The number of rounds and competition prizes are also decided at this stage.

Compartmentalised farm: Co-operative or contract chicken farms which have implemented the animal management and biosecurity measures specified by the OIE and the DLD.

DLD recommendations at cock fighting establishments: The hygiene and biosecurity measures recommended by the DLD for permanent cock fighting stadiums and training arenas are as follows: The establishment should be cleaned, sprayed with disinfectant either before or after the fighting/training day, protected by nets to prevent wild bird contact and kept bird-free for seven days. However, in practice, owners of establishments preferably open their stadiums or training arena on a fixed day in a week (e.g. every Saturday). Disinfectant baths for visitors and vehicles should also be provided. Owners of the establishments should prepare and change the disinfectant on every fighting/training day. Records of visitors and cocks should be kept, and fighting cock passports should be checked.

Equipment: Equipment used during the cock fighting activities, such as bamboo coops, blanket, cages, feed, and first aid equipment.

Factor(s): The factors of interest are those factors associated in some way with cock fighting activities that can plausibly mechanically carry HPAIV H5N1 after becoming contaminated at a cock fighting establishment. Fighting cocks, humans, vehicles and equipment were considered as factors of interest in this study. Although migratory and resident wild birds were originally considered as factors of interest, they were not observed within the cock fighting establishments, and therefore were excluded.

Fighting cocks: Cockerels of over 8 months of age which have been selected and trained for cock fighting activities.

Geographical catchment area: The geographical area from within which visitors of a cock fighting establishment come.

Humans: People involved in the cock fighting activities can be classified into 4 groups:

- Fighting cock owners – *people that have reared and taken their own fighting cocks to cock fighting activities;*
- Spectators – *people that visit cock fighting establishments to watch the cock fighting activities;*
- Fighting cock buyers – *people that visit cock fighting establishments to buy and sell fighting cocks;*
- Fighting cock trainers – *those people responsible for training, preparing and caring for fighting cocks during the cock fighting activities.*

Migratory wild birds: Migratory wild birds which may migrate into Thailand

Resident wild birds: Non-migratory wild birds which are permanently resident within Thailand.

Vehicles: Vehicles are used for transporting human, fighting cocks and equipment for the cock fighting activities. For the purposes of this study, vehicles were classified as cars, trucks or motorbikes.

Risk Question

"What is the risk per year for a province in Thailand of viable HPAI virus (HPAIV) subtype H5N1 being released at least once into the buffer zone surrounding a compartmentalised broiler chicken farm via activities associated with cock fighting?"

Note that, this study only assessed the risk of the virus being released into a buffer zone by this particular mechanism, but not whether this results in spread of the virus within the buffer zone.

Hazard

This was defined as highly pathogenic avian influenza virus (HPAIV), subtype H5N1. Infection of chickens with this virus can result in sudden death, respiratory distress, neurological signs, gastrointestinal infections and reduced egg production. Following introduction into a flock, a mortality rate of more than 10% is commonly observed. Duck and geese are also susceptible to infection and may also show a variety of clinical signs, including depression, decreased appetite, ruffled feathers, swollen head and corneal opacity (DLD 2006; Buranathai 2007).

Buffer zones have been placed around compartmentalised poultry farms in order to reduce the risk of entry of HPAIV and therefore possible infection of poultry with the virus, which would have severe economic effects on farmers as well as being a human health risk due to the potential of transmission to humans. A variety of risk management measures have been defined for buffer zones to prevent initial incursion of the virus (NaRanong 2007).

Risk Pathways

Four pathways were considered in the release assessment, the first of which was the movement of fighting cocks (both HPAIV-infected cocks into the cock fighting establishments and HPAIV-contaminated cocks out of the establishments). Additionally, movement of contaminated humans (owners, trainers, spectators and buyers), vehicles and associated equipment into and out of the cock fighting establishments were also considered. The detailed risk pathways for release of viable HPAIV H5N1 into buffer zones associated with each of these mechanisms are outlined in Figures 1 – 4.

Figure 1. Risk pathway diagram for release of HPAIV H5N1 through fighting cocks into the buffer zone surrounding compartmentalised farms.

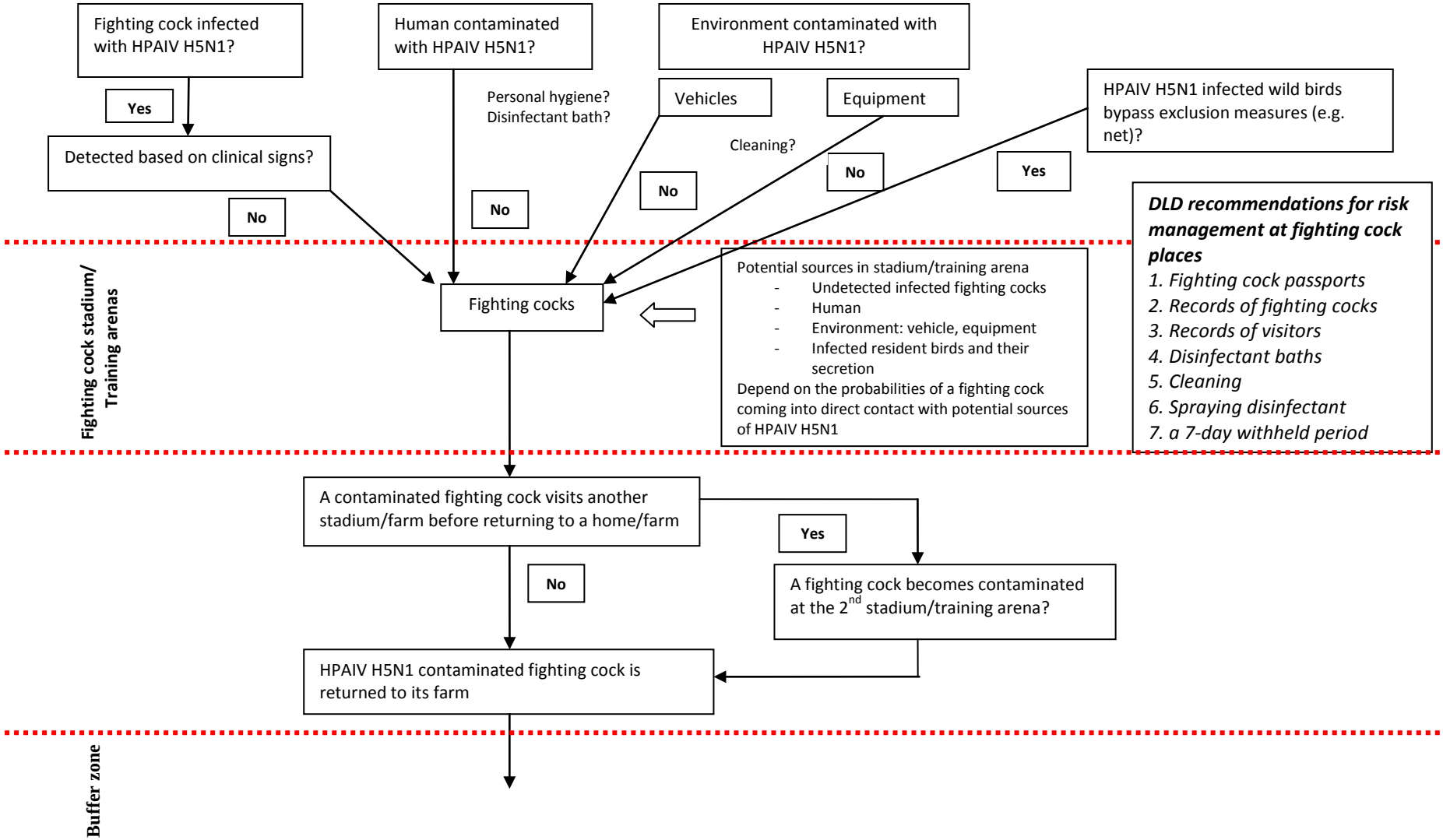


Figure 2. Risk pathway diagram for introduction of HPAIV H5N1 through humans into the buffer zone surrounding compartmentalised farms.

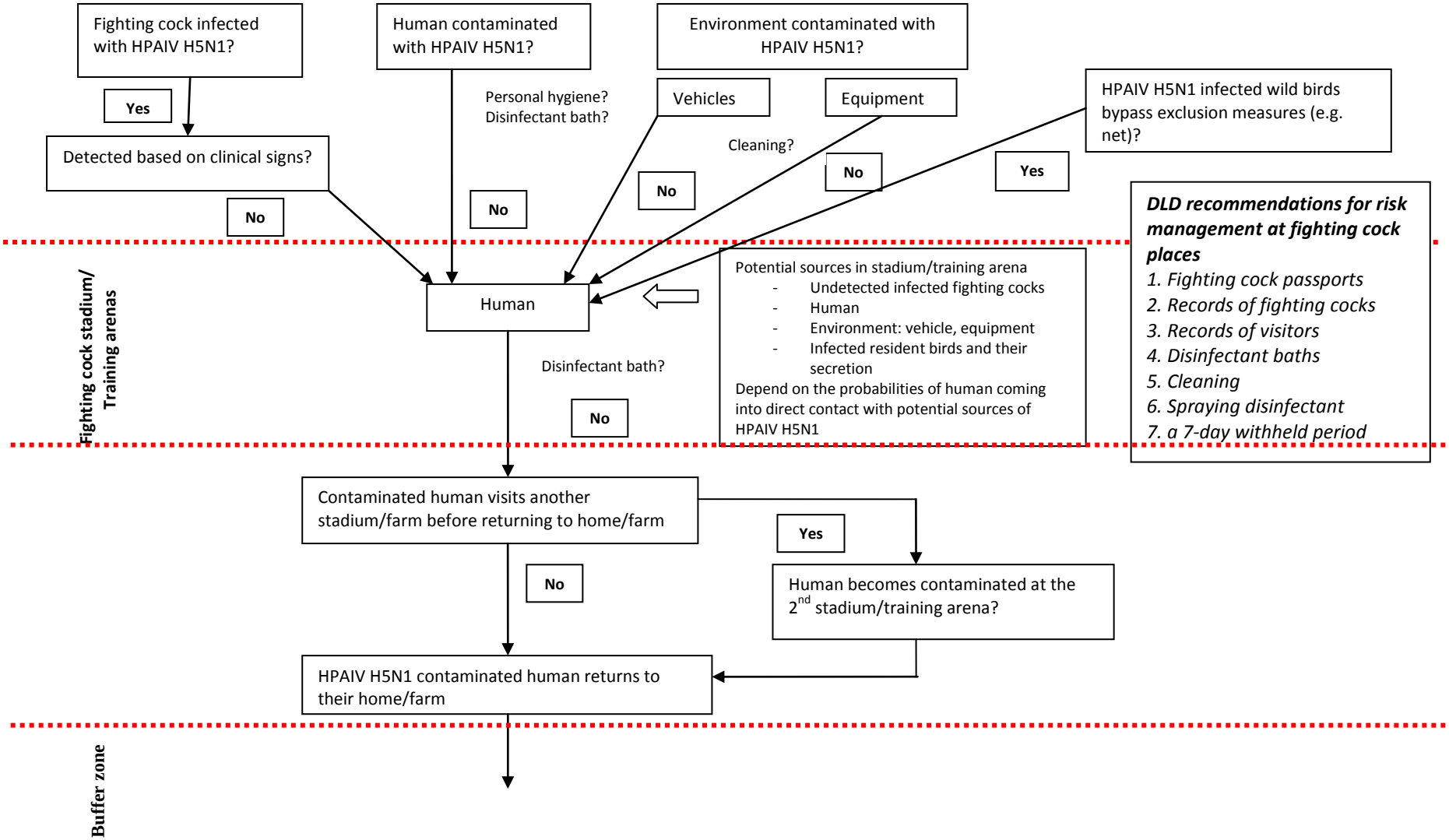


Figure 3. Risk pathway diagram for introduction of HPAIV H5N1 through vehicles into the buffer zone surrounding compartmentalised farms

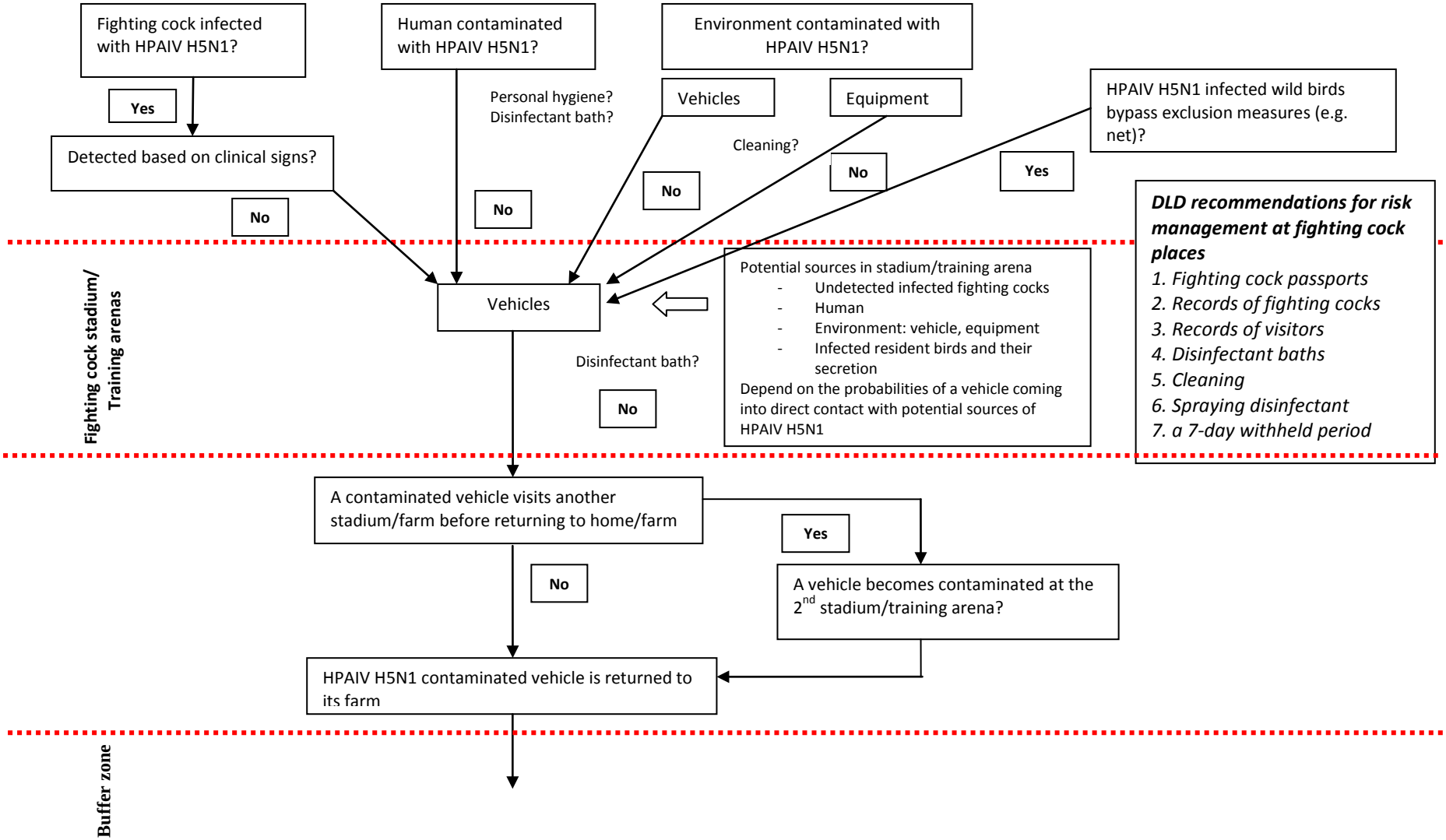
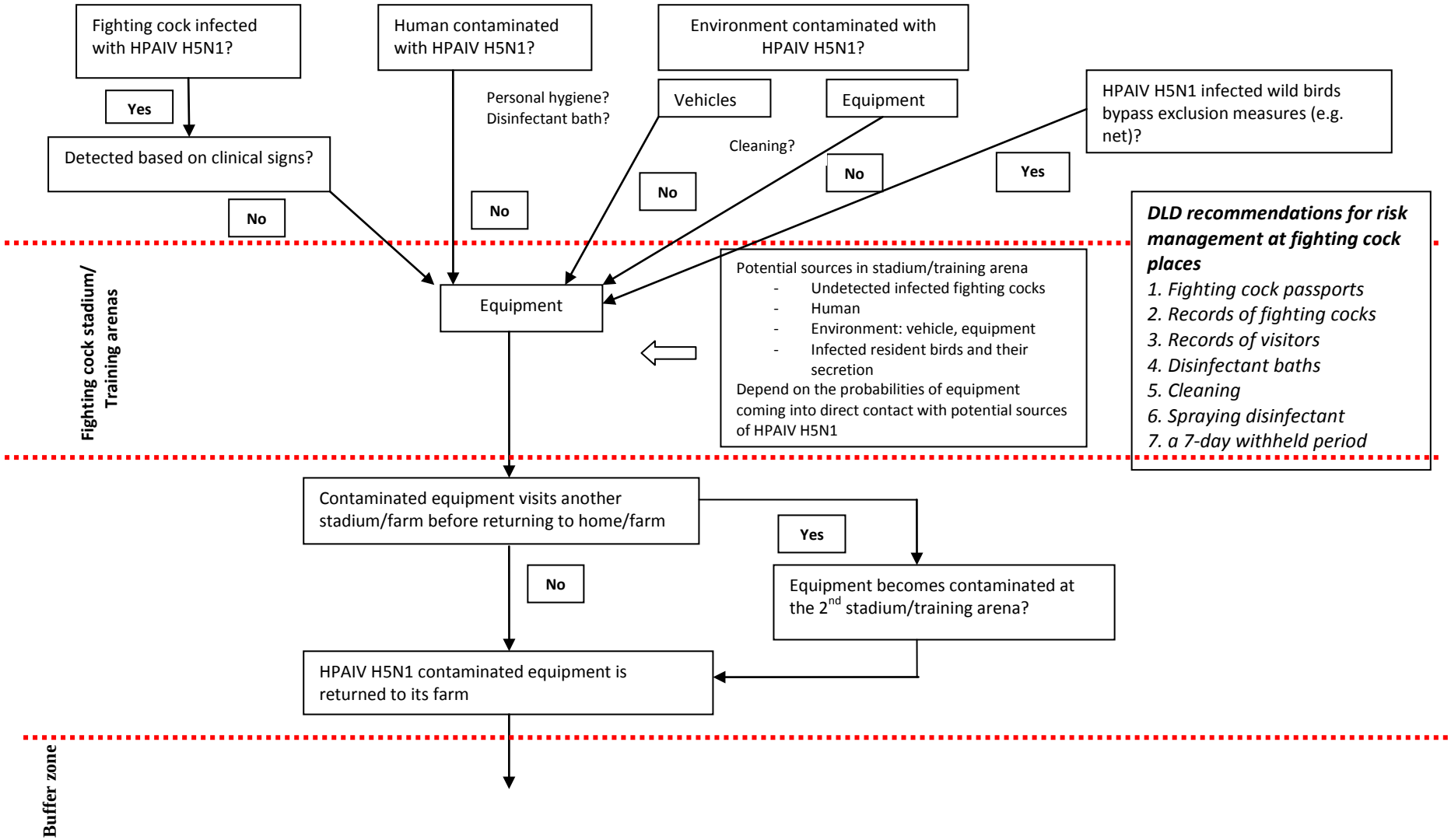


