Avian Influenza in Suphanburi Province, Thailand: Assessment of Transmission Dynamics and Interventions in the Local Poultry Sector

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Dedication

This dissertation is dedicated to my mother, Karen, who has always encouraged me in school and who has been a constant friend thoughout my life. She is a spirited and loving woman who has helped me become the confident, independent and passionate person I am today.

Research Abstracts

Chapter 2: Risk factors for exposure to influenza A viruses, including subtype H5 and H7 viruses, in Thai free-grazing ducks

Free-grazing ducks (FGD) have been associated with highly pathogenic avian influenza (HPAI) H5N1 outbreaks and may be a viral reservoir. In July-August 2010, we assessed influenza exposure of Thai FGD and risk factors thereof. Serum from 6254 ducks was analysed with enzyme-linked immunosorbent assays (ELISAs) to detect antibodies to influenza A nucleoprotein (NP), and hemagglutinin H5 protein. Eighty-five per cent (5305 ducks) were seropositive for influenza A. Of the NP-seropositive sera tested with H5 assays (n=1423), 553 (39%) were H5 ELISA-positive and 57 (4%) suspect. Twelve per cent (74 of 610) of H5 ELISA-positive/ suspect ducks had H5 titers ≥1:20 by hemagglutination inhibition. Risk factors for influenza A seropositivity include older age, poultry contact, flock visitors and older purchase age. Study flocks had H5 virus exposure as recently as March 2010, but no HPAI H5N1 outbreaks have been identified in Thailand since 2008, highlighting a need for rigorous FGD surveillance.

Chapter 3: Seroprevalence of H5N1 antibodies among poultry-exposed persons in Central Thailand, 2008

There is little seroprevalence data from Suphanburi Province, Thailand which had the highest cumulative number of HPAI H5N1 outbreaks during Thailand's second outbreak wave in 2004. The goal of this work was to investigate the seroprevalence of and risk factors for antibodies to HPAI H5N1 in poultry owners living Suphanburi Province. Seroprevalence was 6.3% (23 seropositive of 363) in poultry owners with no known history of H5N1 infection living in HPAI H5N1 outbreak areas. Single persons and those working with farmed chickens were at increased risk of seropositivity. This may be because unmarried persons perform more poultry-related tasks and at a higher frequently than those with a spouse with whom to share the work. Single persons in our study had a significantly lower household size than those married or divorced (3.8 and 4.7 persons, respectively, p=0.01), however we do not have data regarding household members' roles in poultry-related tasks. Blood samples taken from the 30 seropositive individuals after a 28-month period yielded no antibody titers ≥1:160. Continued serological monitoring of both

humans and animals is necessary to identify subclinical poultry cases and human exposures. In addition, education of poultry owners and other in close proximity to poultry should be provided in Thailand, even when the risk of exposure seems small.

Chapter 4: Use of personal protective measures by Thai households in areas with avian influenza outbreaks

This study assessed the use of protective equipment and hand hygiene measures by poultry-owning households during activities involving poultry contact in Suphanburi Province, Thailand. Surveys conducted in 2008 included questions regarding poultry-related activities and protective measures used during an HPAI outbreak (2005) and three years after the study location's last reported outbreak (2008). For both time periods, poultry owners reported limited use of personal protective equipment during all activities and inconsistent hand washing practices after carrying poultry and gathering eggs. This is the first time that personal protective equipment-use in Thailand has been quantified for a large study group. These data are important for ongoing characterization of HPAI risk and for the crafting of educational messages.

Chapter 5: Using agent-based modeling of the village poultry sector in Thailand to identify measures that control influenza transmission while mitigating negative socioeconomic impacts

Outbreaks of avian influenza in and transmission among domestic poultry holdings are facilitated by the movement of poultry and humans and the transport of equipment, vehicles and other vectors. In the local Thai poultry sector there are few biosecurity measures to prevent the spread of infectious diseases among flocks. We developed an agent-based model (ABM) of the local poultry sector to simulate contacts among persons that own poultry and conduct poultry-related activities. Using this model, we were able to identify opportunities for the transmission and control of HPAI H5N1. The model was developed using data from in-depth interviews with free-grazing duck, backyard, and farmed poultry owners, as well as egg and poultry traders. Within the ABM, inter-flock transmission is guided by an SEIR model. The median model outbreak duration over 500 simulations was 49 days, and the median number of flocks infected in each simulation was 86 (25% of flocks). The reproduction number (R_f) calculated for the baseline model scenario was 1.4. Transmission among flocks was dominated by visiting among poultry-

owning neighbors and, less so by indirect transmission from roads traveled by infectious FGD flocks. R_f was brought < 1 when the probability of transmission by visiting was decreased (representing improved biosecurity), and when FGD flock movement between the owner's home and the rice field was minimized.

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Chapter 1: Literature Review

Avian Influenza

Influenza A Viruses

Biology of Influenza A Viruses

Avian influenza (AI) viruses are type A influenza viruses of the Orthomyxoviridae family. They are negative-sense RNA viruses, with eight gene segments that code for 10 viral proteins. The viral surface proteins include hemagglutinin (HA), neuraminidase (NA) and membrane ion channel (M2), and the internal proteins include the nucleoprotein (NP), matrix protein (M1), and the polymerase basic proteins 1 and 2 (PB1 and PB2). The viruses also have two nonstructural proteins (NS1 and NS2). The surface of the virus is a lipid membrane, from which the HA, NA and M2 proteins emerge. These proteins are responsible for binding to host cells (HA), cleavage of membrane components for release of replicated virions (NA), and triggering viral uncoating (M2). The HA and NA proteins are responsible for the antigenicity of the influenza virus (Webster et al., 1992; Suarez, 2008). The Orthomyxoviridae family also includes influenza types B and C, which are human pathogens and not known to affect avian species (Suarez, 2008).

Influenza viruses are named and identified with a "subtype" by their specific HA and NA types. There are sixteen identified HA types (1-16) and nine NA types (1-9). The nomenclature for influenza viruses includes this subtyping and five other steps, as summarized by Suarez (2008): 1) antigenic type (A, B or C); 2) host of isolated virus; 3) geographic origin; 4) unique laboratory reference number; 5) year of isolation; 6) HA and NA subtypes. For example, a novel influenza virus found in chickens in Texas in 2004 was named A/chicken/Texas/298313/2004 (H5N2).

Virus Evolution and Reassortment

The evolution of influenza viruses is primarily facilitated by the occurrence of antigenic drift (the accumulation of point mutations) and antigenic shift (gene reassortment) (Webster et al.,

1992; Suarez, 2008). Point mutations result from a lack of "proofreading" by RNA polymerases, and virus replication yields many variants as a result (Webster et al., 1992). The variants with beneficial mutations survive, and those with deleterious mutations do not. It has been proposed that human influenza A viruses undergo positive Darwinian selection through antigenic drift, selecting for variants that will allow the virus to escape the host immune response (Fitch et al., 1991). Most changes are seen in the surface proteins, and most changes to the HA protein occur in the antigenic codons of the HA gene, likely to evade host immune response (Plotkin and Dushoff, 2003). Accordingly, it has been estimated that the HA gene of human influenza viruses evolves three times faster than the NS gene (Fitch et al., 1991). HA is instrumental in the ability of the virus to penetrate the host cell membrane (Webster and Rott, 1987).

Antigenic shift, which is most common in human populations, is the emergence of a novel virus, either through transmission of a novel virus directly from another species or by transmission of a virus with genetic reassortment of influenza A viruses from one or multiple species (Cox and Uyeki, 2008).

Pathogenicity of Avian Influenza Viruses

Avian influenza viruses are classified as either highly pathogenic avian influenza (HPAI) or low pathogenicity avian influenza (LPAI). Prior to 2005, HPAI viruses were not identified in wild birds, but rather, the HPAI viruses exhibited an evolution of wild-type avian influenza viruses, adapting to domestic poultry and circulating for a period of time sufficient to allow mutation into high pathogenicity (Suarez, 2008). The distinction between high and low pathogenicity is based on the virulence of the virus isolates. HPAI viruses have the ability to cause acute and significant disease in poultry species, and, though this is occasionally a characteristic of LPAI viruses, it is not common. Avian influenza viruses traditionally cause mucosal disease, affecting the respiratory and gastrointestinal tracts. Significant disease caused by HPAI viruses is a result of systemic infection that is not limited to mucosal tissues.

Classification an AI virus as HPAI or LPAI is experimentally determined based on the intravenous virulence of the virus in chickens. Testing protocols have been established by the World Organization for Animal Health (OIE) (World Organization of Animal Health, 2011). In addition to the intravenous testing, isolates with H5 and H7 subtypes, which are the only subtypes known to have high pathogenicity potential, should be sequenced for specific mutations in the

cleavage site of the HA gene. Such mutations are the best molecular predictors of high pathogenicity. H5 and H7 viruses without intravenous pathogenicity that have HA cleavage site mutations similar to those seen in virulent viruses are considered to be HPAI viruses. H5 and H7 viruses without intravenous pathogenicity or the HA mutations are considered LPAI viruses. All H5 and H7 viruses are notifiable to the OIE (World Organization of Animal Health, 2011).

Reservoirs and Transmission of Avian Influenza Viruses

Influenza A viruses are maintained in nature by wild avian species, namely waterfowl and shorebirds. In such species, the virus replicates in the gastrointestinal mucosa, and transmission is via fecal contamination of water and the surrounding environment (Webster et al., 1978). By and large, the role of host and reservoir is played by species in the Anseriformes and Charadriiformes orders (Stallknecht and Shane, 1988; Stallknecht and Brown, 2008). While the Anseriformes (ducks, geese and swans) and Charadriiformes (gulls, terns and shorebirds) groups contain birds of many types, avian influenza viruses tend to display association with specific families and species. It has been determined that most virus isolations in the Anseriformes order have been from dabbling and diving ducks, with the majority of these in the mallard species. Similarly, in the Charadriiformes order, most of the isolates have come from the families containing sandpipers, turnstones, gulls and terns (Stallknecht and Brown, 2008). The distribution of avian influenza subtypes has a tendency to vary within groups of birds and within species (Webster et al., 1992). For example, one study showed that subtypes H3, H4 and H6 are the most frequently isolated subtypes from ducks in North America (Krauss et al., 2004a), and it is reported that the most common isolates of Charadriiformes are H3, H9, H11 and H13 (Krauss et al., 2004b; Swayne, 2008). Avian influenza viruses have been isolated worldwide and from many species of birds.

Influenza viruses are shed into the environment mainly in the feces of infected birds. Though there has been some identification of LPAI viruses in the respiratory tract of infected birds, the majority of virus replication occurs in the gastrointestinal tract. Susceptible birds are infected with the virus as they drink or eat in the contaminated environment (Kim et al., 2009). The fecal-oral transmission of influenza viruses is thought to contribute to the higher prevalence of infection in dabbling ducks than in diving ducks, as they are surface water feeders (Olsen et al., 2006; Kim et al., 2009). Prevalence of influenza infection in wild ducks is highest in the fall

marshalling areas, rather than the areas of winter or spring migration, or along the migration routes (Munster et al., 2007). This is a time when there are many young, immunologically naïve ducks present in the flocks. The prevalence of influenza in wild duck populations can vary with the size of the population, location of sample collection and time of collection (Kim et al., 2009). Figure 1.1 shows the prevalence of influenza infection in relation to the movement and behavior of wild ducks.

Infection of an individual bird is dependent upon many variables, including the size of the inoculum, exposure route, virus antigenicity, host adaptation of the virus and host immunity. Exposure can lead to no infection, infection without clinical signs, or mild to severe disease (Figure 1.2) (Brugh and Johnson, 1987; Swayne, 2008; Kwon and Swayne, 2010). Exposure is influenced by environmental persistence as well as the mechanical spread of the virus by humans, equipment and other vectors (Brugh and Johnson, 1987).

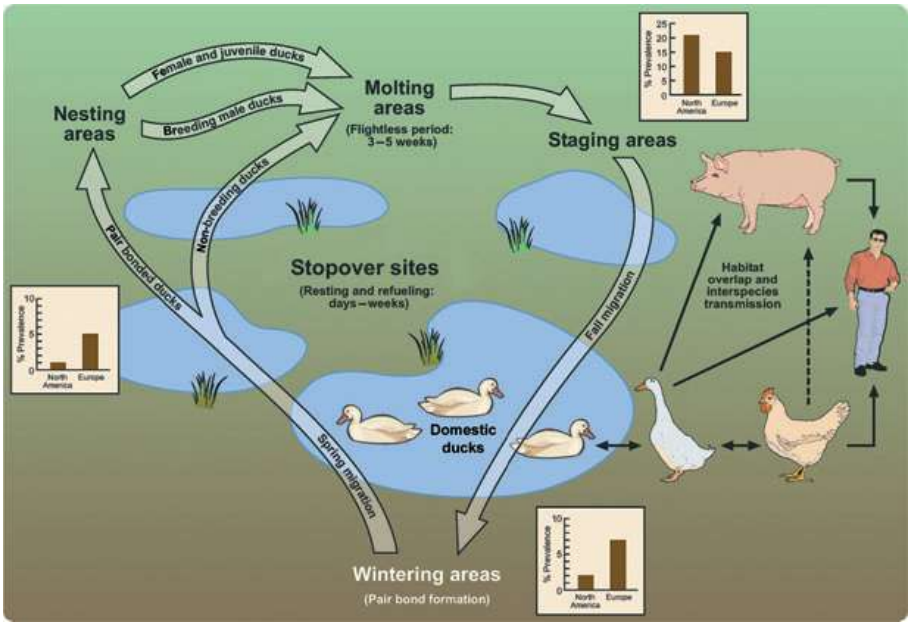

Figure 1. 1 Overview of the annual movement and behavior of migratory ducks and their role in interspecies transmission. During spring and fall migration, the ducks rest and feed for a few days to weeks at numerous stopover sites (wetlands, lakes, or ponds) along the migration route. The length of stay and the aquatic habitat allows the transmission of influenza viruses to and from the domestic duck populations. Domestic ducks that become infected are likely to maintain the virus locally and increase the probability of its spread to other species. In the diagram, solid arrows indicate confirmed routes of transmission of LPAI and/or HPAI viruses between species. The dashed line represents a probable but unconfirmed route of

transmission. The graphs indicate the average prevalence of low-pathogenic avian influenza in North American and European duck populations during 3 stages of the annual migration (Olsen et al., 2006; Wallensten et al., 2007). Figure and description from (Kim et al., 2009). Reproduced with permission.

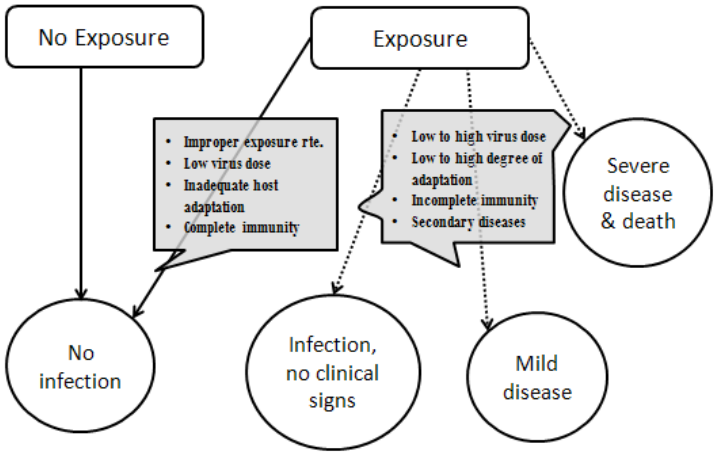

Figure 1. 2 Schematic depiction of the consequences of avian influenza exposure. Adapted from (Swayne, 2008). Reproduced with permission.

Interspecies Transmission of Avian Influenza Viruses

Transmission of avian influenza viruses from the natural reservoir to other wild and domestic species does occur (Fig 1.1), however interspecies transmission of avian influenza viruses is less common than intraspecies transmission, as viruses become adapted to the species in which they circulate. Infections of diverse species of bird with the same virus can have different outcomes (Alexander, 1982). This may have to do with the tissue distribution and quantity of virus sialic acid-inked receptors to which the HA protein binds (Kuchipudi et al., 2009). Avian influenza viruses typically prefer to bind N-acetylneuraminic acid-α2,3-galactose (α2,3Gal) and human and swine viruses to α2,6Gal. The most frequent type of interspecies transmission occurs within taxonomic families (e.g. chickens to quail), but it has been known to occur between different orders (e.g. wild ducks to turkeys) and even between taxonomic classes (e.g. birds to swine, birds to humans) (Swayne, 2008). Kuchipudi and others found that while the gastrointestinal tract of chickens and turkeys expresses only α2,3Gal receptors, both species have both receptor types throughout various organs (Kuchipudi et al., 2009). Chickens have α2,6Gal receptors at a higher density in the trachea and ducks have more α2,3Gal receptors, potentially

presenting a role for chickens as intermediate hosts for avian viruses that may affect humans. The respiratory tract of the pig bears both α2,3Gal and α2,6Gal receptors, allowing it to become infected with viruses that preferentially bind with each receptor type (Ito et al., 1998). This puts the pig in a unique position to influence the maintenance, transmission and reassortment of various influenza A viruses (Kida et al., 1994; Brown, 2000).