Figure 4. Risk pathway diagram for introduction of HPAI H5N1 virus through equipment into the buffer zone surrounding compartmentalised farms



Data Collection

Data needed

Table 1 describes the data needs and data sources for the different steps of the risk pathway. A separate model was used for each factor of interest (as detailed in figures 1-4), and these models are intended to evaluate the probability that HPAIV can be released into the buffer zone by a particular risk pathway (i.e. the release assessment). These models do not assess the probability of HPAIV transmission to poultry flocks in the buffer zone (the exposure and consequence assessment). Data used in the risk estimation were obtained from review of scientific papers, DLD records and the field study.

Table 1. Data required and data sources for risk pathway steps

Events	Data required	Sources of data
Cock fighting establishment becomes contaminated with HPAIV H5N1 through at least one of the following pathways:	*Probability of cock fighting stadium/training point being contaminated with HPAIV H5N1*	
Fighting cocks		
Location of fighting cock farm	- Presence and prevalence of HPAIV H5N1 in vicinity of farm - Number of fighting cocks on farm - Poultry management factors: free-ranging, mixed species of poultry, quarantine measures and environmental sterilization - Testing for HPAIV H5N1 before movement	- DLD records - Questionnaires* - DLD records & questionnaires*
Frequency of cock fighting activities	- Frequency of fighting activity and numbers of fighting cocks participating in fighting activities	- Questionnaires*
Humans		
Location	- History of HPAI H5N1 disease outbreaks in area	- DLD records - Questionnaires*
Hygiene	- Personal hygiene and sanitation measures of people involved in cock fighting activities	
Environment		
Stadium/training places	- History of HPAI H5N1 disease outbreaks in area - Environmental sterilization: - Method and frequency of cleaning - Types of detergents used - Prevention of resident bird access and pest control (netting)	- DLD records - Questionnaires* and field observation* - Field observation*
Equipment		
Transporting birds	- Method and frequency of cleaning cages - Proportion of birds sharing cages with other birds	- Field observation*
The cock fighting activity	- Method and frequency of cleaning	- Questionnaires*

Events	Data required	Sources of data
	equipment, (e.g. bamboo coops, blanket, feed and first aid equipment) before, during and after the fighting activity	
Rest/waiting for the fight	- Method and frequency of cleaning of cages/equipment - Proportion of fighting cocks sharing cages or equipment with other birds	- Field observation*
Vehicles	- Types of vehicles and their intended use - Method and frequency of cleaning - Proportion visiting another bird farm on the same day as the fighting activities - Duration of journey between household and cock fighting establishment	- Questionnaires*
Resident birds	- Presence and species of resident birds in vicinity of stadium - History of HPAI H5N1 disease outbreaks in area - Morbidity and mortality of HPAI H5N1 in resident birds - Probability of resident birds entering the stadium	- Field observation* - DLD - Siengsanan et al., 2009, Chen et al., 2006 - Field observation*
Contamination of factors of interest (fighting cocks, humans, vehicles and equipment) with HPAIV H5N1 from a contaminated cock fighting establishment from at least one of the following sources:	*Probability of contamination of factors of interest (fighting cocks, humans, vehicles and equipment) with HPAIV H5N1 from a contaminated stadium or training place (estimated separately for each risk pathway).*	
Contaminated fighting cocks **- A HPAIV H5N1-infected fighting cock is not detected during routine surveillance prior to entering the cock fighting establishment** **- A fighting cock is contaminated with HPAIV H5N1 through contaminated media in the cock fighting establishment**	- History of HPAI H5N1 disease outbreaks in area - Number of fighting cocks being taken to the cock fighting establishments - Methods of selecting and preparing fighting cocks for matches or training - Proportion of fighting cock passports checked for HPAIV H5N1 test results - Proportion of a fighting cock being selected for the cock fighting competition or training - Proportion of fighting cocks coming into contact with people - Proportion of fighting cocks sharing equipment with other birds - Proportion of fighting cocks coming into contact with another vehicle than their own	- DLD - Questionnaires* - Questionnaires* - Field observation*
Human **- Presence of virus on the clothes and shoes** **- Contamination of humans with HPAIV H5N1 from contaminated media**	- Probability of human's clothes and shoes being contaminated with HPAIV H5N1 - Virus survival on clothes and shoes - Proportion of people using the disinfectant baths - Proportion of people contacting other fighting cocks - Proportion of people contacting other people - Proportion of people sharing vehicles - Probability of a person contacting another person or sharing another's equipment	- Questionnaires* - Shortridge et al., 1998, Sedlmaier et al., 2009, Lu et al., 2003 - Lombardi et al., 2008 - Field observation* - Field observation* - Field observation* - Field observation

Events	Data required	Sources of data
Environment		
Stadium/training arenas **- Virus survival on the ground** **- Factors of interest are contaminated by HPAIV H5N1 from the ground**	- Virus survival on the ground - Proportion of fighting cocks contacting other fighting cocks' faeces - Proportion of humans contacting bird faeces - Proportion of vehicles contacting bird faeces - Proportion of equipment coming into contact with bird faeces	- Shortridge et al., 1998, Sedlmaier et al., 2009, Lu et al., 2003 - Field observation*
Equipment **- Virus survives on the equipment** **- Equipment is contaminated with HPAIV H5N1 through contaminated factors of interest**	- Virus survival on equipment - Proportion of fighting cocks sharing equipment with other fighting cocks - Probability of humans coming into contact with HPAIV-contaminated equipment - Probability of vehicles coming into contact with HPAIV-contaminated equipment	- Shortridge et al., 1998, Sedlmaier et al., 2009, Lu et al., 2003 - Field observation*
Vehicles **- Virus survives on vehicles** **- A vehicle is contaminated with HPAIV H5N1 from contaminated factors of interest**	- Virus survival on vehicles - Proportion of fighting cocks transported to and from the cock fighting establishment in the same vehicle as fighting cocks owned by other people - Proportion of people sharing vehicles to and from cock fighting establishments - Proportion of all equipment carried to and from the cock fighting establishments by vehicles owned by other people	- Shortridge et al., 1998, Sedlmaier et al., 2009, Lu et al., 2003 - Field observation*
Infected resident birds **- Infected resident birds bypass the cock fighting establishment** **- A factor of interest is contaminated with HPAIV H5N1 from infected resident birds in the cock fighting establishment**	- Presence, species and number of resident birds in proximity of stadium - History of HPAIV H5N1 in area - HPAIV H5N1 morbidity and mortality in resident birds - Presence and number of resident birds at the stadium - Proportion of fighting cocks coming into direct contact with resident birds at the cock fighting establishment - Proportion of humans coming into direct contact with resident birds at the cock fighting establishment - Proportion of vehicles coming into direct contact resident birds at the cock fighting establishment - Proportion of equipment coming into direct contact with resident birds at the cock fighting establishment	- Field observation* - DLD records - Siengsanan et al., 2009, Chen et al., 2009 - Field observation*
Entry of contaminated factor of interest (fighting cocks, humans, vehicles and equipment) to a household or farm within a buffer zone	*Probability of HPAIV H5N1-contaminated factors entering households or farms within the buffer zone*	

Events	Data required	Sources of data
A contaminated factor is transported back to its original location	- Duration and route of transportation - Frequency of fighting cocks participating in cock fighting activities - Frequency of humans participating in cock fighting activities - Frequency of vehicles participating in cock fighting activities - Frequency of equipment participating in cock fighting activities - Duration of sunlight exposure - Percentage reduction in virus quantity as a result of sunlight exposure - Proportion of fighting cocks visiting another cock fighting establishment or poultry farm after leaving the first cock fighting establishment - Proportion of humans visiting another cock fighting establishment or poultry farm after leaving the first cock fighting establishment - Proportion of vehicles visiting another cock fighting establishment or poultry farm after leaving the first cock fighting establishment -Proportion of equipment being taken to another cock fighting establishment or poultry farm after leaving the first cock fighting establishment	- Questionnaires* - Lu et al., 2003 - Questionnaire* and Field observation*

**the cock fighting activity survey comprises of a face-to-face interview with questionnaire and field observation at the participating establishments (see Appendix C)*

Description of the model

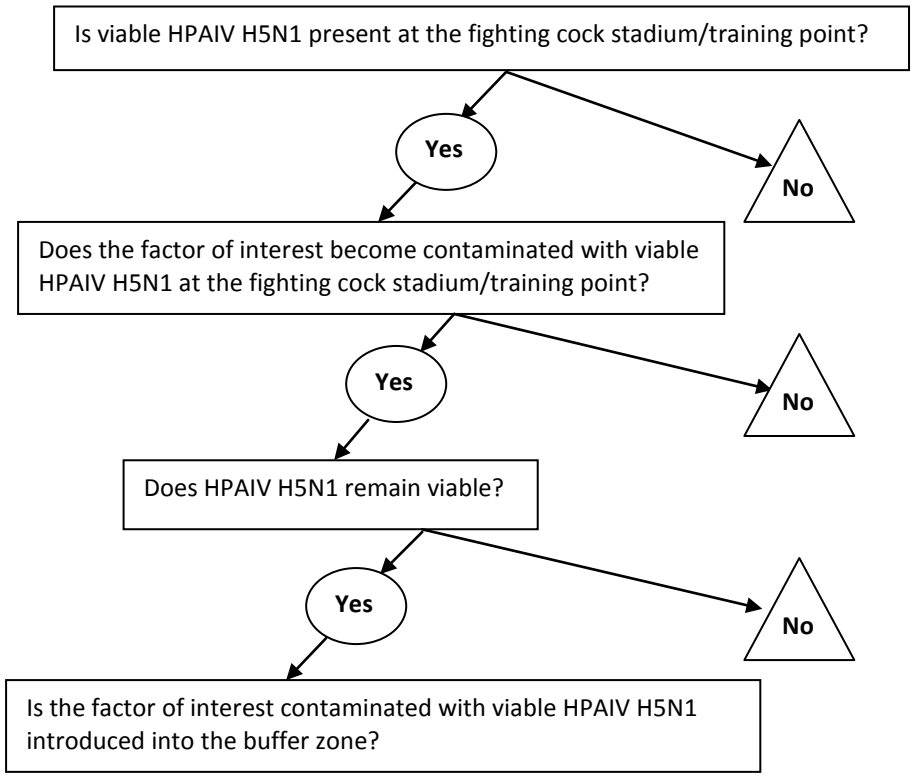
The outcome of interest for each pathway of interest was the probability of release of HPAIV H5N1 into at least one of a province's buffer zones surrounding compartmentalised commercial broiler chicken farms ($P1_x$). A three stage risk pathway (based on that shown in figure 5) was used to estimate the risk of release of HPAIV associated with each pathway of interest using the following equation:

$$P1_x = 1-[1-(Pc \times Pf \times Ps)]^n$$

X relates to a pathway of interest (fighting cocks, humans, vehicles or equipment). Pc is the probability that viable HPAIV H5N1 is present within a cock fighting establishment as a result of the presence of infected fighting cocks or contaminated humans, vehicles or equipment; Pf is the probability that a particular pathway becomes contaminated with viable HPAIV H5N1 at the cock fighting establishment; Ps is the probability of release of HPAIV H5N1 into a buffer zone surrounding a compartmentalised commercial broiler chicken farm from a cock fighting establishment via a particular pathway. n is the frequency of the

event associated with a particular pathway. This study assumed for there to be 100 such events for each particular pathway per province and fighting day, i.e. the number of humans, vehicles etc from a buffer zone that visit a cock fighting establishment and return on the same day. These estimates were based on data from the DLD, which indicates that there are 10 compartmentalised broiler chicken farms, i.e. buffer zones, in province A and 20 in province B. Within each buffer zone, there is a relatively low number of farming households as individual farmers generally need a relatively large area for rice production (province A) or livestock farms (province B). The maximum number of households assumed to participate in cock fighting activities per buffer zone in provinces A and B was 3 and 4, respectively. For the purposes of the current study, it was assumed that each buffer zone contained 5 households participating in cock fighting activities in some way and that the number of buffer zones was 20 per province (thereby modelling a 'worst case' scenario), resulting in 100 households being involved per province and fighting day.

Figure 5. Generic risk pathway for release of HPAIV H5N1 into a buffer zone



Next, the risk of introduction of HPAIV H5N1 into a buffer zone associated with each pathway over the period of a year ($P2_x$) was calculated by using the following equation:

$$P2_x = 1-([1-P1]^F)$$

F is the frequency of cock fighting activities in a year. According to the DLD recommendations, the permanent cock fighting establishments should not open more than once a week. Therefore, this frequency was assumed to be 52. However, the field study indicated that different establishments may open on different days to avoid competing with other establishments for customers. As a worst case scenario, F could therefore be 365 for humans and vehicles assuming that farmers can visit cock fighting establishments every day of the year. However, individual fighting cocks (and therefore also their equipment) cannot participate at this frequency, as they require time for recovery after each match. The field study showed that each of the households involved in cock fighting activities owned several cocks, increasing the possible frequency of engagement in fighting activities. Based on this, it was decided that the maximum annual frequency of fighting activities per household would be 104 (i.e. two per week per household).