Over time, influenza A viruses may develop the ability to bind well (adapt) to receptors on host cells of non-reservoir species, including humans, making it a zoonotic pathogen of considerable importance. It has been shown that the 1918 "Spanish influenza" pandemic H1N1 virus was an avian origin virus that adapted to humans (Reid et al., 1999). The two subsequent pandemic viruses, "Asian influenza" H2N2 in 1957 and "Hong Kong influenza" H3N2 in 1968 were reassortants of human and Eurasian avian origin viruses (Hsieh et al., 2006), and the 2009 H1N1 pandemic virus is composed of human, avian and swine origin gene segments (Garten et al., 2009; Trifonov et al., 2009).

Direct infection of humans with non-human-adapted avian influenza viruses has been rarely and only sporadically reported (Table 1.1), and the only subtypes known to have directly infected humans are H5, H7 and H9 (Cox and Uyeki, 2008). H7 and H9 viruses have occasionally caused clinical signs (usually conjunctivitis) after occupational exposure (Koopmans et al., 2004; Bosman et al., 2005; Du Ry van Beest Holle,M. et al., 2005; Meijer et al., 2006). Notably, after the 2003 poultry outbreak of H7N7 in the Netherlands, approximately 50% of those with poultry contact had antibodies to the virus, and it was estimated that 1000 people may have been infected with AI (Bosman et al., 2005). Subclinical seropositivity to H9N2 viruses, which are endemic in Asia, was identified in nearly 10% of poultry workers and 4% of general citizens tested in Guangdong Province, China (Lu et al., 2008). More widespread and serious have been the infections caused by highly pathogenic H5N1 viruses, beginning in 1997 and continuing to this day as part of the multi-continental epizootic of poultry infection.

Table 1. 1 Cases of infection of humans with avian influenza viruses, excluding HPAI H5N1 in Asia and elsewhere. Adapted from (Cox and Uyeki, 2008). Reproduced with permission.

Virus	Location(s)	Year(s)	No. of cases [ages affected]	Clinical Findings
H7N2 LPAI	United States	2002, 2003	1, 1 [adults]	ILI
H7N2 LPAI	United Kingdom	2007	4 [adults]	ILI, lower respiratory tract disease, conjunctivitis
H7N3 LPAI	United Kingdom	2006	1 [adult]	Conjunctivitis
H7N3 LPAI	Canada	2004	1 [adult]	Conjunctivitis
H7N7 LPAI	United Kingdom	1996	1 [adult]	Conjunctivitis
H9N2 LPAI	Hong Kong SAR of China	1999, 2003, 2007	2, 1, 1 [children]	ILI
H9N2 LPAI	China	1998, 1999	4, 1 [adults, children]	ILI
H7N3 HPAI	Canada	2004	1 [adult]	Conjunctivitis
H7N7 HPAI	Netherlands	2003	89 (1 death) [primarily adults]	ILI, conjunctivitis, severe pneumonia with ARDS

Transmission Dynamics of Avian Influenza

The incubation period (period between infection and clinical signs) of avian influenza infection in birds varies with the type of virus, host species and route of exposure, but is approximately 3 days (sometimes as little as one day) in naturally infected chickens and 14 days in chicken flocks (Easterday et al., 1997; United States Department of Agriculture, 2007). Because avian species can shed virus prior to displaying clinical signs, the incubation period is not the most accurate measure for assessing influenza activity, although it is the most observable. Both the OIE and the United States Department of Agriculture (USDA) consider a 21-day

incubation period when determining flock freedom from avian influenza (United States Department of Agriculture, 2007; World Organization for Animal Health, 2011b). With this strategy, which is primarily utilized in regulations of poultry movement, it is reasoned that 21 days would be the longest period that may occur between introduction of disease to a bird or flock and the onset of clinical signs.

The infectious period is the time through which birds are actively shedding virus into the environment. Shedding time varies with the type and dose of virus, species affected and age of the birds. A general infectious period estimate for LPAI viruses is 7 to 10 days (Swayne, 2008). Potentially of greater importance when dealing with large holdings, village flocks or other premises such as live bird markets, is the potential for sustained viral persistence within the population, driven by low-level infection in large groups of birds or the continued influx of naïve or young birds (Swayne, 2008). Viral transmission is based upon the number of susceptible animals, rather than the number of infectious individuals, and, as the susceptible pool decreases, transmission is slowed and can be maintained at a low and undetectable level. Periods of stress, such as transport and flock mixing, or an influx of large numbers of naïve birds may initiate clinical illness and more intense viral shedding. Such persistence has been noted in chickens over periods as long as 8 weeks and 6 months, with varying levels of seropositivity in the flocks during that time (Ziegler et al., 1999). In closed systems, such persistence may occur through a combination of flock transmission and environmental survival.

Avian Influenza in Domestic Poultry

While influenza infections in domestic poultry can be subclinical, there are often observed clinical signs and production losses (Suarez, 2008). In all poultry species, LPAI viruses usually cause mild to severe respiratory symptoms, decreased intake of feed and water and a drop in egg production. LPAI viruses can sometimes lead to high mortality, especially if infection is complicated by secondary pathogens (Swayne and Pantin-Jackwood, 2008). Diagnostic testing of dead birds is a sensitive way to identify infection within flocks (Suarez et al., 2006). Clinical signs in gallinaceous poultry include coughing, sneezing, rales and rattles, epiphora, occasional diarrhea, decreased egg production, and behavior changes including huddling, lethargy and inappetance (Swayne and Pantin-Jackwood, 2008).

HPAI viruses cause severe morbidity and mortality by inducing systemic disease in gallinaceous species but typically little or no disease in ducks and geese (Swayne and Pantin-Jackwood, 2008). Since 2002, one exception has been the Asian HPAI H5N1 viruses, which have established the ability to cause disease in ducks and species of wild birds (Ellis et al., 2004; Pantin-Jackwood and Swayne, 2007). Peracute clinical signs of HPAI infection in gallinaceous poultry include sudden death before the onset of other signs or 24 hours of recumbency, obtundity and dehydration prior to death (Swayne and Pantin-Jackwood, 2008). Clinical respiratory disease is less common with HPAI. In layers, there is a severe drop in egg production, including complete termination of production by day six. In the acute course of infection, chickens may be infectious for 3-7 days prior to death, sometimes experiencing neurologic signs. Velogenic Newcastle disease is a differential diagnosis for this type of infection.

Routes of Influenza Transmission in Domestic Poultry Populations

Swayne describes the five routes by which avian influenza viruses can be introduced into a poultry population: 1) direct exposure to infected birds; 2) exposure to equipment or materials contaminated with virus-containing feces or respiratory secretions; 3) movement of the virus by human shoes or clothing; 4) virus-contaminated water; and 5) airborne movement of virus (Swayne, 2008). While direct contact is the most effective route of transmission, exposure to fomites or contaminated water commonly leads to infection in domestic poultry populations (Alexander, 1982). The most important sources of virus to domestic poultry populations were itemized by Halvorson as poultry manure, respiratory secretions, live poultry, dead poultry, unwashed eggs and contaminated people or equipment. The amount of gross contamination plays a large role in transmission by these routes (Halvorson, 2008). In a summary of the epidemiology of avian influenza written in 1987, Brugh and Johnson remarked that, “Mechanical transmission by anything that may walk, crawl or fly from farm to farm must be considered a possibility but confirmation is difficult” (Brugh and Johnson, 1987).

<u>Movement of Humans and Equipment</u>

During the 1983-84 H5N2 outbreak in Pennsylvania, people and equipment were found to be the most likely source of infection for flocks (Fichtner, 1987). This included visitors such as neighbors, friends and relatives. Virus was isolated from egg flats, dust, flies, chicken carcasses, exhaust fans and elsewhere on infected premises. Also during that outbreak, virus was isolated

from the albumen, yolk and shell surface of eggs from both clinically and non-clinically affected farms (Cappucci et al., 1985). The initial surveillance methods for this outbreak included reporting of sick flocks by owners and service representatives, and those who had experience in AI diagnosis would assist their neighbors. Biosecurity measures were poorly implemented, and it is thought that this community-based surveillance may have helped the spread of AI throughout the area (Fichtner, 1984). This early phase of the outbreak was also characterized by a tendency of flock owners to observe the severity of the disease before reporting infection in their flock. Lectures and media attention focused on biosecurity were important in the outbreak response (Fichtner, 1984). These types of findings were not limited to the Pennsylvania H5N2 outbreak. During the 2002 Virginia outbreak of LPAI H7N2, movement of dead poultry to an offsite rendering plant had the strongest epidemiologic association with infection, indicating that poultry farms were becoming infected via contaminated vehicles or humans (Akey, 2003).

Surface Water

Surface water contaminated with influenza viruses from wild birds has led to infections in domestic poultry and domestic swine populations (Hinshaw et al., 1979; Markwell and Shortridge, 1982; Sivanandan et al., 1991). Influenza virus has also been found to persist on the feathers of infected chickens and ducks and be a potential source of infection (Yamamoto et al., 2007; Yamamoto et al., 2007; Busquets et al., 2010; Yamamoto et al., 2010).

Airborne Spread

Empirical evidence for airborne spread of influenza viruses is still lacking, but anecdotal and epidemiologic evidence does exist. While it is thought that airborne transmission is not a primary contributor to between-flock transmission, it can occur if poultry manure or other materials are exposed to wind (Bowes et al., 2004). During the H5N2 outbreak in Pennsylvania in the early 1980s, a flock located 4 meters from a roadway was infected with the virus and showed signs 3 days after a truck hauling infected birds passed on the way to slaughter (Johnson, D.C., unpublished data from (Brugh and Johnson, 1987)). Also during that outbreak, epidemiologic evidence suggested that contaminated litter spread on fields provided an exposure to nearby flocks, and air sampling returned 5 of 6 H5N2-positive samples at a distance of 3-6 meters downwind of an affected flock and 1 of 12 positive samples at 45-85 meters (Brugh and Johnson, 1987). During an H7N3 outbreak in Canada in 2004, researchers used air samplers to detect

viable virus inside one chicken barn and influenza virus nucleic acids 800 meters away from the barn in a field (Schofield et al., 2005), although no other samples in the study were positive. Investigators of an equine influenza virus outbreak in Australia make a case for aerosol transmission of virus to barns over 1-2 km, and there are anecdotal reports of virus spread up to 8 km (Huntington and Victoria. Dept. of Agriculture and Rural Affairs, 1990; Davis et al., 2009). Recent studies have shown that respiration, coughing and sneezing (by pigs and humans) can produce aerosolized virus particles, although such transmission of influenza A viruses is poorly understood and may be dictated by conditions such as temperature, humidity and the characteristics of individual viruses (Lowen et al., 2007; Lowen et al., 2008; Tellier, 2009).

Influenza in Minnesota turkeys

As domestic ducks are thought to attract wild ducks, premises with mixed poultry have long been considered important potential sources of infection (Brugh and Johnson, 1987) and thus should be monitored for evidence of virus circulation and mutation, especially in cases where all in-all out production is not practiced. One example that describes the risk posed by wild duck contact is the experience of turkey farming in Minnesota, a key location for duck marshalling prior to southern migration (Halvorson, 2008). Prior to the late 1990s, many turkeys in Minnesota (the largest turkey producing state in the United States) were raised outdoors, and the industry suffered from over 100 introductions of avian influenza resulting from contact between turkeys and wild ducks. These introductions of influenza led to infection in over 1000 flocks. Instrumental research by Halvorson and other identified transmission of avian influenza viruses from wild ducks to turkeys (Halvorson et al., 1983), and after the daunting emergence of HPAI H5N1 in Hong Kong in 1997, the turkey industry largely transitioned to indoor production systems. Since that time, only five flocks have been infected with avian influenza. Pigs have also long been known to be a source of influenza A viruses for domestic poultry (Andral et al., 1985; Swayne, 2008) and are now the primary source of influenza A viruses in Minnesota turkeys (Halvorson, 2008).

The susceptibility of ducks to infection with avian influenza has led some to believe that they could be a useful sentinel population for avian influenza isolates carried by wild birds. One recent study in Europe involved the monitoring of five sentinel duck flocks as well as wild birds for infection with influenza A viruses (Globig et al., 2009). Researchers monitoring for influenza

in sentinel ducks considered this a useful adjunct to surveillance of wild birds. The spread of influenza viruses by migratory ducks to resident duck populations at stopover sites is likely, and it has been shown that resident or domestic duck populations have the capability to cause infection in neighboring domestic poultry (Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005; Gilbert et al., 2006; Olsen et al., 2006). Ducks, then, may provide a means for monitoring viruses circulating in wild birds that may pose a threat to domestic poultry.

Environmental Persistence of Influenza A Viruses

Persistence of Influenza A Viruses in Water

The fecal-oral transmission of avian influenza makes water in the surrounding area an important player in the spread of viruses from one bird to another. Influenza A viruses have been shown to have the ability to persist in the environment for varying time periods, depending upon the individual virus isolate characteristics and the water temperature, pH and salinity (Stallknecht et al., 1990; Brown et al., 2007b; Brown et al., 2009). Early studies using distilled water estimated that low pathogenicity viruses had the ability to survive for up to 100 days at 17^{o}C, depending on salinity, pH and isolate type. More recent studies have been conducted with both LPAI and HPAI (H5N1) viruses, showing variability among viruses of the same subtype, isolated from different birds at different times, and between HPAI H5N1 isolates (Brown et al., 2007b; Brown et al., 2009). These studies indicate that survival is influenced by the interaction of temperature and salinity, as well as being inversely proportional to the two conditions individually. With a salinity of 0ppm, Brown *et al.* (2007) estimated that LPAI viruses could persist up to 667 days in water 17^{o}C, while corresponding viruses persisted only up to an estimate of 118 days at 28^{o}C. In addition, the isolates estimated to persist longest in the colder water did not necessarily persist longest in the warmer water. The same study predicted that HPAI H5N1 isolates could persist up to 158 days at 17^{o}C and up to 30 days at 28^{o}C, at 0ppm. Overall, the HPAI H5N1 virus isolates did not survive as long as did wild-type isolates, but the variability between the isolates indicates that there is more to be learned about their persistence in the environment. In addition, the authors of this study note that the role of the environment may be quite different for HPAI H5N1 viruses than for LPAI viruses, due in part to this decreased survival time, as well as the increased tendency for respiratory virus replication and transmission.

Avian influenza viruses have been isolated and analyzed from bodies of water frequented by waterfowl (Ito et al., 1995; Zhang et al., 2006; Lang et al., 2008). These studies were carried out in cold-weather climates, Alaska and Siberia, which are ideal for maintenance of viral particles. In one study, sampling the sediment of three Alaskan ponds over a nine month period yielded a detection rate of over 50% (Lang et al., 2008). These ponds were frequented by thousands of waterfowl daily during the fall and spring. Virus was still readily isolated from sediment over the winter months. Another study suggested that influenza A viruses deposited by birds can be preserved in Siberian lake ice until the following spring when the ice melts and the birds return (Zhang et al., 2006). Results indicated that areas most frequented by waterfowl provided samples with the highest concentrations of avian influenza A virus. Though it is unknown how duck populations acquire their yearly avian influenza infections, it is speculated that virus may survive the winter in frozen waters and re-infect the ducks in the spring (Webster et al., 1992).

Persistence of Influenza A Viruses in Fecal Material

Virus in naturally contaminated materials may persist for hours to months (Vrtlak and Kapitancik, 1967). High moisture and low temperature may prolong persistence in feces, and in a controlled study, HPAI H5N2 persisted in wet feces for 35 days and in dry feces for 9 days at 4^{o}C (Beard et al., 1984). Wet feces kept at 25^{o}C could only support virus viability for 2 days, and dry feces at that temperature for only 1 day. Virus concentrations were as high as 10^{9} ELD 50/gram in the feces used in that study, produced by chickens experimentally infected with a lab-derived H5N2 Pennsylvania isolate (Beard et al., 1984). During the Pennsylvania H5N2 outbreak, virus was isolated in one barn from wet manure 105 days after depopulation (Fichtner, 1984; Fichtner, 1987). In the control of that outbreak, composting had been recommended to get rid of infectious manure, but as virus was identified in some compost piles, exposure of the contaminated litter to the sun (and thus complete drying) was thought to be the most effective way to inactivate virus. While ultraviolet light can kill influenza virus that is exposed, it is not efficacious for the inactivation of virus contained within fecal material (or compost piles), and it plays only a minor role in the disinfection process for poultry houses (Birnbaum and O'Brien, 2008).