Finally, the risks associated with each factor were combined in order to estimate the overall annual risk of HPAIV release into at least one of a province's buffer zones through at least one of the pathways considered (P3), using the following equation:

$$P3 = 1- [(1-P_{\text{fighting cock}}) \times (1-P_{\text{human}}) \times (1-P_{\text{equipment}}) \times (1-P_{\text{vehicle}})]$$

It is recognised that many of the parameters used for this model are subject to substantial uncertainty. In this risk assessment it was decided to not model uncertainty and variability separately, since the model would otherwise have become much more complex and therefore difficult to communicate to stakeholders.

Model parameters

1. Probability that cock fighting establishments become contaminated with HPAIV H5N1

Six episodes of HPAI H5N1 disease outbreaks have been reported in Thailand since the disease was first reported in 2004 (Poovorawan 2007; Tiensin 2009). The DLD has implemented control measures and an active surveillance programme (the "X-ray programme") in order to improve early detection of infection in poultry (Tiensin *et al*. 2007). All poultry are required to have tested negative for HPAIV H5N1 based on reverse-

transcriptase polymerase chain reaction and viral isolation applied to cloacal swabs prior to movement. Since fighting cocks are potentially moved relatively frequently, the DLD introduced a compulsory system of registration based on a "passport". As part of this scheme, fighting cocks must be tested monthly at the local DLD office for HPAIV H5N1 and prior to any movement the DLD has to be presented with an up-to-date passport with negative test results (Buranathai 2007; Tiensin 2009). The DLD also recommends cock fighting establishments to check fighting cock passports, record the names and addresses of people entering and leaving, and record the numbers of fighting cocks and owners.

At the cock fighting establishments, the DLD also recommends the implementation of hygiene measures, specifically the use of disinfectant footbaths for all humans and vehicles entering (which should be changed for each day of cock fighting activities), cleaning and disinfectant use throughout the establishment and on any vehicles after the matches, and ensuring the establishment is kept bird-free for at least seven days after each match (DLD 2006). Note that this number of days in effect means that cock fighting establishments can only open less than once a week, which in practical terms means that owners will tend to not comply and they will be opened at least once a week. HPAIV may be mechanically carried into the buffer zone of compartmentalised commercial poultry farms from cock fighting establishments by contaminated fighting cocks, humans, vehicles and equipment. Although wild and resident birds may also acquire HPAIV infection and spread the virus (Siengsanan 2009), neither wild nor resident birds were observed in any of the cock fighting establishments during the cock-fighting activities survey, and so this potential pathway of virus release was excluded from this assessment.

The probability of a cock fighting establishment becoming contaminated with HPAIV depends both on the prevalence of HPAIV in its geographical catchment area and on hygiene and biosecurity measures in place within the establishment. These will be discussed in more detail below.

HPAIV prevalence: In this study, we focused on four potential risk pathways: fighting cocks, humans, vehicles and equipment. It was assumed that the prevalence of HPAIV infection in fighting cocks taken to the establishments or HPAIV contamination of the factors of interest was equal to the HPAIV prevalence in poultry in each province, as calculated from the results of the 'X-ray' HPAI surveillance programme. Based on the 'X-ray' programme in 2008, all 117,524 pooled samples taken from a total of 2,628,512 bird flocks (samples were

pooled across several flocks) were negative to AIV - resulting in 99.5% confidence that the AI prevalence was less than 0.001%. The 'Betabuster' software programme (University of California, Davis) was used to convert these values into alpha (a) and beta (b) parameters. These were then used in the model to define the beta probability distribution for representing the prevalence of HPAIV in the different disease and risk management scenarios. For scenario no. 1, it is assumed that HPAIV H5N1 prevalence is present at a level lower than the intensive active surveillance and control in the province (current situation) is capable of detecting, the probability distribution for HPAIV prevalence was assumed to be: prev = 1.67E-07 (1.71E-06 – 9.95E-05)(a = 1 and b = 29 955.8). For the two other scenarios assuming epidemic occurrence of HPAI H5N1 disease outbreaks with or without risk management procedures being applied at cock fighting establishments based on DLD-hygiene and biosecurity recommendations, data on reported daily HPAI outbreaks and the number of poultry flocks in both provinces of interest in 2004 were used to estimate the HPAI outbreak prevalence. Due to the HPAI prevalence being estimated at a flock level, it was assumed that all fighting cocks in a flock were infected once a flock became infected with HPAIV. The HPAIV prevalence was modelled using a betaPERT probability distribution with a minimum, most likely and maximum values of 0, 3.26E-05 and 6.19E-04, respectively.

Hygiene and biosecurity measures in cock fighting establishments: As mentioned above, cock fighting establishments should meet DLD recommendations relating to biosecurity in these establishments in order to be registered and allowed to operate. Commonly used disinfectants such as phenolic disinfectants, quaternary ammonia compounds, peroxygen compounds and iodine/acid disinfectants have been reported to be 100% effective in the inactivation of avian influenza (AI) viruses (Suarez 2003; Lombardi 2008). Based on field observation, if the disinfectant baths are correctly filled and used, or the cock fighting establishment is sprayed with freshly prepared disinfectant obtained from the DLD, the effectiveness of these in inactivating AI viruses would be expected to be around 100%. The proportion of cock fighting establishments adopting effective cleaning practices was modelled using a beta probability distribution, as shown in Table 2.

Table 2. Beta distribution parameters used in the model

Beta (s+1, n-s+1)	s		n	
Proportion of cock fighting establishments which are effectively cleaned	*Number of cock fighting establishments being cleaned, sprayed with disinfectant and closed for 6-days*	*2*	*Number of cock fighting establishments visited by the risk assessment (RA) team*	*15*
Proportion of cock fighting establishments with disinfectant baths in use	*Number of cock fighting establishments having disinfectant baths in use*	*2*	*Number of cock fighting establishments visited by the RA team*	*15*
Proportion of cock fighting establishments in which fighting cocks shared vehicles owned by other people	*Number of cock fighting establishments in which fighting cocks shared vehicles owned by other people*	*0*	*Number of cock fighting establishments visited by the RA team*	*15*
Proportion of fighting cock owners with fighting cock passports	*Number of fighting cock owners with fighting cock passports*	*0*	*Number of respondents with their own fighting cocks (i.e. number of fighting cock owners)*	*193*
Proportion of humans in close contact with fighting cocks	*Number of fighting cock owners and trainers*	*263*	*Number of respondents*	*526*
Proportion of people who shower and change clothes before leaving home	*Number of people reporting showering and changing clothes before leaving home*	*526*	*Number of respondents*	*526*
Proportion of vehicles cleaned before transporting fighting cocks	*Number of fighting cock owners cleaning their vehicles before transporting fighting cocks*	*0*	*Number of fighting cock owners amongst respondents*	*193*
Proportion of equipment cleaned before use	*Number of fighting cock owners cleaning their equipment before use*	*0*	*Number of fighting cock owners amongst respondents*	*193*
Proportion of equipment shared with other fighting cocks	*Number of fighting cock owners or trainers sharing equipment with other owners or trainers*	*0*	*Number of fighting cock owners or trainers amongst respondents*	*263*
Proportion of humans visiting a second cock fighting establishment	*Number of fighting cock owners or trainers visiting a second cock fighting establishment for fighting or training on the same day*	*15*	*Number of fighting cock owners or trainers amongst respondents*	*263*
Proportion of humans visiting a poultry farm after having been at a cock fighting establishment	*Number of people visiting a household with backyard chickens on the way home from a cock fighting establishment*	*50*	*Number of respondents*	*526*
Proportion of vehicles exposed to sunlight for more than 30 minutes during the journey	*Number of vehicles driving for more than 30 minutes during the journey.*	*78*	*Number of vehicles*	*526*

Fighting cocks: Experience suggests that owners of fighting cocks take good care of their birds and will bring only healthy cocks to fighting or training events (Paul 2010). Although HPAIV H5N1 infection in chickens has been shown to cause sudden death with 100% mortality within 2-4 days of infection, no clinical signs were apparent prior to this (Saito 2009). At the cock fighting establishments, fighting cock passports are checked in

order to ensure HPAIV testing is up-to-date. As passports are compulsory, fighting cocks are not allowed to enter cock fighting establishments if their owner fails to show an up-to-date passport. The proportion of fighting cock owners with correct fighting cock passports was modelled using a beta probability distribution (Table 2). Workers at the cock fighting establishments inspect all fighting cocks for any clinical signs of HPAI H5N1, following the DLD case definition. This case definition has been reported to have a high sensitivity (95-100%) in the early detection of disease (DLD 2006). The sensitivity of this screening was therefore modelled using a betaPERT probability distribution with a minimum, most likely and maximum values of 0.95, 0.99 and 1.0, respectively.

Human, vehicles and equipment: It was assumed that showering and changing clothes, cleaning vehicles and cleaning cock fighting equipment before visiting the cock fighting establishments would completely remove any HPAIV contamination. The proportion of people following each of these procedures was modelled using beta probability distributions (Table 2).

2. Probability of contamination with HPAIV H5N1 at a cock fighting establishment for each risk pathway considered

In the model, cock fighting establishments could become contaminated with HPAIV through the entry of HPAIV-infected fighting cocks or HPAIV contaminated people, equipment or vehicles, thereby influencing the risk of contamination for the risk pathways considered here. Note that it is considered highly unlikely that HPAIV could be released into a cock fighting establishments through infected fighting cocks or wild birds, and therefore these mechanisms were not represented here.

Fighting cocks come into contact with other fighting cocks during a match. Therefore the probability of contact between fighting cocks is dependent on the probability that a fighting cock is selected for a fighting or training match during the stage of comparison of fighting cocks. Based on the interview survey conducted with the owners of fighting cocks and the establishments, it was observed that all fighting cocks brought to the training places did engage in a match (due to prior arrangement between owners), whereas stadium owners reported that only approximately 80% of fighting cocks arriving at the stadiums could be matched with a suitable combatant and therefore went on to fight. The probability of being matched with a suitable combatant was modelled using a betaPERT probability

distribution with a minimum, most likely and maximum value of 0.8, 0.9 and 1.0, respectively.

Only fighting cock owners and trainers are allowed to come into direct contact with fighting cocks, and this occurs only during the matching comparison for identification of potential combatant pairs. The estimate of the probability of humans coming into direct contact with fighting cocks was modelled as the proportion of humans who were fighting cock owners and trainers, using a beta distribution (Table 2).

The probability of a fighting cock coming into direct contact with equipment belonging to another owner was modelled as the proportion of equipment which was shared between owners, using a beta distribution (Table 2). The probability of contact between fighting cocks and vehicles was estimated similarly, with the proportion of vehicles which were shared between owners carrying fighting cocks to a fighting cock establishment being modelled using a beta distribution (Table 2).

3. Probability that risk pathways linking cock fighting establishments with buffer zones around compartmentalised commercial poultry farms become contaminated with HPAIV H5N1

DLD recommends that disinfectant foot- and wheel- baths for use by all visitors and their vehicles should be used when exiting a fighting cock establishment. Although these would be expected to remove contamination with HPAIV H5N1 completely, compliance with the use of these was observed to be poor during the survey. Therefore, the probability of removal of HPAIV H5N1 was modelled based on the observed use of these baths, using a beta distribution (Table 2).

After exiting a cock fighting establishment, each risk pathway may also become contaminated by visiting another cock fighting establishment or a household with backyard chickens. The proportion of fighting cocks taken to each of these was individually modelled using beta distributions (Table 2). It was assumed that the risk of HPAI contamination from visiting a second fighting establishment was the same as that at the first establishment, and that any backyard chickens contacted would have the same prevalence of HPAIV infection as the province

Sunlight exposure during transport will have the effect of reducing HPAIV contamination, with direct exposure for a 30 minute period being expected to inactivate

HPAI viruses completely (Songserm 2006). However, this UV virus destruction depends on both the degree of exposure to the light source and the medium which the virus is in contact with – meaning that only viruses on certain surfaces exposed to sunlight will be inactivated in this way (Chumpolbanchorn 2006; Shahid, Abubakar et al. 2009). In the model, the duration of travelling was used as a proxy for the duration of sunlight exposure, and the probability of sunlight exposure of more than 30 minute duration was modelled using a beta distribution (Table 2). The effectiveness of sunlight exposure for reducing HPAIV contamination may reach 100% only in certain environmental conditions (such as high relative humidity and temperature) (Weber and Stilianakis 2008) – at room temperature, four hours of continuous UV exposure may be required to inactivate the virus, whereas at 56^{o}C, only 30 minutes of UV exposure is required (Chumpolbanchorn 2006; Shahid, Abubakar et al. 2009). Additionally, observations made during the field study revealed that the risk pathways considered here may not be exposed to direct sunlight for the whole duration of transport (due to both the method of transportation and variations in local climate). Based on these observations, a subjective assessment of the likely effectiveness of at least 30 minutes of transportation on reducing viral contamination was made. This parameter was modelled using a betaPERT distribution with a mode of 60%, a minimum of 50% and a maximum of 70%.

Model Analysis

A stochastic simulation model was developed using Monte Carlo simulation software (@Risk version 5.5; Palisade Corp., Ithaca, NY) linked to a spreadsheet software (Excel; Microsoft, Redmond, WA). The model structure and parameters were entered with their appropriate distributions and Monte Carlo simulation was used to estimate the output distributions. The model was run using three disease and risk management scenarios: (1) absence of evidence of infection, but HPAIV H5N1 may be present at a level lower than the intensive active surveillance in the province is detectable capacity of with current control interventions and management practices ('current situation' scenario); (2) epidemic occurrence of HPAI H5N1 outbreaks in the province without implementation of DLD-recommended hygiene and biosecurity measures at the cock fighting establishments; and (3) epidemic occurrence of HPAI H5N1 outbreaks in the province with implementation of these hygiene and biosecurity measures. For the 'current situation' scenario, the modal HPAI

prevalence in the province poultry population was set to zero as there was no report of HPAIV infection during the study period. For the two outbreak scenarios, the HPAI prevalence in the area was taken from data on reported daily outbreaks collected in both provinces of study in 2004. Both these risk management scenarios assume that none of the poultry sector-wide control measures (such as depopulation and movement restrictions), intended for use in the event of an outbreak of HPAI, are implemented by the DLD. This makes the model more parsimonious and allows a clearer interpretation of the effect of DLD-recommended hygiene and biosecurity control measures at cock fighting establishments on the risk of HPAIV transmission. A sensitivity analysis was carried out to identify the most important determinants of the risk of the HPAIV H5N1 release and the impact of uncertainty in the model inputs by measuring the correlations between the variability of the output and variability of the input factor. If an input with high correlation is identified, it suggests that the factor greatly influences the variability of the output. The model was run for each risk management scenario with 10,000 iterations and sensitivity analysis was performed to investigate the stability of the output distributions.

Table 3. Summary of the risk management scenarios and the model assumptions

Assumption	Absence of evidence of infection, HPAI H5N1 may be present at lower-than-detectable level of prevalence (current situation)	Epidemic occurrence of HPAI H5N1 disease outbreaks in province without the DLD-hygiene and biosecurity measures specific to cock fighting	Epidemic occurrence of HPAI H5N1 disease outbreaks in province with the DLD-hygiene and biosecurity measures specific to cock fighting
HPAIV H5N1 prevalence: The prevalence of HPAIV H5N1 infection in fighting cocks taken to the establishments or HPAIV H5N1 contamination in the factors of interest was equal to the HPAIV H5N1 prevalence in poultry in the province.	***1.67E-07 (1.71E-06-9.95E-05***	***3.79E-05 (1.19E-05, 3.09E-043)***	***3.79E-05 (1.19E-05, 3.09E-043))***
Frequency of each factor of interest within buffer zones in a province, -i.e. fighting cocks, humans, vehicles and equipment visiting cock fighting establishments per day.	***100***	***100***	***100***
Frequencies of each factor participating in the cock fighting establishments per year			
Best *(open once a week)*	***52***	***52***	***52***
Worst *(open every day)*	***365 (humans, vehicles) 104 (fighting cocks, equipment)***	***365 (humans, vehicles) 104 (fighting cocks, equipment)***	***365 (humans, vehicles) 104 (fighting cocks, equipment)***

Results

For the 'current situation' (no reported HPAI H5N1 outbreaks of but possibly undetected HPAIV H5N1 in the province), the model predicts that, out of four risk pathways considered here, the fighting cock risk pathway poses the highest risk of HPAIV H5N1 release into at least one of a province's buffer zones of compartmentalised commercial broiler chicken farms. Assuming cock fighting establishments are opened once a week, the modal annual risk of releasing HPAIV H5N1 into the buffer zone is estimated to be to be about once in 1,500 years. There is a 95% chance that it occurs once every 59 years (Table 5). Although the modal risk was <0.0007 when the fighting cock establishment opened once a week, this increased three-fold when the establishments opened every day (Table 4). If the cock fighting establishments open every day, the modal risk of at least one virus release is once every 562 years with a 95% chance that such an event occurs less than once every 18 years.