Removal of Influenza Virus from Contaminated Environments

Avian influenza viruses are susceptible to multiple disinfectants as a result of their viral envelope. In addition to disinfectants, the virus is susceptible to heat, and heating a barn to 90-100^{o}F for one week is recommended as a first step for disinfection (Halvorson and University of Minnesota. Agricultural Extension Service, 1984). After the heating step, the premise must be cleaned, including removing all manure and litter, and then disinfected. The building should then be left empty for 2-3 weeks before bringing in a new flock. In its *Standard Operating Procedures for HPAI Response*, the Food and Agriculture Organization (FAO) recommends the burning, burial or composting of bird carcasses, contaminated feed and litter (deep, so as to avoid unearthing by scavenging animals) (Food and Agriculture Organization, 2011b). Chemical disinfectants recommended to inactivate avian influenza viruses (each with their own advantages and disadvantages) include soaps and detergents, iodine, peroxygen compounds, ethanol, hypochlorites, sodium dichlorotriazine trione, phenols, cresols, quaternary ammonium compounds, aldehydes, and various acids and bases (Birnbaum and O'Brien, 2008; Food and Agriculture Organization, 2011b). The use of personal protective equipment (PPE) by those conducting the cleaning and disinfection is critical, as there are both zoonotic and chemical exposure risks involved (Birnbaum and O'Brien, 2008).

Highly Pathogenic Avian Influenza

Highly pathogenic avian influenza viruses were long known in Europe as "fowl plague", a disease that had a tendency to cause mild disease in some flocks of chickens and other poultry before turning into a more virulent disease and then returning to the mild form after a period of time (Kaleta and Rulke, 2008). Fowl plague was first differentiated from fowl cholera in 1878, and it was not until 1955 that the causative agent was described as an influenza virus. To date, only H5 and H7 viruses have ever been characterized as highly pathogenic and, as such, they are classified as notifiable by the OIE (World Organization for Animal Health, 2011b).

How avian influenza viruses develop increased pathogenicity is not completely understood, but they appear to require extended periods of circulation in domestic poultry populations, undergoing selection pressures that facilitate mutations leading to increased virulence. On a molecular level, to attain infectiousness, all AI viruses require the HA protein to be cleaved into HA1 and HA2 (Webster and Rott, 1987; Suarez, 2008). For LPAI viruses, this cleavage occurs extracellularly, however HPAI viruses have a multiple basic amino acid insertion

at the HA cleavage site, allowing cleavage by proteases found within most host cells, and thus generating an infectious virus particle immediately upon release from the cell (Kuroda et al., 1986; Webster and Rott, 1987; Stieneke-Grober et al., 1992; Steinhauer, 1999; Suarez, 2008). This cleavage is the most important characteristic of high pathogenicity, as it allows the virus to infect and replicate in multiple atypical cell types, such as neurologic, cardiac and muscle cells (Suarez, 2008). The transition of an AI virus from low to high pathogenicity, and the associated molecular changes, has been observed in at least three cases, Pennsylvania in 1983, Mexico in 1994 and Italy in 1999 (Kawaoka and Webster, 1985; Webster and Rott, 1987; Perdue et al., 1997; Zanella et al., 2001).

As mentioned previously, HPAI viruses are identified by their ability to cause high mortality in 4-6 week-old specific pathogen-free chickens (World Organization for Animal Health, 2011b). As a result, HPAI viruses do not necessarily result in high pathogenicity in other poultry species. For example, long before fowl plague was identified as avian influenza, it was known that it did not have the same pathogenicity for ducks as for gallinaceous species (Kaleta and Rulke, 2008). As will be discussed below, the low pathogenicity characteristics of HPAI viruses in ducks and other species is inconsistent and can change with time and genetic evolution of the virus.

Highly Pathogenic H5N1 Epizootic

History of HPAI H5N1

The likely progenitor virus of the HPAI H5N1 influenza viruses that are now endemic in parts of Asia was isolated in 1996 from dead domestic geese in Guangdong Province of China (Xu et al., 1999). HPAI H5N1 first came to the attention of the world in 1997 after widespread infection of domestic poultry in Hong Kong and multiple human fatalities. These early viruses are now known as clade 0. At the time of the outbreaks, Hong Kong had a dense poultry sector with approximately 120,000 poultry sold at live bird markets daily, sourced from both Hong Kong farms and farms in southern mainland China (Sims et al., 2003). The virus was eradicated from Hong Kong through depopulation of live bird markets and poultry farms and the cessation of poultry trade (Sims et al., 2003). Beginning in 2001, other viruses containing the HA gene of the original 1996 goose virus were identified in Hong Kong and caused intermittent infection of

poultry and wild birds until 2003 (Ellis et al., 2004). Prior to that time, there had been no identified cases of HPAI H5N1 in free-living birds. In late 2003, the virus (now known as clade 1) was isolated in China, Cambodia, Laos, Thailand and Vietnam in close succession (Sims et al., 2005). In the years since, HPAI H5N1 emerged in other Asian countries as well as some European and African nations, affecting poultry, wild birds or both. Genetic evidence from wild bird surveillance prior to 1997 indicates that HPAI H5N1 originated from LPAI H5 viruses of wild birds, perhaps ducks migrating from northern Japan (Okazaki et al., 2000; Duan et al., 2007). Neurologic symptoms and a large die-off of migratory geese in the Qinghai Lake area of China in 2005 was the first time that sustained transmission of HPAI H5N1 was seen in migratory waterfowl, and the dominant isolate from this event (clade 2.2) was subsequently identified in the Middle East, Western Asia, Europe and Africa (Chen et al., 2005; Sims and Brown, 2008). The genetic make-up of the viruses isolated from the geese indicate that the virus causing the migratory bird outbreak was introduced at a single time point by domestic poultry in southern China (Chen et al., 2005).

From late 2003 through today, HPAI H5N1 has been the largest epizootic of HPAI to date, primarily existing as an avian disease with occasional spillover into the human population. Global avian mortality is estimated to be hundreds of millions of birds, from both infection and instituted control measures. The virus has evolved into multiple genotypes, and today can be classified into 9 clades with many first, second and third-order clades, based on nucleotide divergence in the HA gene (Guan et al., 2002; Li et al., 2004; Chen et al., 2006; Guan et al., 2009; World Health Organization, 2011b). Antigenic cross-reactivity among these clades is variable, although monoclonal and polyclonal antibody responses to heterologous antigen are not as consistent as they are for homologous antigen (Chen et al., 2006; Li et al., 2011). HPAI H5N1 is considered endemic by the FAO in Bangladesh, China, Egypt, Indonesia and Vietnam and is detected sporadically in other countries (FAO-OIE-WHO, 2011).

Routes of Long-Distance HPAI H5N1 Movement

Though it is known that HPAI H5N1 can cause illness and death of wild birds, little is known about their role in local and long-distance virus circulation. There is limited evidence of the carriage of H5N1 over migratory routes (Feare, 2007). Since the start of wide-scale spread of HPAI H5N1 in late 2003-early 2004, much effort has been put into determining whether the virus

is most likely to have spread via migratory and other wild birds, by transport of poultry and poultry products or by transport of infected captive wild birds. The FAO has identified trade and movement of animals as potential risk factors in the spread of HPAI (Food and Agriculture Organization, 2008), and research has shown that transmission has likely been driven by a combination of poultry transport and wild bird movement (Kilpatrick et al., 2006; Normile, 2006; Beato and Capua, 2011). The initial simultaneous incursion of HPAI H5N1 throughout Southeast Asia in late 2003 and early 2004 is hypothesized to have resulted from poultry transport, whereas movement of the virus into Europe (clade 2.2 viruses) was likely a result of the movement of migratory birds (Kilpatrick et al., 2006).

Risk Factors for HPAI H5N1 Spread in Domestic Poultry

There are many published studies describing spatial and flock-level risk factors for virus persistence and poultry infection with HPAI H5N1, most of which were conducted in Southeast Asia. Environmental factors that have been found to increase risk for H5N1 outbreaks are low elevation and rice-cropping (wetland) areas (Gilbert et al., 2006; Gilbert et al., 2007; Pfeiffer et al., 2007; Gilbert et al., 2008; Paul et al., 2009), whereas non-rice cropping areas have been found to decrease the risk of outbreaks (Henning et al., 2009a). In spatial analyses, poultry-related factors that have been found to increase risk for H5N1 outbreaks are free-grazing duck density, and, less reliably, the distribution of native chickens and fighting cocks (Gilbert et al., 2006; Gilbert et al., 2007; Gilbert et al., 2008; Paul et al., 2009; Tiensin et al., 2009). Ducks raised in a farm setting were not found to be associated with outbreaks (Gilbert et al., 2008). Despite the fact that most outbreaks occurred in chicken flocks, the strongest association of HPAI outbreaks has been found with the density of free-grazing ducks. This finding has since garnered much attention, as free-grazing ducks are likely to have direct and indirect interactions with wild birds due to a shared habitat, presenting opportunities for exchange of viruses.

Spatial analyses in Thailand have also identified anthropogenic risk factors including human population density, high road density, short distance to highway junctions, short distance to a large city and presence of a slaughterhouse in the area (Gilbert et al., 2006; Paul et al., 2009; Tiensin et al., 2009). Roads have been implicated as risk factors for HPAI H5N1 spread in other countries as well (Ward et al., 2008; Rivas et al., 2009; Loth et al., 2010; Yupiana et al., 2010). In the United States, vehicular transport of turkeys infected with avian influenza viruses is known to

have lead to infection of farms along the traveled roads ((Johnson, D.C., unpublished data from (Brugh and Johnson, 1987)); David Halvorson, personal communication 2011).

The first outbreaks of HPAI H5N1 in Hong Kong occurred in poultry farms and in live bird markets (Sims et al., 2003). Since that time, live bird markets have been identified as important locations for the maintenance and transmission of HPAI H5N1 viruses (Chen et al., 2006; Sims, 2007). Virus isolations from market poultry have frequently come from healthy poultry (Sims et al., 2003; Li et al., 2004; Chen et al., 2006). The presence of multiple species of birds in one area, especially when there are no clinical signs to suggest a need for bird separation, can lead to virus exchange (Sims and Brown, 2008). Additionally, poultry and equipment that leave the markets can facilitate spread to and from farms.

At the flock level, risk factors for small farms in Vietnam were found to include lack of vaccination or only one vaccination, visitors on the farm premise, geese on the farm and sharing of scavenging areas with other flocks by poultry that were free to roam, although the small sample size utilized in this work resulted in imprecise risk estimates (Henning et al., 2009b). On-farm traffic, including visitors, poultry purchase and farm workers living off-site were identified in other risk factor studies (Kung et al., 2007; Fasina et al., 2011), as was the accessibility of farms to feral animals such as dogs and cats (Biswas et al., 2009a) and direct sale of poultry to retail markets (Kung et al., 2007). Poultry traders onsite and the exchange of egg trays were found to be risk factors for HPAI H5N1 infection (Biswas et al., 2009a; Desvaux et al., 2011), and contact between ducks and other poultry, has been identified as a risk factor for backyard chicken HPAI H5N1 outbreaks (Biswas et al., 2009b).

Ducks and HPAI H5N1

Avian influenza viruses, including HPAI viruses, do not commonly cause clinical signs of disease in ducks. This finding changed significantly, however, with the emergence of HPAI H5N1 in Asia. Prior to 2002, no duck deaths were identified as a result of HPAI H5N1, and infection, which included respiratory and gastrointestinal tract shedding, was subclinical (Shortridge et al., 1998; Perkins and Swayne, 2002; Sims, 2007). However, beginning in 2002, viruses were isolated that were capable of causing death in experimentally infected ducks (Ellis et al., 2004; Sturm-Ramirez et al., 2004; Lee et al., 2005; Nguyen et al., 2005; Sturm-Ramirez et al., 2005). The viruses often displayed neurotropism, with infected ducks showing signs of torticollis

and seizures, and affected the respiratory tract, pancreas, adrenal glands and myocardium (Swayne and Pantin-Jackwood, 2008). Despite this ability to cause severe signs, H5N1 pathogenicity in ducks differs by viral isolate, and some infections can lead to few clinical signs of disease despite the shedding of significant amounts of virus (Chen et al., 2004; Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005; Songserm et al., 2006; Jeong et al., 2009). HPAI H5N1 has been isolated on numerous occasions from asymptomatic ducks (Li et al., 2004; Songserm et al., 2006; Buranathai et al., 2007; Amonsin et al., 2008). In October 2004, 100% of the duck flocks in Thailand were sampled, yielding a 39% positivity rate by virus isolation (Buranathai et al., 2007).

Ducks have been found to shed HPAI H5N1 viruses preferentially (at higher titers) from the respiratory tract, rather than from the gastrointestinal tract, as is typical in infection other influenza A viruses (Ellis et al., 2004; Sturm-Ramirez et al., 2004; Hulse-Post et al., 2005). While the role of this shedding route in transmission is not yet understood (Kim et al., 2009), these findings support the use of pharyngeal swabs as well as the more traditional cloacal swabs in duck surveillance activities (Keawcharoen et al., 2008). The duration of virus shedding has differed in experimental conditions, with one study showing shedding for as long as 17 days (Hulse-Post et al., 2005), others for as long as 2-5 days (Shortridge et al., 1998; Perkins and Swayne, 2002), and one for 10 days (Sturm-Ramirez et al., 2004). In addition to direct duck sampling at 10 days post-infection, drinking water was also found to be positive for viable virus at day 10 (Sturm-Ramirez et al., 2004). Hulse-Post, *et al.* (2005) reported that in ducks shedding virus for an extended period of time, viruses once highly pathogenic H5N1 to the ducks can revert to relative nonpathogenicity, while remaining highly pathogenic to chickens (Hulse-Post et al., 2005). This finding indicates that the HPAI H5N1 viruses may be moving toward stability in ducks. Extended shedding can facilitate the development of humoral immunity to the viruses, thus allowing the potential for genetic drift in ducks that have recovered from infection (Hulse-Post et al., 2005; Songserm et al., 2006). Asymptomatic shedding combined with successful virus replication makes ducks (a natural host species) a potential reservoir and source of H5N1 infection for other poultry.

The pathogenicity and transmissibility of HPAI H5N1 viruses can vary with duck species, age and the viral isolate, and prevalence measurements can be dependent upon the duck population size and time of collection (Hanson et al., 2003; Kwon et al., 2005; Brown et al.,

2006; Brown et al., 2007a; Pantin-Jackwood et al., 2007). In one study conducted on North American duck species, wood ducks displayed a higher susceptibility to two H5N1 isolates, as well as a longer viral shedding time, than did the mallard, northern pintail, blue-winged teal and red-head ducks (Brown et al., 2006). The wood duck was the only species to display morbidity or mortality. Further study of this species revealed that the infectious dose of H5N1 for the wood duck is less than that for chickens (Brown et al., 2007a). Immunologically naive young ducks are more likely to become infected and shed virus longer (Webster et al., 1992; Pantin-Jackwood et al., 2007).

HPAI H5N1 Zoonotic Risk and Human Cases

As of April 12, 2012, there have been 602 human cases in 15 countries (World Health Organization,). The crude case fatality rate is high at 59%, but this rate varies by country (Van Kerkhove et al., 2011). Transmission from poultry to humans is apparently not a frequent occurrence, as there is often only a single confirmed case in an area, and there is little evidence of widespread subclinical infection (Appendix A), despite large numbers of infected poultry in close proximity to many humans. It is likely that, as in poultry (Figure 1.2), the risk of infection is influenced by the size of the inoculum, the frequency and intimacy of contacts and the host immune response (Swayne, 2007; Swayne, 2008). The gender and age of human H5N1 cases has varied by country (Van Kerkhove et al., 2011). In 2007, over 50% of cases were in persons younger than 40 years of age (World Health Organization, 2007c), and after a large number of very young cases in Egypt in 2010-2011, the median age of infection is 5 years (World Health Organization, 2010b). There does not appear to be a gender predisposition for infection. Most cases of HPAI H5N1 in humans have involved symptoms of fever, cough, pneumonia and hypoxia, with progression to acute respiratory distress syndrome (Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus, 2008; Van Kerkhove et al., 2011).

While the risk factors for infection are incompletely understood, transmission of HPAI H5N1 to humans is most likely caused by direct contact with fecal material from infected poultry or contact with contaminated secretions, blood or organs. An early case-control study (January 1998) including 15 of the first human cases of H5N1 in Hong Kong revealed that cases had a 4.5-fold higher odds of contact with poultry in the week prior to the onset of symptoms (95% CI 1.2,

21.7) (Mounts et al., 1999). None of the cases or controls in that study had been to live poultry farms, only markets, and nearly all of the cases had been previously healthy. Contact with sick or dying poultry prior to disease onset, either by preparation of ill poultry for consumption or indirect contact with ill household or neighborhood poultry, has been found to be the most common risk factor for infection (Areechokchai et al., 2006; Dinh et al., 2006). Consumption of unhealthy poultry products has also been identified as a risk factor (Dinh et al., 2006), and consumption of uncooked duck blood has been investigated as a cause of human infection in Vietnam (Centers for Disease Control and Prevention, 2008a).