Table 4. Results of the quantitative risk assessment of the annual probability of releasing HPAIV H5N1 into at least one of a province's buffer zones surrounding compartmentalised broiler chicken farms in Thailand under three disease and risk management scenarios.

Risk pathway of interest	Absence of evidence of infection, HPAI H5N1 may be present at lower-than-detectable level of prevalence (current situation)			Epidemic occurrence of HPAI H5N1 disease outbreaks in province without risk management specific to cock fighting*			Epidemic occurrence of HPAI H5N1 disease outbreaks in province with risk management specific to cock fighting*		
	5%	Mode	95%	5%	Mode	95%	5%	Mode	95%
Cock fighting establishments - Open once a week									
Fighting cock	*1.19E-04*	*1.98E-04*	*0.01*	*7.74E-04*	*2.86E-03*	*0.03*	*8.75E-07*	*1.62E-06*	*2.29E-04*
Human	*3.47E-05*	*4.23E-05*	*3.47E-03*	*2.24E-04*	*1.04E-03*	*0.012*	*1.95E-06*	*6.31E-06*	*3.17E-04*
Vehicles	*3.58E-06*	*2.05E-05*	*1.23E-03*	*4.54E.-5*	*9.10E-05*	*7.85E-03*	*8.22E-08*	*2.70E-07*	*3.87E-05*
Equipment	*1.29E-05*	*1.71E-05*	*4.4E-03*	*8.18E-05*	*8.18E-05*	*0.016*	*1.06E-06*	*1.21E-06*	*5.78E-04*
Overall	**2.12E-04**	**6.55E-04**	**0.017**	***1.42E-03***	***4.59E-03***	***0.06***	**8.02E-06**	**6.86E-05**	**1.07E-03**
Cock fighting establishments - Open every day									
Fighting cock	*2.38E-04*	*3.97E-04*	*0.02*	*1.55E-03*	*5.72E-03*	*0.06*	*1.75E-06*	*3.24E-06*	*4.52E-04*
Human	*2.43E-04*	*8.96E-04*	*0.024*	*1.57-E-03*	*7.29E-03*	*0.079*	*1.37E-05*	*4.43E-05*	*2.12E-03*
Vehicles	*2.51E-05*	*1.44E-04*	*8.63E-03*	*3.18E-04*	*6.39E-04*	*0.054*	*5.77E-07*	*1.90E-06*	*2.72E-04*
Equipment	*2.58E-05*	*3.42E-05*	*8.77E-03*	*1.54E-04*	*1.64E-04*	*0.031*	*2.12E-06*	*2.23E-06*	*1.55E-03*
Overall	**6.70E-04**	**1.78E-03**	**0.056**	***4.56E-03***	***0.014***	***0.20***	**2.93E-05**	**4.85E-05**	**3.76E-03**

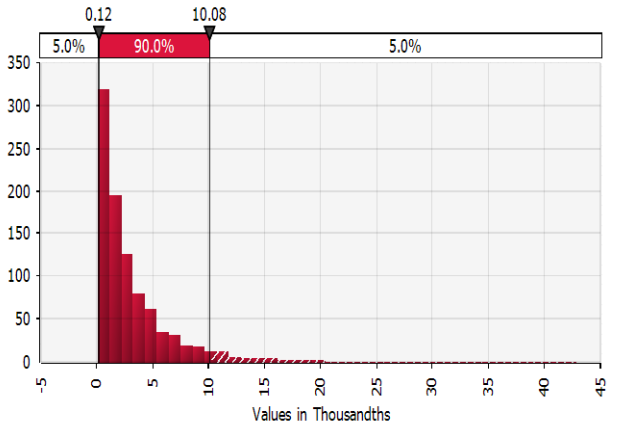
**management: hygienic and biosecurity management at the cock fighting places recommended by the DLD*

Table 5. Number of years between releases of HPAIV H5N1 via cock fighting associated activities

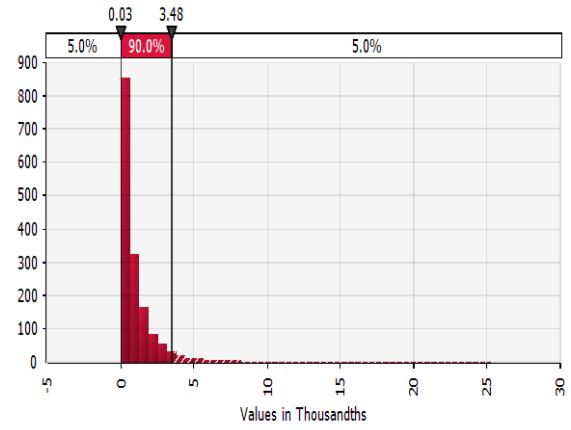
Scenarios	Frequency of opening cock fighting establishments	5th Percentile	Mode	95th Percentile
Absence of evidence of infection, HPAI H5N1 may be present at lower-than-detectable level of prevalence	*Once a week*	59	1,527	4,673
	Every day	18	562	1,493
Epidemic occurrence of HPAI H5N1 disease outbreaks in province without risk management specific to cock fighting *	*Once a week*	16	218	876
	Every day	5.1	72	220
Epidemic occurrence of HPAI H5N1 disease outbreaks in province with risk management specific to cock fighting*	*Once a week*	936	14,577	124,660
	Every day	267	20,618	34,167

** hygienic and biosecurity management at cock fighting establishments recommended by the DLD*

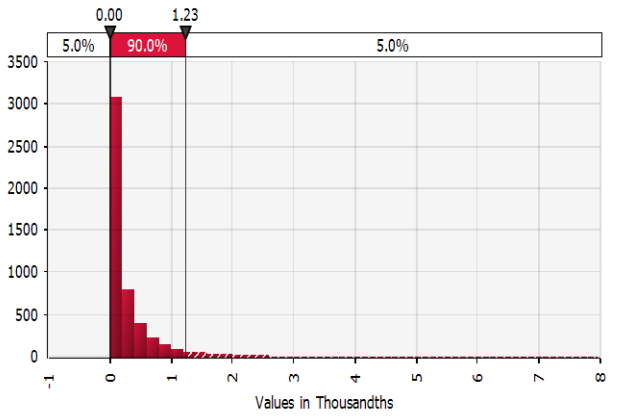
Figure 6. Probability of releasing HPAIV H5N1 into at least one buffer zone within a province per year for each risk pathway considered and their combination, assuming the 'current situation' disease and risk management scenario and cock fighting establishments being open once a week.



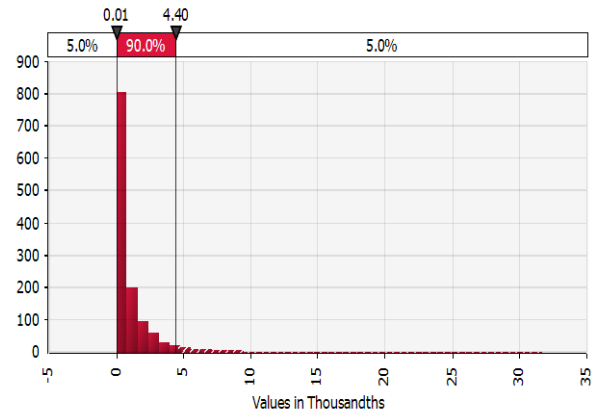
a) Fighting cock risk pathway



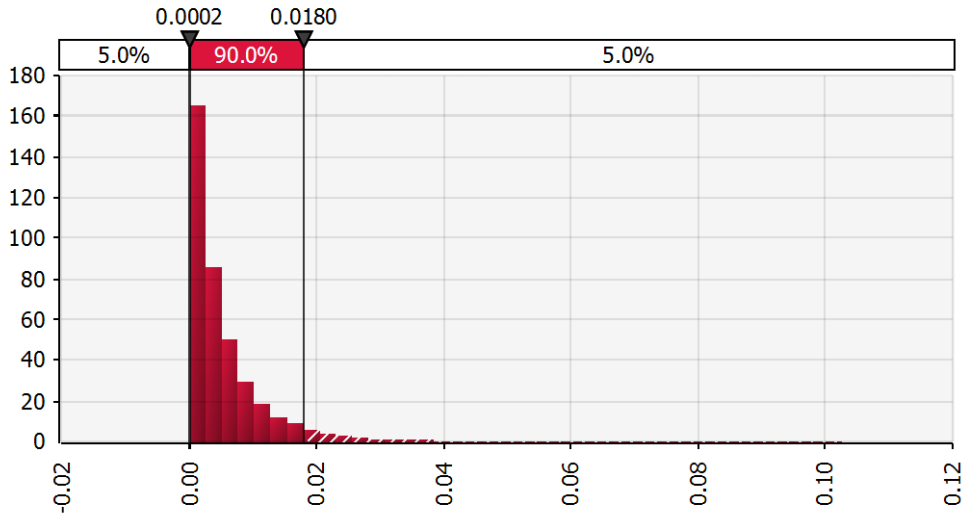
b) Human risk pathway



c) Vehicle risk pathway

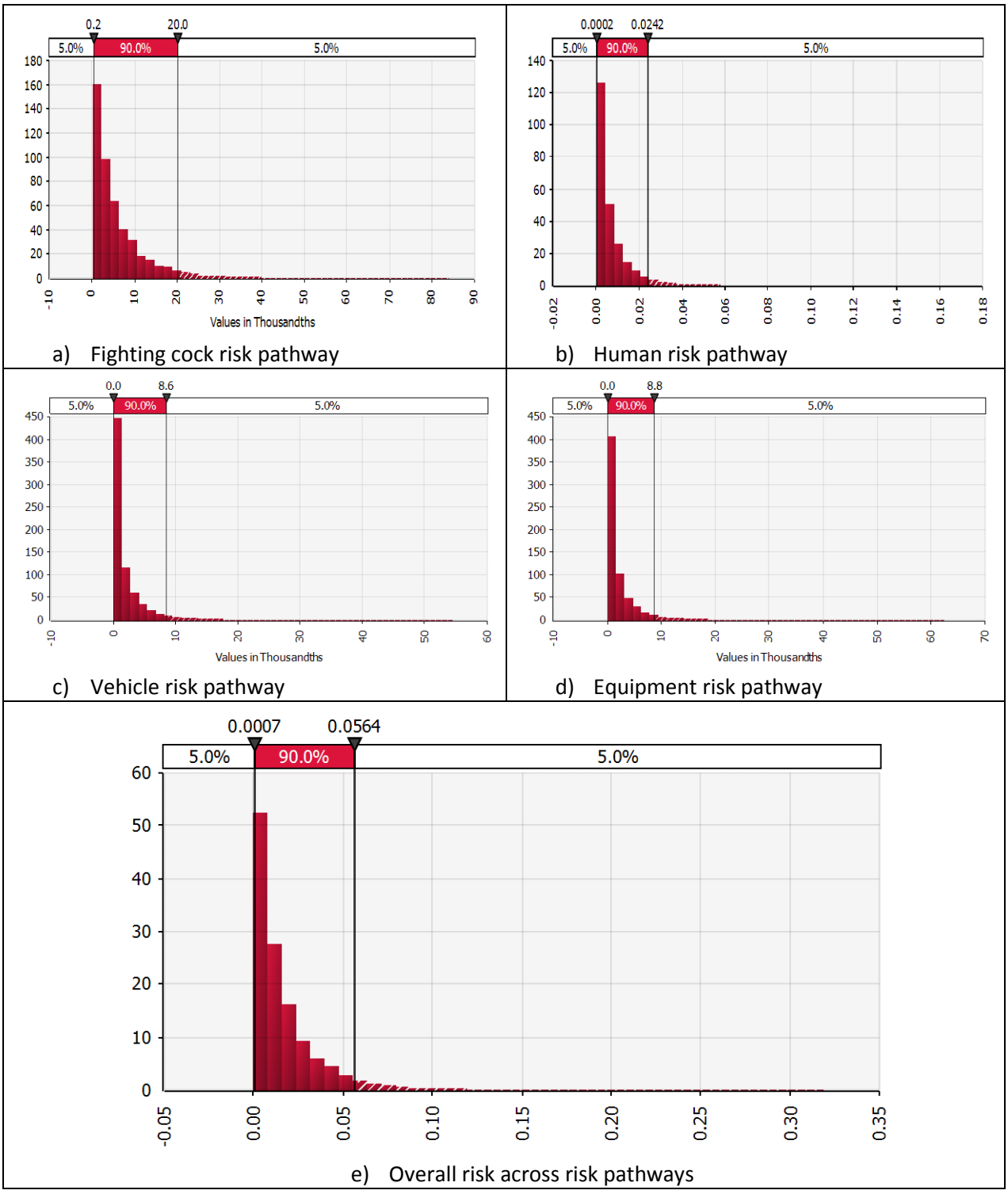


d) Equipment risk pathway



e) Overall risk across risk pathways

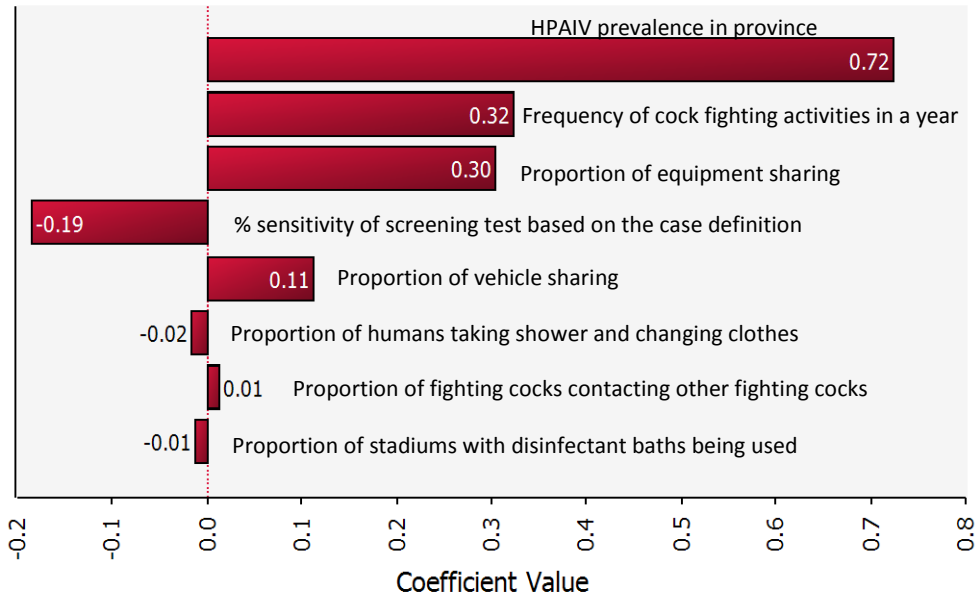
Figure 7. Probability of releasing HPAIV H5N1 into at least one buffer zone within a province per year for each risk pathway considered and their combination, assuming the 'current situation' disease and risk management scenario and cock fighting establishments being open every day.



Under the risk management scenario of epidemic occurrence of HPAI H5N1 disease outbreaks in the province, the risk of HPAIV H5N1 release into at least one buffer zone within a province became less than five in a thousand (<0.00459) in the absence of any control measures at cock fighting establishments when the establishments opened once a week-meaning that the virus could be expected to be released at least once every 218 years, and a 95% chance of less often than one virus release every 16 years. With the same epidemic HPAIV background risk, strict implementation of DLD-recommended control measures at the cock fighting establishments mitigated this risk considerably. The modal risk became one virus release every 14,577 years with a 95% confidence limit of one such event less often than every 936 years assuming that the cock fighting establishments are opened once a week. If the cock fighting arenas are opened every day, the modal risk became one virus release every 20,618 years with a 95% chance of less often than one such release every 267 years.