The role of indirect contacts such as aerosolized virus, water and feces is incompletely understood, but for persons who live in close contact with poultry, these types of contact may provide unpredictable and perhaps even continuous exposure to virus. Environmental exposure at live poultry markets has been a risk factor for infection, even without direct contact with poultry (Yu et al., 2007; Wan et al., 2011). Contact with fertilizer containing feces has been proposed as a risk (Kandun et al., 2010), and two Cambodian studies identified swimming or bathing in community ponds to be associated with seropositivity (Vong et al., 2009; Cavailler et al., 2010). The role of multiple or sustained exposures, or of dose-response, on transmission and immune response is unknown (Cox and Uyeki, 2008).

Occupational exposures, where persons have been exposed to HPAI H5N1 through culling of infected poultry, have been shown to occur (Bridges et al., 2002; Schultsz et al., 2009). Rarely, wild birds have been implicated as the most likely route of exposure for human infections with HPAI H5N1 (Gilsdorf et al., 2006; Cai et al., 2009).

At this time, the primary mode of human infection with HPAI H5N1 is from infected poultry to humans, and there is little evidence of transmission among human contacts (Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus, 2008). Where such transmission has occurred, it has been unsustained. Healthcare worker studies have shown varied results, with an early study finding 8 of 217 (3.7%) healthcare workers in direct contact with human H5N1 cases and 2 of 309 (0.7%) without such contact to be seropositive (Buxton Bridges et al., 2000). In that study, six of the seropositive workers also had poultry exposure. Subsequent studies have revealed no

evidence of transmission to healthcare workers (Apisarnthanarak et al., 2005; Schultsz et al., 2005).

Studies identifying clusters of disease within households are often limited by the presence of similar environmental exposures (i.e. poultry) (Auewarakul, 2008), however, limited reports have described probable human-to-human transmission after close contacts between an infected person and a caretaker or relative (Ungchusak et al., 2005; Wang et al., 2008). Transmission of avian influenza viruses, including HPAI H5N1, across the species barrier has the potential to lead to reassortment of avian and human influenza viruses, yielding viruses that are more easily transmitted from human to human and could have pandemic potential. Evidence has shown that some avian influenza viruses have been transmitted from human to human after contact with infected poultry. In one study of poultry farmers and families showed that 59% of household contacts with no poultry exposure had antibodies to an H7N7 outbreak virus in the Netherlands in 2003 (Bosman et al., 2005). One early study of HPAI H5N1 cases found that 12% (6 of 51) of household contacts of confirmed human clinical cases of H5N1were seropositive for the virus as well but had no symptoms (Katz et al., 1999). Despite this finding, there is currently very little human-to-human transmission and no evidence of sustained human-to-human transmission of HPAI H5N1 (FAO-OIE-WHO, 2011). Evolution of the virus has not lead to an increase in public health threat (World Health Organization, 2011a).

The human antibody response to HPAI H5N1 exposure is thought to be similar to that of seasonal influenza. After the onset of clinical symptoms, neutralizing antibodies in humans infected with HPAI H5N1 have been detected at 14 days and are found to increase after 20 days (Katz et al., 1999). One study of confirmed human cases found that positive antibody titers are established between 2 and 3 weeks after disease onset, and may last up to 5 years (Kitphati et al., 2009). The same study identified cross-neutralizing antibodies to clade 1 viruses with 96% HA protein homology and differing syalic acid receptor affinities. Cross-reactivity has been shown elsewhere and has been quantified in some cases (Rowe et al., 1999; World Health Organization, 2008a; Boon and Webby, 2009; Ducatez et al., 2011; Li et al., 2011). Boon *et al*. summarized information from the WHO demonstrating that the contemporary H5N1 viruses show approximately a four-fold drop in HI titer between clades (World Health Organization, 2008a; Boon and Webby, 2009), and that genetic and antigenic diversity are apparently correlated. Such

information is necessary to inform the development of a universal vaccine for people in geographic locations with H5N1 viruses of differing clades and sub-clades, however, there appears to be considerable variation in reactivity among such viruses and antibodies. Monoclonal antibody studies indicate that there may be some antigenic characteristics conserved across all H5N1 clades, but these may not be dominant when immune response is assessed via polyclonal measures (i.e. hemagglutination inhibition) (Kaverin et al., 2002; Wu et al., 2008). There is also some evidence of neuraminidase cross-reactivity between antibodies to human N1 (e.g. seasonal H1N1 influenza) and the N1 antigen of H5N1, although the phylogenetic differences between these viruses are great (Sandbulte et al., 2007; Pichyangkul et al., 2009).

HPAI H5N1 in Thailand

Poultry Production in Thailand

Prior to 2004, Thailand was one of the world's largest exporters of chicken meat and producers of domestic ducks (Department of Livestock Development, Thailand, 2003; Tiensin et al., 2007). The Central and Eastern regions of Thailand contain most of the country's poultry production (Tiensin et al., 2005). Poultry are raised in four types of production systems (Table 1.2): Sector 1, where poultry live in high-biosecurity closed systems, with all aspects of production and transport completed internally (vertical integration); Sector 2, contract farms, in which poultry are in closed facilities with basic physical barriers; Sector 3, characterized by low biosecurity open houses with or without netting where poultry may be allowed outside of the housing (includes free-grazing duck flocks); and Sector 4, the backyard or village setting, where poultry roam freely in human domestic settings (Tiensin et al., 2005; Songserm et al., 2006; Food and Agriculture Organization, 2011a). While 90% of the country's poultry are produced in the first two sectors, this includes only 1-5% of the flocks, with the rest being raised in constant potential contact with people and other animals (Rushton, 2005; Heft-Neal et al., 2009). Approximately 50% of all chickens in the country are raised in Central Thailand, followed by the northeast region (NaRanong, 2007).

In the twenty years prior to the first HPAI outbreaks in Thailand, the poultry sector had been undergoing a restructuring, with the development of large integrated farms owned by large companies with export goals and production contracts (Rushton, 2005; NaRanong, 2007). In

addition, commercial farms with corporate suppliers in Sector 2 were developing biosecurity and structural advancements, including evaporative cooling, which facilitated production in Thailand's hot climate. These changes allowed Thailand to become the fourth largest poultry exporter in the world in 2002 (USDA). Despite these changes, the domestic poultry market continued to be supplied in part by smaller farms in Sector 3, and local poultry markets continued to receive meat from these farms and the backyard sector. Commercial farms in Sector 3 usually have from 100-1,000 birds and include chicken layer farms, duck farms, and some native chicken and quail farms. In this sector, chickens are kept caged in open houses, and ducks are kept in open housing but with the ability to leave the house to swim in adjacent ponds.

Table 1. 2 Poultry production systems in Thailand, as determined by FAO. Reproduced with permission from (Tiensin et al., 2005).

Poultry Production	Biosecurity	Market Orientation	Example
Sector 1	High	Commercial	Industrial integrated system: all components of the production chain (e.g. hatchery, feedmill, poultry farm, slaughterhouse, processing plant, transportation) owned by company with strictly implemented procedures for biosecurity
Sector 2	Moderate to high	Commercial	Semivertical integrated system (or contract farming system): poultry houses owned by the farmer but chicks, feed and veterinary service supplied by private company. Birds kept indoors with basic physical barriers and hygiene to prevent contact with other animals
Sector 3	Low	Commercial, local, or live-bird market	Layer farm with caged birds in open sheds or free-roaming birds that spend time outside the shed
Sector 4	None	Local	Village or backyard poultry: birds freely roam the village around people and other animals, including cockfighting

In Thailand, households typically have backyard flocks of up to 30 or 50 native chickens, most with both hens and roosters that may be used for cock fighting. These backyard flocks are

free to roam around the yard and frequently will roam amongst backyard poultry from other households. There are not good estimates of how many chickens are raised in the backyard sector, because only flocks with 100 or more birds are registered, but they may make up 75% of poultry flocks in Thailand, which is 2.1 million flocks (Safman, 2010). Birds and eggs produced in this backyard system are either eaten at home or sold on rare occasions, such as at the Chinese New Year (see Chapter 6). Fighting cocks, which are raised as backyard poultry, are estimated to number 1-6 million birds in Thailand (Safman, 2010).