Figure 8 shows the results of sensitivity analysis for risk management scenario assuming absence of evidence of infection, but HPAI H5N1 may be present at a lower-than-detectable level of prevalence (‘current situation’). The results for the sensitivity analysis for each of the other risk management scenarios assuming epidemic presence of HPAI H5N1 outbreaks in the province are shown in the appendixes. In the absence of outbreaks of HPAI H5N1 disease (but very low undetected HPAIV H5N1 prevalence in province), the prevalence of HPAIV H5N1 infection in the province had the highest correlation with the risk estimate (c=0.72). The frequency of cock fighting activities per year and the proportion of fighting cocks which shared equipment during fighting activities both also had moderately positive correlations with the probability of introduction of HPAIV into the buffer zones of compartmentalised broiler farms. The sensitivity of the screening test used was negatively and the proportion of vehicles shared was moderately positively correlated with the HPAI H5N1 risk release estimate.

Figure 8. Tornado graph presenting the correlation between variation in model input parameter probability distributions and the output distribution for risk of HPAIV H5N1 release into at least one buffer zone within a province per year through at least one of the 4 risk pathways considered, assuming the disease and risk management scenario 'Absence of evidence of infection, HPAI H5N1 may be present at a lower-than-detectable level of prevalence'



Assuming epidemic occurrence of HPAI H5N1 outbreaks within the province, Table 5 shows the correlation coefficients between the input parameter distributions for various risk management measures and the output distribution of risk of HPAIV H5N1 release. Provision of disinfectant baths was found to have the greatest effect on the final estimate, although frequency of opening of cock fighting establishments also had a moderate effect.

Table 6. Results of sensitivity analysis for selected risk management measures to be applied in cock fighting establishments assuming epidemic occurrence of HPAI H5N1 disease outbreaks in the province (correlation coefficients express relationship between variation in input and output parameter distributions)

Risk management measure	Assumption	Correlation coefficient
Cock fighting establishments		
Proportion of establishments making use of disinfectant footbaths	The effectiveness of disinfectant baths in inactivating AI viruses is 100% and compliance with footbath use, if present in the establishment, is 100%	-0.44
Frequency of opening	In the best case: all risk pathways apply once a week In the worst case: Humans and vehicles visit cock fighting establishments every single day during a year, whereas fighting cocks and equipment visit the establishment twice weekly	0.31
Fighting cocks		
Inspection of fighting cocks at entry to establishment	Sensitivity of screening test in detecting infected birds is 95-100% (modelled as shown in Table 2)	-0.06
Presentation of valid fighting cock passport	Fighting cocks are not allowed to enter cock fighting establishments if their owner fails to present an up-to-date passport.	-0.12
Number of fighting cocks within a buffer zone	The minimum and maximum numbers of fighting cocks across all buffer zones in a province are 0 and100, respectively.	0.05
Humans		
Showering and changing clothes	Showering and changing clothes before visiting the cock fighting establishments is 100% effective in removing HPAIV	-0.02
Vehicles		
Cleaning of vehicles	Cleaning of vehicles is 100% effective in removing HPAIV	-0.01
Cock fighting equipment		
Cleaning of equipment	Cleaning of equipment is 100% effective in removing HPAIV	-0.08

Discussion

The model described here indicates that, in the absence of HPAI H5N1 disease outbreaks but assuming very low undetected HPAIV H5N1 prevalence in the province, the probability of releasing HPAIV H5N1 via at least one risk pathway associated with cock fighting activities into at least one buffer zone of compartmentalised broiler chicken farms

within a province per year was about once in 1,500 years with a 95% chance of less often than one virus release every 59 years. This risk estimate has to be classified as very low, if not negligible, suggesting that in the current situation fighting activities are very unlikely to result in introduction of virus into buffer zones of compartmentalised broiler farms. Such a very low estimate is mainly due to a very low background prevalence of HPAIV H5N1 in the provinces. This risk was found to be strongly associated with the prevalence of HPAIV infection in the province, the frequency of cock fighting activities, and the probability of contact between fighting cocks through shared equipment at the cock fighting establishments.

Assuming epidemic occurrence of HPAI H5N1 outbreaks, the annual risk of viral release into at least one buffer zone within a province increases by ten times in the absence of control measures, but it still remains very low in absolute terms. If the condition and parameters assumed here remained constant, there is a 95% chance that virus is released into at least one buffer zone less than once every 16 years (assuming cock fighting establishments being open once a week). Similarly, increasing the frequency of cock fighting activities increased the risk of HPAIV H5N1 release both, in the absence of any disease outbreaks in the province (but assuming very low undetected HPAIV H5N1 prevalence) and in the presence of epidemic outbreaks with DLD-recommended biosecurity measures specific to cock fighting in place. The model estimated that there was a 95% chance that a virus release occurs less than once every 18 years (for the former scenario) and became once every 5 years (for the latter scenario) when assuming the cock fighting establishment being open daily. Although not directly modelled here, the frequency of cock fighting activities would also be expected to influence the level of virus contamination within the establishments (due to a build-up of contamination during and between days).

Although the proportion of cocks exposed to shared equipment was found to have a large effect on the final risk estimate, equipment used during cock fighting activities contributed less to the overall risk of HPAIV introduction into the buffer zones due to a lower likelihood of equipment being shared between fighting cocks. All fighting cock owners reported that they had their own equipment which they were unlikely to share with other owners' belongings because they were highly concerned about the safety of their cocks. It can be assumed that this behaviour will reduce the risk of contamination of equipment from other cocks. However, if virus contamination of equipment did take place, dissemination of

infection would be likely, as equipment was reported as rarely being cleaned either before or after fighting activities (see Appendix C).

Although DLD recommendations currently recommend that cock fighting establishments should be opened less than once a week, a lack of compliance with this recommendation was observed– particularly for fighting cock stadiums. An enforcement of the DLD-recommended bird-free period of 7 days would be expected to reduce the risk of transmission of HPAIV H5N1 associated with cock fighting activities. Although approximately 80% of the training places included in the study reported that they maintained a bird free period of at least 6 days after cock fighting activities, only 33% of all stadiums complied with this recommendation (see Appendix C). As the maintenance costs of stadiums are higher than those of training points, it can be assumed that these will open more than once a week in order to increase income, especially given that the perceived risk of HPAIV H5N1 introduction currently is considered to be low amongst the farming population in Thailand.

In the presence of epidemic HPAI H5N1 disease and implementation of the DLD-recommended bird-free period of 7 days of fighting cock establishments, cock fighting may still occur daily within the province due to individual establishments opening on different days in a week. This may even result in fighting cock owners visiting more establishments during a week than if each establishment was opened every day. In the presence of epidemic HPAI H5N1 disease outbreaks, DLD recommended biosecurity measures specific to cock fighting establishments should be strictly implemented in each individual place in order to reduce the risk of virus release into the buffer zones. Assuming epidemic occurrence of HPAI H5N1 outbreaks with DLD-recommended risk management specific to cock fighting establishments reduced the risk of at least one virus release considerably; there is a 95% chance that at least one virus release occurs less often than every 936 years (assuming weekly opening of cock fighting establishments) or 267 years (assuming daily opening of cock fighting establishments). If full compliance with these measures cannot be ensured, cock fighting activities would pose a considerable risk of virus incursion into buffer zones. In this case, enforced closure of cock fighting establishments during an outbreak of HPAI H5N1 in the country may be considered as a control option. However, the difficulties associated with restriction or cessation of cock fighting also need to be taken into account, as these recommendations may be difficult to enforce and instead may encourage illegal cock fighting activities and therefore result in a loss of regulatory control and the ability to

conduct disease surveillance in this context. These potential problems therefore require further consideration before making policy recommendations.

Live birds, humans, vehicles and equipment have all been identified as potential mechanical carriers of HPAIV H5N1 (Serratosa 2007; Zepeda 2007). Of all risk pathways considered, fighting cocks carried the greatest risk of HPAIV release into a buffer zone when the cock fighting establishments opened once a week and humans carried the greatest risk when the establishments opened every day. This is due to humans being able to participate in the cock fighting activities every single day, whereas fighting cocks can only visit the establishments at a maximum frequency of twice a week. However, both fighting cocks and humans would be expected to have a greater level of contact with HPAIV than other risk pathways, along with fewer measures being used for removal of virus contamination. The finding that human movement played a greater relative role in the final risk estimates when the frequency of opening of cock fighting establishments was increased is a cause for additional concern. Although not accounted for in this release assessment, people associated with cock fighting activities can easily move around the country, and therefore potentially can disseminate the virus over large areas if they are contaminated (Ungchusak 2005; Peiris 2007; Chunsuttiwat 2008).

The model predicted that the fighting cock risk pathway poses the highest annual risk of HPAIV H5N1 release, out of all four risk pathways considered here. In the presence of epidemic occurrence of outbreaks within the province throughout the year, fighting cocks can play a role in releasing the virus into a buffer zone, and there is a 95% chance that virus is released into at least one farm's buffer zone less than once every 5 years (assuming fighting places being open daily).The number of fighting cocks in geographic areas has recently been identified as a risk factor for HPAI H5N1 disease outbreaks in Thailand, but its cause-effect relationship is still unknown, thus fighting cocks may be a confounder for other unmeasured causal risk factors (Gilbert, Xiao et al. 2007; Tiensin 2009; Paul 2010; Souris 2010). But it is still warranted to recommend that the density of fighting cocks kept within buffer zones and their movements is monitored more closely.

Vehicles consistently had the smallest influence on the overall risk of HPAIV H5N1 introduction into the buffer zones. This is expected, as vehicles are less likely to both become contaminated and to maintain this contamination than the other factors investigated. All vehicles in this study were reported to be parked in an area separate from

the cock fighting establishment (in accordance with DLD recommendations), and as such were also less likely to come into contact with other fighting cocks and humans (see Appendix C). The infrequent sale or exchange of fighting cocks at the fighting establishments also reduced the likelihood of the vehicles coming into contact with other cocks. Additionally, avian influenza viruses are known to not persist on vehicle surfaces due to their hard and non porous composition and exposure to sunlight and heat (Chumpolbanchorn 2006; Songserm 2006).

Maintenance of appropriate biosecurity measures has been recognised as a vital tool in the control of avian influenza viruses within poultry populations (Koch 2006; Zepeda 2007). These measures, if correctly implemented, would be expected to reduce virus entry into the establishments, virus persistence within the establishments and virus exit from the establishments. In the case of the current DLD recommended hygiene and biosecurity measures, HPAIV contamination of humans and vehicles would be expected to be reduced on entry and exit to and from the establishments through the use of disinfectant foot and wheel baths, viruses within the establishments would be expected to be removed by cleaning and inactivated by disinfection, and the use of a 6-day bird-free period after each cock fighting event would prevent viral accumulation in the environment (DLD 2006; Serratosa 2007).

Assuming epidemic occurrence of HPAI H5N1 disease outbreaks in a province, the risk estimates produced by the model were weak negatively correlated with the proportion of establishments with footbaths in use. However, direct observation at the cock fighting establishments identified a low compliance with footbath use by people, with only one in five establishments having an operating footbath (see Appendix C). If cock fighting establishments were to remain open during an outbreak of HPAI H5N1 disease in a province, it is of great importance that footbath use be encouraged, possibly through educational campaigns and continued monitoring of footbath use at the establishments.

As mentioned above, humans and fighting cocks were at a relatively high risk of exposure to HPAIV H5N1 during fighting activities. Although current DLD measures, if followed, would be expected to remove HPAIV contamination from shoes prior to exit from the cock fighting establishments, they would be less effective at removal of contamination from clothing or the fighting cocks themselves. It is therefore advised that feasible methods of removal of viral contamination from these factors at exit from the cock fighting

establishments are investigated. It should be noted here that the current model did not account for infection of fighting cocks with HPAIV H5N1 at the cock fighting establishments, only for contamination. The irreversible nature of HPAIV H5N1 infection in chickens would make hygiene-based measures aimed at removing virus on exit from the establishment ineffective, and therefore measures aimed at early identification of infected birds and minimisation of contamination of cock fighting establishments should be maintained.

Hygiene and biosecurity measures within the buffer zones should also be considered so as to minimise the risk of exposure and transmission, should HPAIV H5N1 be realised into a buffer zone. Also as mentioned above, efforts should be made to monitor the number of fighting cocks within these zones, in order to ensure that the numbers remain relatively low.

Sensitivity analysis demonstrated that the estimated HPAIV infection prevalence in the province, the frequency of cock fighting activities, and sharing of equipment between fighting cocks were strongly associated with the risk estimates created, of which frequency of fighting activities and sharing of equipment have been discussed above. Control of HPAIV H5N1 infection prevalence in a province will be the most effective method of controlling the risk of HPAIV H5N1 introduction into the buffer zones from cock fighting activities, as is to be expected. HPAIV H5N1 surveillance and proactive control measures should therefore continue to ensure early detection of HPAIV H5N1 infection in the country (Buranathai 2007). Fighting cock passports provide a method of screening and monitoring of individual birds for infection [all fighting cock owners are required to show negative HPAIV H5N1 test results prior to the movement of their birds (Tiensin, Nielen et al. 2007)]. It is therefore recommended that consideration be given to the instigation of a compulsory passport scheme for fighting cocks, as this would serve to both reduce the risk of HPAIV H5N1 dissemination and provide a method of monitoring the number of fighting cocks in the country.

The model used in this study does not incorporate HPAI H5N1 control measures implemented for fighting cocks during the event of an outbreak in the country, such as movement restriction. Therefore, the results are very likely to be an overestimate of the true risk (Tiensin 2005; Auewarakul 2008). In the event of an HPAI H5N1 outbreak, movement restrictions would be expected to greatly reduce the risk of HPAIV H5N1 introduction into buffer zones, and should be relatively easy to enforce (due to the value of fighting cocks,

owners may also be less likely to move them illegally during an outbreak due to the well-known risk of infection).

Study Limitations

Due to limited data availability with respect to several parameters, this model had to be based on a number of assumptions. It was assumed that cock fighting establishments could become contaminated with HPAIV H5N1 through the entry of HPAIV-infected fighting cocks or HPAIV contaminated people, equipment or vehicles, and the factors of interest could become contaminated through exposure to these. Regarding HPAIV contamination/infection of these risk pathways, a 'worst case' scenario was assumed - i.e. that the HPAIV 'prevalence' associated with the risk pathways of interest was equal to the HPAIV H5N1 infection prevalence in the province and that within each infected flock all fighting cocks would be infected with HPAIV H5N1. It was also assumed that approximately 100 households within buffer zones were participating in cock fighting activities in each province. This number is likely to be an overestimate. Considering all the above assumptions, this model is likely to overestimate the risk of introduction.

Additional assumptions were made in relation to the effectiveness of disinfectants and the efficacy of cleaning vehicles and cock fighting equipment, which were assumed to remove all virus contamination. HPAIV H5N1 contamination was also assumed to be completely removed by sunlight exposure during transportation for longer than 30 minutes, showering and changing of clothes or cleaning vehicles or cock fighting equipment before visiting the cock fighting establishments. These assumptions would all be expected to underestimate the true risk.

Conclusions

Based on this study, given current practices at cock fighting establishments and in the absence of evidence of infection, but recognising that HPAI H5N1 may be present at a lower-than-detectable level of prevalence, there is a 95% chance that the annual risk of introducing HPAIV H5N1 infection into at least one buffer zone of compartmentalised broiler chicken farms through at least one risk pathway associated with cock fighting activities occurs less often than once in every 59 years assuming that the cock fighting establishments only opened once a week. This risk was increased three-fold by more frequent opening, which was reported to occur in many of the stadiums visited as part of the cock fighting survey

conducted to provide background information for this risk assessment. Although changing the current DLD recommendation of opening cock fighting establishments only every 7 days to a legal requirement might reduce the risk, if compliance cannot be enforced it may lead to an increase in illegal cock fighting activities and therefore result in an increased risk.

The model also predicted that assuming epidemic occurrence of HPAI H5N1 disease outbreaks in the country, implementation of DLD-recommended hygiene and biosecurity measures at the cock fighting establishments considerably reduced the risk of HPAIV H5N1 introduction into the buffer zones. However, compliance with these measures was rarely observed at the cock fighting establishments visited as part of the survey described in Appendix C, possibly as a consequence of no case of HPAI H5N1 having been reported in the last few years. It is believed that these measures had been strictly implemented during previous outbreaks of HPAI H5N1 together with various control measures applied across the poultry sector by the DLD, e.g. pre-emptive culling, movement restriction, etc. (Buranathai 2007). If full compliance with recommended risk mitigation measures at cock fighting establishments cannot be achieved during an outbreak, consideration should be given to prohibition of cock fighting activities during such periods.

Amongst the factors considered here that were associated with cock fighting, movement of fighting cocks and humans posed the greatest risk of HPAIV H5N1 release into the buffer zones, as these risk pathways would be expected to have greatest opportunity for contact with virus. There are currently no measures in place for effective removal of viral contamination from human clothing or fighting cocks prior to exit from the establishments. Therefore, whilst it is advised that current control measures at the cock fighting establishments be maintained and enforced (particularly the use of disinfectant footbaths), further research on methods of effectively removing viral contamination from fighting cocks and human clothing is warranted. The number of fighting cocks within the buffer zones should be closely monitored, possibly through the use of an obligatory passport scheme for these birds.

References

Auewarakul, P. (2008). The Past and Present Threat of Avian Influenza in Thailand, Springer Science+Business Media, LLC.

Buranathai, C., Amonsin, A., Chaisigh, A., Theamboonlers, A., Pariyothorn, N. and Poovorawan, Y. (2007). "Surveillance activities and molecular analysis of H5N1 highly pathogenic avian influenza viruses from Thailand, 2004-2005." Avian Diseases 51: 194-200.

Chumpolbanchorn, K., Suemanotham, N., Siripara, N., Puyati, B. and Chaichoune, K. (2006). "The effect of temperature and UV light on infectivity of avian influenza virus (H5N1, Thai field strain) in chicken fecal manure." Southeast Asian J Trop Med Public Health 37(1): 102-105.

Chunsuttiwat, S. (2008). "Response to avian influenza and preparedness for pandemic influenza: Thailand's experience." Respirology 13 (Suppli.1): S36-S40.

DLD (2006). Avian Influenza Control in Thailand, Agricultural Coooperative Publisher of Thailand.

Gilbert, M., X. Xiao, et al. (2007). "Avian influenza, domestic ducks and rice agriculture in Thailand." Agriculture, Ecosystems & Environment 119(3-4): 409-415.

Koch, G., Elbers, A.R.W. (2006). "Outdor ranging of poultry: a major risk factor for the introduction and development of High-Pathogenicity Avian Influenza." NJAS 54(2): 179-194.

Lombardi, M. E., Ladman, B.S., Alphin, R.L. and Benson, E.R. (2008). "Inactivation of Avian Influenza virus using common detergents and chemicals." Avian Diseases 52: 118-123.

NaRanong, V. (2007). "Structural changes in Thailand's poultry sector and its social implications." Poultry in the 21st Century: 29 pages.

OIE (2005). Guidelines for risk analysis. Paris, Office International des Epizooties.

Paul, M., Tavornpanich, S., Abrial, D., Gasqui, P., Charras-Garrido, M., Thanapongtharm, W., Xiao, X., Gilbert, M., Roger, F. and Ducrot, C. (2010). "Anthropogenic factors and the risk of highly pathogenic avian influenza H5N1: prospects from a spatial-based model." Vet Rec 41(28).

Peiris, J. S. M., de Jong, M.D. and Guan, Y. (2007). "Avian Influenza Virus(H5N1): a Threat to Human Health." Clinical Microbiology Reviews 20(2): 243-267.

Poovorawan, Y. (2007). "Molecular Epidemiology of Avian Influenza H5N1 in Thailand." ScienceAsia 33 Supplement 1: 87-90.

Saito, T., Watanabe, C., Takemae, N., Chaisingh, A., Uchida, Y., Buranathai, C., Suzuki, H., Okamatsu, M., Imada, T., Parchariyanon, S., Traiwanatam, N. and Yamaguchi, S. (2009). "Pathogenicity of highly pathogenic avian influenza viruses of H5N1 subtype isoloated in Thailand for different poultry species." Veterinary Microbiology 133: 65-74.

Serratosa, J., Ribo, O., Correia, S. and Pittman, M. (2007). "EFSA Scientific Risk Assessment on animal health and welfare aspects of avian influenza (EFSA-Q-2004-075)." Avian Dis 51: 503-507.

Shahid, M., M. Abubakar, et al. (2009). "Avian influenza virus (H5N1); effects of physico-chemical factors on its survival." Virology Journal 6(1): 38.

Siengsanan, J., Chaichoune, K., Phonaknguen, R., Sariya, L., Prompiram, P., Kocharin, W., Tangsudjai, S., Suwanpakdee, S., Wiriyarat, W., Pattanarangsan, R., Robertson, I., Blacksell, S.D. and Ratanakorn, P. (2009). "Comparison of outbreaks of H5N1 Highly Pathogenic Avian Influenza in wild birds and poultry in Thailand." Journal of Wildlife Disease 45(3): 740-747.

Songserm, T., Jam-on, R., Sae-Heng, N. and Meemak, N. (2006). "Survival and stability of HPAI H5N1 in different environments and susceptibility to disinfectants." Developments in Biologicals 124: 254.

Souris, M., Gonzalez, J.P., Shanmugasundaram, J., Corvest, V. and Kittayapong, P. (2010). "Retrospective space-time analysis of H5N1 Avian influenza emergence in Thailand." International Journal of Health Geographics 9(3).

Suarez, D. L., Spackman, E., Senne, D.A., Bulaga, L., Welsch, A.C. and Froberg, K. (2003). "The effect of various disinfectants on detection of avian influenza virus by real time RT-PCR." Avian Diseases 47: 1091-1095.

Tiensin, T., Ahmed, S.S.U., Rojanasthien, S., Songserm, T., Ratanakorn, P., Chaichoun, K., Kalpravidh, W., Wongkasemjit, S., Patchimasiri, T., Chanachai, K., Thanaphontham, W., Chotinan, S., Stegeman, A. and Nielen, M. (2009). "Ecologic risk factor investigation of clusters of Avian Influenza A (H5N1) virus infection in Thailand." The Journal of Infectious Diseases 199: 1735-43.

Tiensin, T., Chaitaweesub, P., Songserm, T., Chaisingh, A., Hoonsuwan, W., Buranathai, C., Parakamawongsa, T., Premashthira, S., Amonsin, A., Gilbert, M., Nielen, M., And Stegeman, A. (2005). "Highly pathogenic avian influenza H5N1, Thailand 2004." Emerging Infectious Diseases 11: 1664-1672.

Tiensin, T., M. Nielen, et al. (2007). "Geographic and temporal distribution of highly pathogenic avian influenza A virus (H5N1) in Thailand, 2004-2005: an overview." Avian Dis 51(1 Suppl): 182-8.

Ungchusak, K., Auewarakul, P., Dowell, S.F., Kitphati, R., Auwanit, W., Puthavathana, P., Uiprasertkul, M., Boonnak, K., Pittayawonganon, C., Cox, N.J., Zaki, S.R., Thawatsupha, P., Chittaganpitch, M., Khontong, R., Simmerman, J.M. and Chunsutthiwat, S. (2005). "Probable Person-to-Person transmission of Avian Influenza A (H5N1." The New England Journal of Medicine 352(4): 333-340.

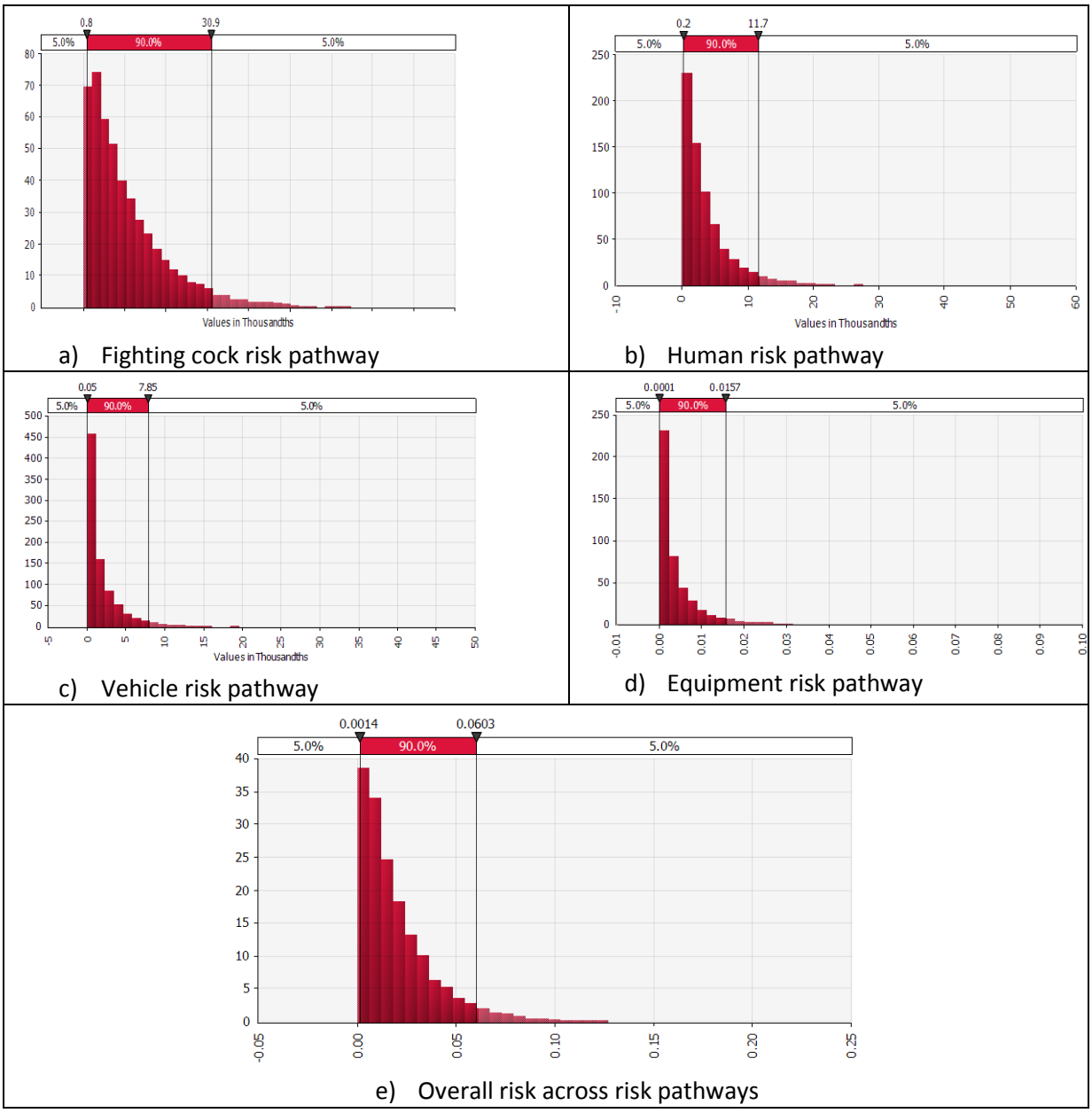
Weber, T. P. and N. I. Stilianakis (2008). "Inactivation of influenza A viruses in the environment and modes of transmission: A critical review." Journal of Infection 57(5): 361-373.

Zepeda, C. (2007). "Highly pathogenic avian influenza in domestic poultry and wild birds: A risk analysis framework." Journal of Wildlife Disease 43(3 (Supplement)): S51-S54.

Appendix A: Probability density functions for the risk estimates associated with the two outbreak risk management scenarios.

Epidemic occurrence of HPAI H5N1 disease outbreaks without the implementation of risk management measures specific to cock fighting establishments.

Figure 9. Probability of releasing HPAIV H5N1 into at least one buffer zone within a province per year for each risk pathway considered and their combination, based on the 'epidemic occurrence of HPAI H5N1 outbreaks in province' without risk management scenario and cock fighting establishments being open once a week.



a) Fighting cock risk pathway

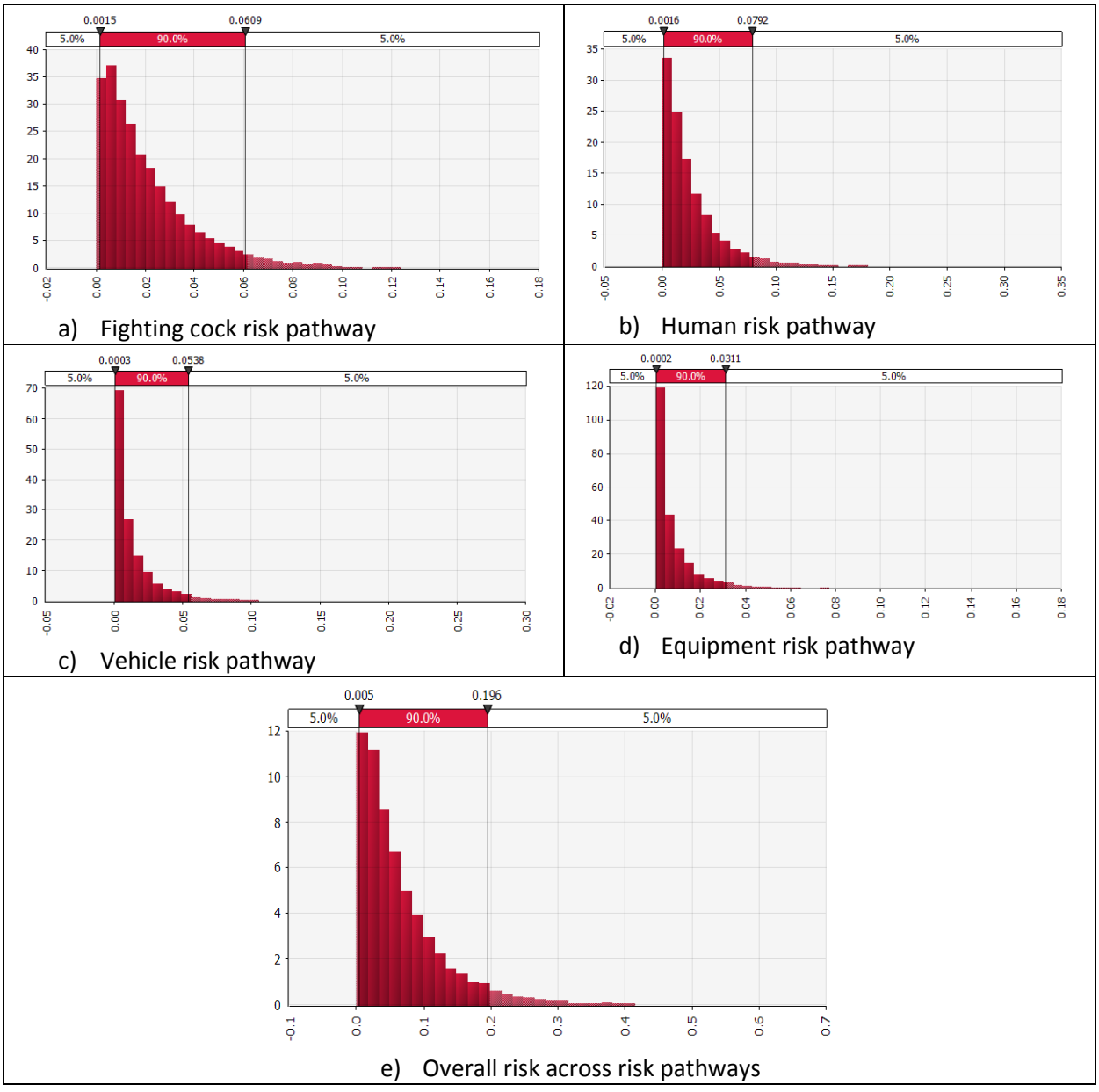
b) Human risk pathway

c) Vehicle risk pathway

d) Equipment risk pathway

e) Overall risk across risk pathways

Figure 10. Probability of releasing HPAIV H5N1 into at least one buffer zone within a province per year for each risk pathway considered and their combination, based on the 'epidemic occurrence of HPAI H5N1 outbreaks in province' without risk management scenario and cock fighting establishments being open every day.



Epidemic occurrence of HPAI H5N1 disease outbreaks with the implementation of the DLD recommended risk management measures specific to cock fighting establishments.

Figure 11. Probability of releasing HPAIV H5N1 into at least one buffer zone within a province per year for each risk pathway considered and their combination, based on the 'epidemic occurrence of HPAI H5N1 outbreaks in province' with risk management scenario and cock fighting establishments being open once a week.

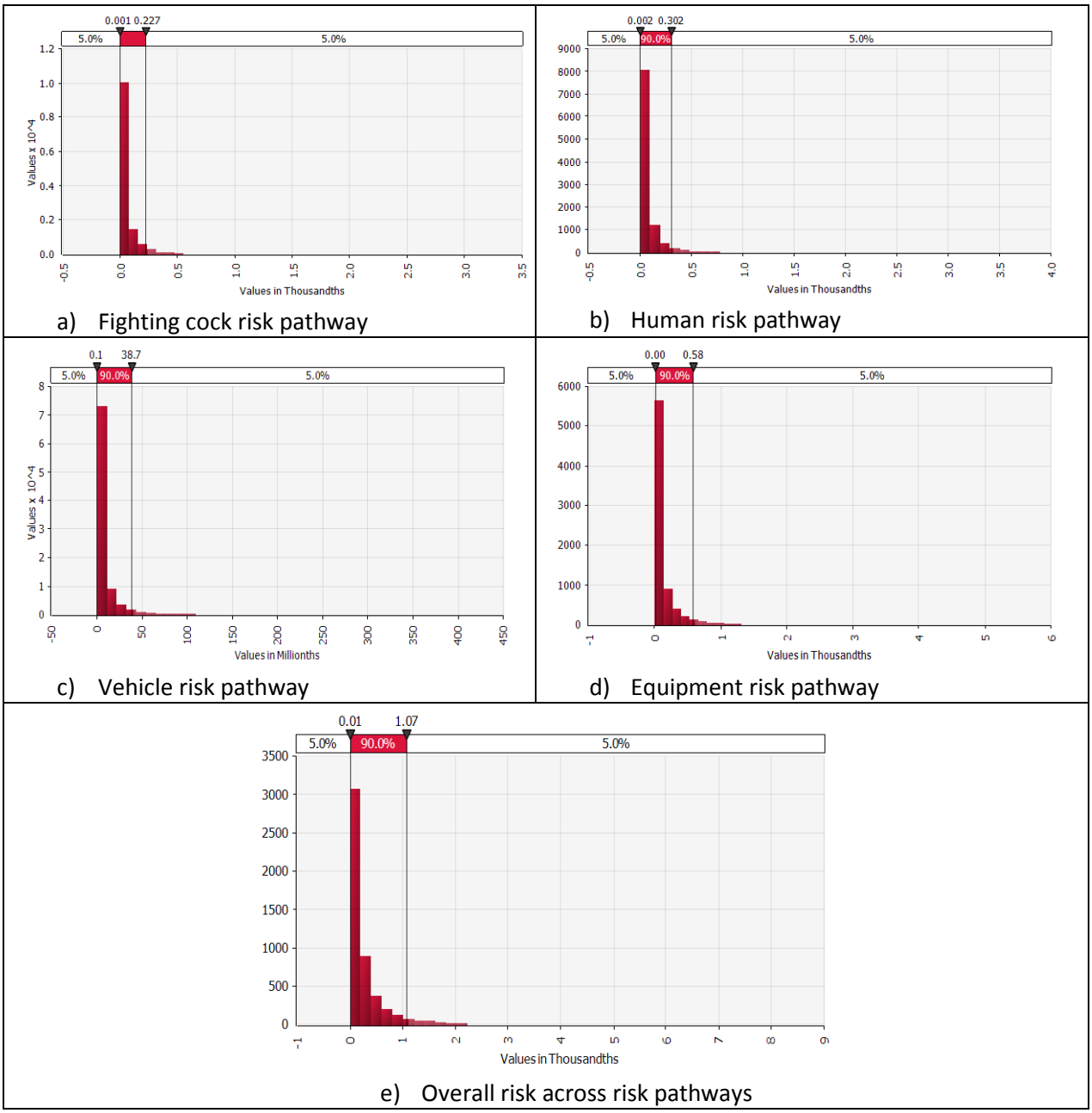
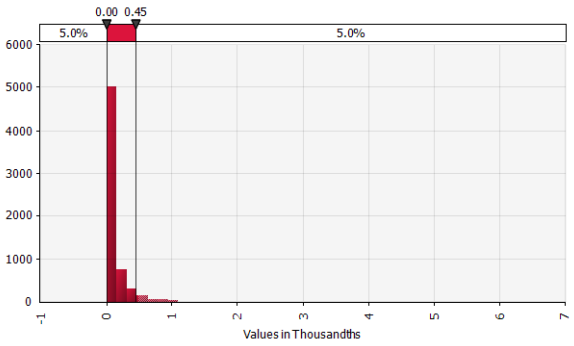
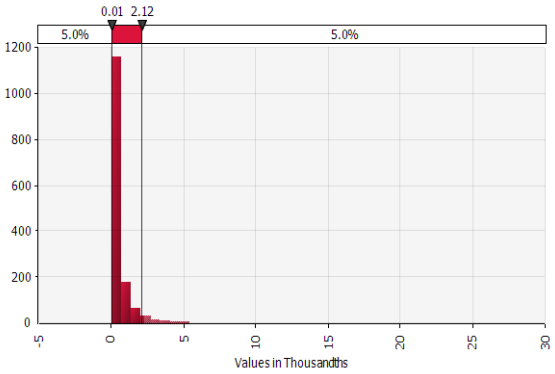


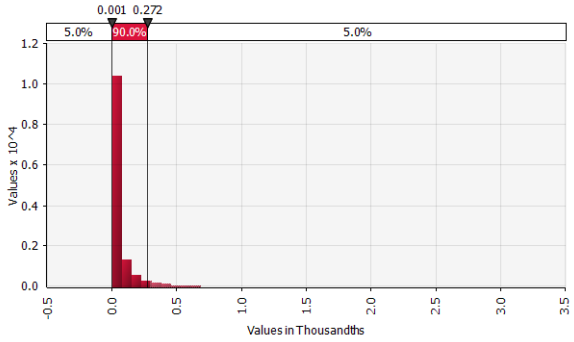
Figure 12. Probability of releasing HPAIV H5N1 into at least one buffer zone within a province per year for each risk pathway considered and their combination, based on the 'epidemic occurrence of HPAI H5N1 outbreaks in province' with risk management scenario and cock fighting establishments being open every day.



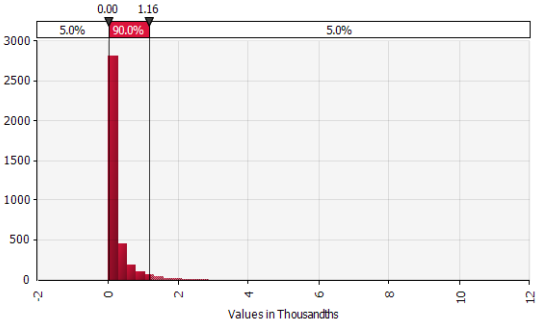
a) Fighting cock risk pathway



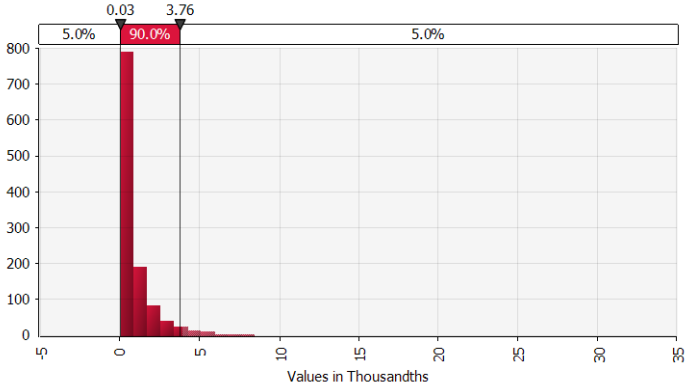
b) Human risk pathway



c) Vehicle risk pathway



d) Equipment risk pathway

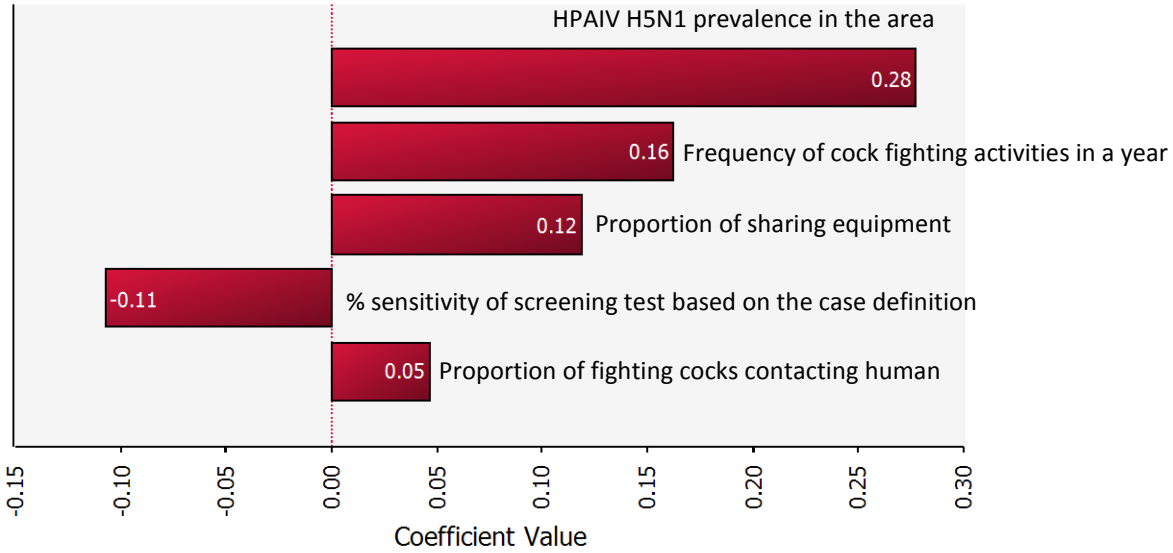


e) Overall risk across risk pathways

Appendix B: Sensitivity analysis: Tornado graphs show the correlation between the input parameters and the overall risk of release of at least one HPAIV contaminated factor into the buffer zone of compartmentalised chicken broiler farms within a province per year.

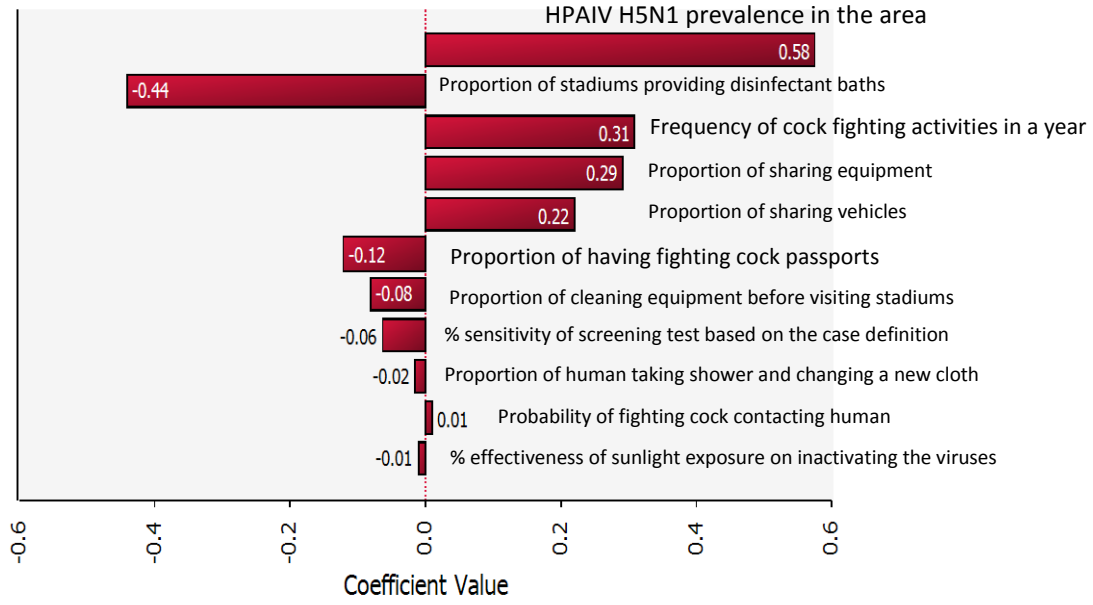
Epidemic occurrence of HPAI H5N1 disease outbreaks without implementation of the DLD recommendation at the cock fighting establishments

Figure 13. Tornado graph presenting the correlation between variation in model input parameter probability distributions and the output distribution for risk of HPAIV H5N1 release into at least one buffer zone within a province per year through at least one of the 4 risk pathways considered, assuming 'epidemic occurrence of HPAI H5N1 disease outbreaks in province' without risk management scenario



Epidemic occurrence of HPAI H5N1 disease outbreaks with implementation of DLD recommended risk management measures specific to cock fighting establishments

Figure 14. Tornado graph presenting the correlation between variation in model input parameter probability distributions and the output distribution for risk of HPAIV H5N1 release into at least one buffer zone within a province per year through at least one of the 4 risk pathways considered, assuming 'epidemic occurrence of HPAI H5N1 disease outbreaks in province' with risk management scenario



Appendix C: Cock Fighting Activity Survey

Field investigations to generate baseline data on fighting cock related activities of farming households and cock fighting establishments in Thailand

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Introduction

Highly pathogenic avian influenza virus (HPAIV) H5N1 has had a significant impact on poultry production and human health in many countries, particularly in South-East Asia, and in China and Egypt. In Thailand, the Department of Livestock Development (DLD) as national veterinary authority implemented a series of control policies since the first outbreak of HPAIV H5N1 was reported in early 2004. In this context, DLD also recommends that poultry farms are converted to compartments (Tiensin 2005; Buranathai 2007). This involves implementation of prescribed biosecurity measures and active surveillance for preventing introduction of avian influenza viruses (AIV) and demonstrating disease freedom (Buranathai 2007). HPAIV H5N1 may be introduced into disease-free areas by movement of live poultry and poultry products as well as mechanical transmission associated with poultry production, e.g. human, vehicles and equipment etc. (Souris 2010).

Keeping fighting cocks is a long-standing tradition in Thailand, particularly in rural areas. Roosters are raised with the aim of participating in cock fighting activities, which involves movements of fighting cocks, human, vehicles and equipment. As a result of such movements, fighting cocks have the potential to disseminate the virus (Tiensin 2005). In accordance with DLD control measures, cloacal swabs from all birds must be taken for virus isolation of AIV in a 7-10 day prior to movement; and a movement permit is granted if all results are negative to AIV (DLD 2006). Since 2005, DLD requires that fighting cocks are subjected to a monthly check for AIV and if moved need to have a unique identification document, called 'passport', confirming that they have been tested negative for HPAIV H5N1 (Buranathai 2007). Specific risk management measures have also been implemented by DLD at the cock fighting establishments, including checking of fighting cock passports, recording of visitors and fighting cocks, use of disinfectant footbaths for all humans and vehicles, cleaning and disinfecting of the establishments, keeping it bird-free for at least six days after

each competition day and preventing entry of wild birds (DLD 2006; Buranathai 2007). Several studies have identified the number of fighting cocks in a geographic area as a risk factor for occurrence of HPAI H5N1 outbreaks, but no cause-effect relationship has been demonstrated and they may therefore well be a confounder for other unmeasured causal risk factors (Gilbert 2006; Tiensin 2009; Souris 2010). Subsequent to a qualitative risk assessment examining the risk of introduction of HPAIV H5N1 into buffer zones around compartmentalised poultry farms in Thailand, it was decided in agreement with DLD to conduct a quantitative risk assessment for introduction of HPAIV H5N1 into these buffer zones through activities associated with cock fighting. Detailed data on cock fighting activities, such as frequencies of each factors participating into the activities and human behaviours related to the cock fighting activities are not available, and the surveys described here were conducted to fill this data gap.

Materials and Methods

Two surveys were conducted, one to describe farmer behaviour in relation to cock fighting activities and the other was about practices employed at cock fighting competitions. The data was collected in 2 provinces, Province A in Northern Thailand and Province B in Central Thailand, between May and July 2009. Both provinces were selected on the basis of having a high density of compartmentalised poultry farms as well as frequent cock fighting activities, compared with other parts of Thailand.

The survey at cock fighting establishments was aimed at describing management practices and the behaviour of people visiting the establishments. Data was collected through direct observation by the investigators based on a questionnaire. A list of cock fighting establishments was obtained from the provincial DLD office for each of the two study provinces, in total 23 and 24 establishments in provinces A and B, respectively. In each province, the managers of active cock fighting establishments were asked for their consent to be included in this survey, allowing direct observation and access to records of participants in the establishments. The owners of 32 cock fighting establishments declined participating in the study, resulting in the enrolment of 15 cock fighting establishments in total. In province A, the sample included six of the 15 districts with each being represented by one establishment. Of the 10 districts in province B, five were represented by one establishment each and two were represented by two establishments each.

The survey of households involved in cock fighting included collection of data based on a face-to-face interview conducted by the investigators on farm characteristics, husbandry practices, personal hygiene and biosecurity measures related to cock fighting activities. The sample size was calculated assuming the proportion of farmers giving a particular response to be 50%, and allowing an error of 5% (with a 95% level of confidence). A total of 384 households was required, and it was decided to have 192 per province. In each province, a sampling frame comprised of records held by the provincial DLD office of all farmers living within buffer zones and records of all visitors kept by the participating cock fighting establishments. Records of visitors to the selected cock fighting establishments were obtained on a single weekend. Assuming that most individuals would visit the cock fighting establishments on every weekend during a year, this data should appropriately represent the vast majority of farmers located in all districts of a province which participate in cock fighting. Based on the obtained records, visitors from the same household were counted as one unit of interest and all visitors living outside the provinces of interest were excluded. This resulted in 258 and 268 farming households from provinces A and B, respectively. It was decided to include all households in the study. The analysis of the data was conducted using cross-tabulation and χ^2 tests were used to test the relationships between qualitative variables for statistical significance.

Results

Cock fighting establishments

From the field survey, no cock fighting establishment was found in any of the buffer zones in the two provinces. All cock fighting establishments visited in province B were swept clean after the fighting activities, whereas in province A, 50% of the establishments did not report sweeping at all (Table 1). The frequency of disinfectant spraying was variable, with one third of all establishments in each province not reporting any disinfectant use. Although disinfectant footbaths for visitors were provided in all permanent establishments, less than 25% of these were observed being used. Although fewer than half of all cock fighting establishments used netting to prevent resident or wild bird access, neither of these types of birds were observed during the cock fighting activities. 40% of all establishments maintained a bird free period of at least six days following cock fighting activities.

Only two establishments, both in province B, reported correct implementation of most hygiene and biosecurity measures (cleaning, disinfecting and withheld 6-day period) as recommended by the DLD.

Table 7. Hygiene and biosecurity measures implemented at cock fighting establishments.

	Province A (6)	Province B (9)
Disinfectant bath in use	0%	33.3%
Surrounded by fence	16.7%	44.4%
Protected by covering net	33.3%	33.3%
Clean sweep of cock fighting establishment		
- **Before cock fighting competition/training**	16.7%	0%
- **After cock fighting competition/training**	33.3%	100%
Frequency of disinfectant spraying		
- **once a month**	16.7%	11.1%
- **twice a month**	16.7%	0%
- **3 times a month**	16.7%	0%
- **once a week**	16.7%	55.6%
Rest period (≥ 7 days)	33.3%	55.6%

The number of establishments is included in parentheses

People involved in cock fighting activities

A total of 526 people responded to the questionnaire (Table 2): 258 from province A and 268 from province B. Less than 15% of all respondents in each province lived within a buffer zone, and less than 10% of all respondents in each province were fighting cock owners living within a buffer zone. Although spectators were the most common group amongst those living outside the buffer zone in both regions (42% of respondents in province A and 61% of respondents in province B), inside the buffer zone, owners of fighting cocks were the most common group – accounting for 69% of respondents in province A and 66% of respondents in province B. This difference in the representation of different types of respondents between the buffer and non-buffer zones was significant in each province (χ^2 test p-value =0.006 for province A and $p<0.0001$ in province B).

About 37% of the respondents in province A had their fighting cocks tested for HPAIV H5N1. Although this question was initially not included in the questionnaires for interviewing households located within the buffer zones of province B, 61% of the remaining 204

households in this province had their fighting cocks tested (Table 3). All tests were negative for HPAI H5N1.

Table 8. Types of respondents in the study.

Province	Outside buffer zone				Inside buffer zone			
	Owners	Buyers	Spectators	Trainers	Owners	Buyers	Spectators	Trainers
A (n=258)	33.7%	3.1%	36.4%	13.2%	9.3%	0	2.3%	1.9%
B (n=268)	23.9%	0	55.2%	10.8%	6.7%	0.4%	2.2%	0.8%

Table 9. Percentage of households reporting that their fighting cocks had been tested for HPAIV subtype H5N1 according to DLD requirements.

Province	Fighting cocks tested for HPAIV H5N1
A (n=238)	36.55%
B (n=204*)	61.27%

*only respondents living outside the buffer zones.

All respondents reported that they spent less than 30 minutes travelling to the cock fighting establishments, and the most popular form of transport overall was the motorcycle. The use of different vehicles differed between the three types of cock fighting establishments. Approximately 70% of respondents at the fighting cock training places in province A used motorcycles for their journey. Trucks were the most commonly used vehicles amongst visitors to stadiums in this province (Table 4). A similar pattern was observed in province B, and in each province these differences were statistically significant (χ^2 test, $p<0.0001$). The average capacity of the stadiums investigated was greater than that of the training places, and people tended to stay in the stadiums for a longer time (on average 7 hours). All permanent establishments were open from 10.00 am until the last match ended (on average 8 hours for stadiums and 6 hours for training places). All respondents reported that they visit to watch a number of matches.

Table 10. Descriptive statistics in relation to visitors of cock fighting establishments.

	Province A			Province B		
	PFCS (n=82)	TFCTP (n=164)	PFCTP (n=12)	PFCS (n=120)	TFCTP (n=54)	PFCTP (n=94)
Mean number of total people at the establishment during the day	150	90	33	106	42	51
Mean journey duration (minutes)	29	24	19	23	25	18
Mean time spent in establishment (hours)	7	6	5	7	4	4
Percentage of visitors arriving by:						
Car	16.7%	6.3%	0%	15.8%	9.4%	8.7%
Truck	50%	18.4%	20%	26.7%	16.9%	11.9%
Motorcycle	26.9%	69.6%	70%	57.5%	71.7%	68.4%
Walking	6.4%	5.7%	10%	0%	1.9%	10.8%

PFCS = Permanent Fighting cock stadiums
TFCTP = Temporary Fighting cock training places
PFCTP = Permanent Fighting cock training places

Less than 10% of owners who were unable to identify a matching combatant for their fighting cock at the first cock fighting establishment travelled to another second establishment. Instead, most owners chose to remain at the establishment in the hope that a suitable fighting cock might arrive later during the day. All those owners reporting travelling on to a second establishment preferred to keep the duration of travel to less than 30 minutes.

The vast majority of fighting cocks were taken home after their last fighting activities, rather than being sold or given to another person (Table 5), and this was not significantly affected by the outcome of the fighting activities (province A: χ^2 test, p=0.17; province B: χ^2 test, p= 0.28). There was a significant difference in the proportion of owners visiting another place after leaving the cock fighting establishment between provinces, with 11% of owners in province A and 32% of owners in province B undertaking this activity (χ^2 test, p<0.0001). Of these people, those in province A were significantly more likely to engage in activities relating to cock fighting than those in province B, with 42% going to buy fighting cocks or seek cocks for breeding, compared to 18% in province B (χ^2 test, p<0.0001).

Table 11. Observed behaviour of fighting cock owners in relation to different scenarios.

	Province A	Province B
If no suitable competitor is found, fighting cock is taken to a second establishment	*(n = 111)* 9%	*(n = 82)* 9.7%
Winning cocks	*(n = 51)*	*(n = 41)*
- Taken home *	98%	97.6%
- Sold/given to another person	2%	2.4%
Losing cocks	*(n = 25)*	*(n = 18)*
- Taken home *	92%	88.9%
- Sold/given to another person	8%	11.1%
Drawing cocks	*(n = 37)*	*(n = 22)*
- Taken home *	97.3%	100%
- Sold/given to another person	2.7%	0%
Owners visiting another place after leaving the cock fighting establishment. Reasons:	*(n = 12)*	*(n = 38)*
- To buy fighting cocks	16.7%	2.6%
- To seek fighting cocks for breeding	25 %	15.8%
- To visit friends	25%	31.6%
- **Other activities**	33.3%	50%

Discussion

Although DLD recommendations currently advise against fighting cock establishments being open more than once a week, a lack of compliance with this recommendation was observed– particularly for stadiums. Although approximately 80% of the training places included in the study reported that they maintained a bird free period of at least 6 days after cock fighting activities, only 33% of all visited stadiums complied with this recommendation. As the maintenance costs of stadiums are higher than those of training points, it is presumed that these may open more than once a week in order to increase income, especially given that the perceived current risk of HPAIV H5N1 introduction is considered low amongst the farming population in Thailand.

In this survey, all participants owned fighting cocks, but some of these would only have attended as spectators. The latter may have been because they owned a new cock that still required time to prepare to be ready for the cock fighting matches, or it was in the 1-6 month period that cocks may need after fighting to recover from fight injuries.

The majority of fighting cocks were returned home by their owners after their last fighting activities on the day regardless of the outcome of the cock fighting matches. (Paul et al (2010) suggest that the relatively high monetary and cultural value of fighting cock may

influence the decision of owners to keep their roosters, even if they were unsuccessful in fighting. As the value of fighting cocks is determined by the level of their fighting skills, owners are inclined to increase the fighting opportunities for their cocks by visiting several fighting or training places during a week, so as that their cock can gain more experience. These movements will increase the risk of HPAIV H5N1 introduction into a disease-free area if cocks either become HPAIV H5N1 infected or contaminated at a fighting or training establishment. Other researchers (Tiensin 2009; Paul 2010) reported that there was a higher incidence of HPAIV H5N1 infection in geographic areas with a high density of fighting cocks. This finding might be associated with the relatively high movement frequency and more complex resulting contact structure of fighting cocks.

If fighting cock owners did not find a suitable competitor at the first establishment, owners usually remained at the same establishment hoping to eventually find a suitable competitor and avoiding a further journey on the same day that might compromise the fighting fitness of their bird. Most visitors, particularly fighting cock owners, reported that they would return to their homes directly after leaving the fighting establishment. Spectators stayed at the establishments to watch all games while fighting cock owners after finishing their matches used the opportunity to identify good cocks for potential purchase. During the comparison stage at the fighting establishments, only fighting cock owners and trainers were allowed to come into direct contact with other people's fighting cocks for checking their weight and height in order to find suitable competitors. This behaviour may lead to contamination of humans or cocks with HPAIV H5N1 should a cock be contaminated or infected. Moreover, people associated with cock fighting activities are more likely to travel around the country than those not involved such activities, and thereby may disseminate the virus over large areas if they are contaminated (Ungchusak 2005; Peiris 2007; Chunsuttiwat 2008).

Human behaviour associated with cock fighting activities in both provinces appeared to be similar, except in relation to the places they visited after attending a competition. Some fighting cock owners in province A reported stopping more frequently on the farms of other fighting cock owners (42%), compared to 18% fighting cock owners in province B. This behaviour is important since fighting cock owners visiting a farm on which an outbreak is already occurring may result in infection of a disease-free fighting cock returning from

competition. Moreover, visiting another farm may introduce the virus into the farms if they or their birds have been contaminated by HPAIV H5N1 at a competition.

All fighting cock owners reported that they brought their own equipment and were unlikely to share it with other owners because they were highly concerned about the safety of their cocks. It can be assumed that this behaviour will reduce the risk of contamination of equipment from other cocks. However, if virus contamination of equipment did take place, dissemination of this would be likely, as equipment was reported as rarely being cleaned either before or after fighting activities.

All vehicles in this study were reported to be parked in an area separate from the cock fighting establishment, and as such were also less likely to come into contact with other fighting cocks and humans. The infrequent sale or exchange of fighting cocks at the fighting establishments also reduced the likelihood of the vehicles coming into contact with other cocks. Avian influenza viruses are known to not persist on vehicle surfaces due to their hard and non-porous composition and the detrimental effect of direct exposure to sunlight and heat (Chumpolbanchorn 2006; Songserm 2006).

Limitations

Due to the small number of cock fighting establishments included in the study, only those establishments whose owners were willing to participate, data only being collected for each on a single occasion (owners of 32 cock fighting establishments declined participation) the data collected on hygiene and biosecurity measures needs to be interpreted cautiously.

Conclusions

Based on this study, only two establishments, both permanent cock fighting training places, implemented most of the recommended hygiene and biosecurity measures (cleaning, disinfecting and withheld 6-day period). Most fighting cocks are brought back to their owners' home after a fight regardless of the outcome of the competition. Fighting cock owners take good care of their cocks, and are particularly concerned about health and fitness of their cocks; they preferably use their own equipment and vehicles and prefer short journeys to the fighting places (less than 30-minutes driving trip). Human behaviours related to cock fighting activities in both provinces appeared to be similar, except in relation to their behaviour after leaving the establishments. Fighting cock owners in province A were significantly more likely to be involved in cock-fighting related activities after leaving the

establishments, (e.g. buying or searching a good cock) than those of province B. The apparently poor compliance with recommended DLD-hygiene and biosecurity measures at the cock fighting establishments described in this survey raises concerns, and efforts should be made rectify that situation, since the data also show that fighting cock associated activities have strong potential for virus spread in the event of an outbreak.

References

Buranathai, C., Amonsin, A., Chaisigh, A., Theamboonlers, A., Pariyothorn, N. and Poovorawan, Y. (2007). "Surveillance activities and molecular analysis of H5N1 highly pathogenic avian influenza viruses from Thailand, 2004-2005." Avian Diseases **51**: 194-200.

Chumpolbanchorn, K., Suemanotham, N., Siripara, N., Puyati, B. and Chaichoune, K. (2006). "The effect of temperature and UV light on infectivity of avian influenza virus (H5N1, Thai field strain) in chicken fecal manure." Southeast Asian J Trop Med Public Health **37**(1): 102-105.

Chunsuttiwat, S. (2008). "Response to avian influenza and preparedness for pandemic influenza: Thailand's experience." Respirology **13 (Suppli.1)**: S36-S40.

DLD (2006). Avian Influenza Control in Thailand, Agricultural Coooperative Publisher of Thailand.

Gilbert, M., Chaitaweesub, P., Parakamawongsa, T., Premashthira, S., Tiensin, T., Kalpravidh, W., Wagner, H., and Slingenbergh, J. (2006). "Free-grazing ducks and highly pathogenic avian influenza, Thailand." Emerging Infectious Diseases **12**(2): 227-34.

Paul, M., Tavornpanich, S., Abrial, D., Gasqui, P., Charras-Garrido, M., Thanapongtharm, W., Xiao, X., Gilbert, M., Roger, F. and Ducrot, C. (2010). "Anthropogenic factors and the risk of highly pathogenic avian influenza H5N1: prospects from a spatial-based model." Vet Rec **41**(28).

Peiris, J. S. M., de Jong, M.D. and Guan, Y. (2007). "Avian Influenza Virus(H5N1): a Threat to Human Health." Clinical Microbiology Reviews **20**(2): 243-267.

Songserm, T., Jam-on, R., Sae-Heng, N. and Meemak, N. (2006). "Survival and stability of HPAI H5N1 in different environments and susceptibility to disinfectants." Developments in Biologicals **124**: 254.

Souris, M., Gonzalez, J.P., Shanmugasundaram, J., Corvest, V. and Kittayapong, P. (2010). "Retrospective space-time analysis of H5N1 Avian influenza emergence in Thailand." International Journal of Health Geographics **9**(3).

Tiensin, T., Ahmed, S.S.U., Rojanasthien, S., Songserm, T., Ratanakorn, P., Chaichoun, K., Kalpravidh, W., Wongkasemjit, S., Patchimasiri, T., Chanachai, K., Thanaphontham, W., Chotinan, S., Stegeman, A. and Nielen, M. (2009). "Ecologic risk factor investigation of clusters of Avian Influenza A (H5N1) virus infection in Thailand." The Journal of Infectious Diseases **199**: 1735-43.

Tiensin, T., Chaitaweesub, P., Songserm, T., Chaisingh, A., Hoonsuwan, W., Buranathai, C., Parakamawongsa, T., Premashthira, S., Amonsin, A., Gilbert, M., Nielen, M., And Stegeman, A. (2005). "Highly pathogenic avian influenza H5N1, Thailand 2004." Emerging Infectious Diseases **11**: 1664-1672.

Ungchusak, K., Auewarakul, P., Dowell, S.F., Kitphati, R., Auwanit, W., Puthavathana, P., Uiprasertkul, M., Boonnak, K., Pittayawonganon, C., Cox, N.J., Zaki, S.R., Thawatsupha, P., Chittaganpitch, M., Khontong, R., Simmerman, J.M. and Chunsutthiwat, S. (2005). "Probable Person-to-Person transmission of Avian Influenza A (H5N1." The New England Journal of Medicine **352**(4): 333-340.