HPAI H5N1 Outbreaks in Thailand

HPAI H5N1 Outbreaks from 2003 to 2006

In Thailand, surveillance for HPAI H5N1 in domestic poultry was initiated in 1997, after its emergence in Hong Kong (Chaisingh et al., 2003). Prior to this, only a single avian influenza virus had been isolated in Thailand, a low pathogenicity H6N9 virus from a pheasant in 1984 (Tantaswasdi et al., 1986). Surveillance has included both passive and active methods, with testing of sick poultry submitted to diagnostic laboratories and healthy birds from abattoirs and aviaries. HPAI was first reported in Thailand in 2004, when the HPAI H5N1 virus was isolated from a traditionally-housed 66,000-chicken layer flock in Bang Pla Ma District of Suphanburi Province, in Central Thailand (World Organisation for Animal Health, 2004). The flock was confirmed as infected on January 23, 2004, three days after the onset of clinical signs. The flock experienced 13% morbidity and 9% mortality, and surviving members of the flock were destroyed. While this outbreak was the first to be reported to OIE, earlier outbreaks occurred in late 2003 in Central and Northern Thailand (Centers for Disease Control and Prevention (CDC), 2004; Keawcharoen et al., 2004; Tiensin et al., 2005), and their occurrence was suppressed and downplayed by the authorities in an effort to avoid financial impact on the large poultry export holdings (Safman, 2010). The identification of the confirmed layer farm coincided with the implementation of cloacal sampling of poultry flocks throughout Thailand (Tiensin et al., 2005). On the same day as the notification of poultry disease to OIE, two human cases of HPAI H5N1 were identified in Suphanburi and Kanchanaburi Provinces (World Health Organization, 2004). These cases were both children who eventually died of the disease.

Since late 2003, there have been seven distinct outbreak periods, with the most recent occurring in October and November of 2008 (Amonsin et al., 2006; Tiensin et al., 2007; Chaichoune et al., 2009; World Organization for Animal Health, 2011c). The first three "waves" of HPAI H5N1 affected the most locations and poultry, with the first wave occurring from late 2003 through May 2004, the second from July 2004 through April 2005, and the third from July through November of 2005 (Figures 1.3 and 1.4). The majority (up to 76%, see Table 1.3) of poultry affected were native chickens raised in backyard holdings, but layer and broiler hens, ducks and geese were also affected (Buranathai et al., 2007; Chantong and Kaneene, 2011). The viruses isolated during the three waves were all clade 1 and clustered with the Vietnamese strains. Over the course of the three waves, over 65 million birds were culled, and approximately USD 135.2 million was spent on flock owner compensation (Buranathai et al., 2007).

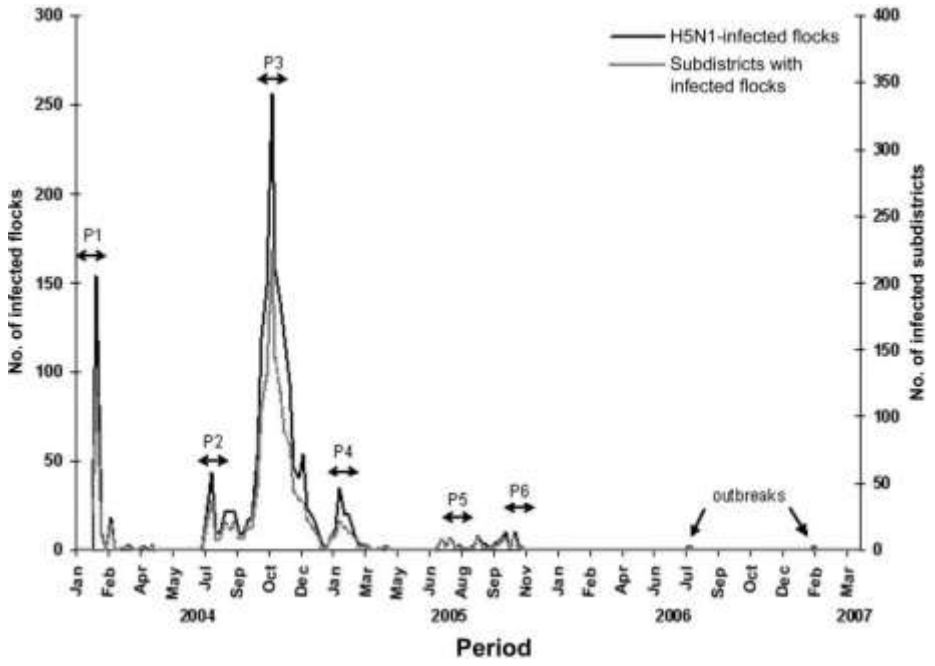

Figure 1. 3 Weekly epidemic curve of the number of flocks infected with highly pathogenic influenza A (H5N1) virus and the number of subdistricts with infected flocks, Thailand, 2004 –2007. P1, January–February 2004; P2, July–August 2004; P3, October–November 2004; P4, January–February 2005; P5, July–August 2005; P6, October–November 2005. Reproduced with permission from (Tiensin et al., 2009).

Table 1. 3 Proportion of affected flock types during the first three HPAI H5N1 outbreaks in Thailand. Generated from data in (Chantong and Kaneene, 2011).

Outbreak	Native Chickens	Broilers	Layer Hens	Ducks	Quail	Other
Jan- May 2004	63.7%	11.6%	10.5%	6.3%	4.7%	3.2%
July 2004- April 2005	57.6%	5.3%	4.7%	28.8%	2.0%	1.5%
July-Nov 2005	76.3%	5.3%	2.6%	6.6%	7.9%	1.3%

Control of HPAI H5N1 in Thailand

Control measures of HPAI H5N1 implemented in Thailand included stamping out, control of poultry movement, improvement of biosecurity and the implementation of door-to-door surveillance campaigns (Buranathai et al., 2007; Auewarakul, 2008; World Organization for Animal Health, 2011c). Stamping out is a widely-used strategy for quickly eliminating a newly introduced disease and involves a combination of zoning, intense surveillance, quarantine and movement restrictions, culling of susceptible animals, safe carcass disposal and disinfection (Geering et al., 2001). During the first wave, the cull-zone around infected premises was set at 5 km, making it very difficult for the government to conduct culling, burning or burying of carcasses and infectious materials, and disinfection of the premises in the time period recommended by the OIE (48-72 hours) (Safman, 2010). In addition, public support for such widespread poultry elimination was lacking, and with such a large radius, it was at times arbitrarily decided whose farm was inside the cull-zone. For the second and third waves, they utilized a 1 km radius, allowing better response time and improving the public's acceptance of control measures.

Vaccination for avian influenza has never been permitted as a disease control measure in Thailand and is illegal in the country (Rushton, 2005). Over the years this has been a point of contention between small poultry producers, including fighting cock owners, and the government, which is seen as putting the lives of small-holder poultry (and their caretakers' livelihoods) at risk in the promotion of large commercial poultry exporters' interests (Safman, 2010). The decision

not to vaccinate differed from that made in other Southeast Asian countries, such as Vietnam, China and Indonesia (Rushton, 2005; Eagles et al., 2009). It is unclear whether the vaccination strategies that these and other countries have utilized are playing a role in the evolution of HPAI H5N1, by providing a partially immunized population for the virus to evade immunologically (Cattoli et al., 2011).

Surveillance Strategies

In October of 2004, the X-ray campaign was initiated for the first time. This country-wide, simultaneous targeted surveillance system included door-to-door surveys to identify sick poultry and humans and testing of every flock (Tiensin et al., 2005). The X-ray surveillance system was a joint endeavor by the Ministry of Agriculture and Cooperatives (and the underlying Department of Livestock Development) and the Ministry of Public Health (World Health Organization, 2007b; Safman, 2010). The campaign functioned through the establishment of Surveillance and Rapid Response Teams (SRRT), made up of grassroots village health volunteers, who worked with trained ministry employees. Information gathered at the subdistrict (tambon) level was fed to the district and then provincial level, where a designated official served as "Mr. Bird Flu", coordinating all activities at that level. In addition to collecting samples for laboratory testing and reporting clinical disease, the SRRT were tasked with providing public health education to poultry owners and for serving as a public relations entity for the government implementing control measures (World Health Organization, Regional Office for South-East Asia, 2006; World Health Organization, 2007b; Safman, 2010). The acceptance of village health volunteers by the community makes them important assets for conducting surveillance in their communities. There were five X-ray campaigns conducted between 2004 and 2006 (Buranathai et al., 2007).

While the outbreaks during 2003-2005 affected nearly all provinces at one or more times, subsequent sporadic outbreaks have occurred largely in the Central and Northern areas of Thailand, where the poultry density is highest (World Organization for Animal Health, 2011c). The most recent HPAI H5N1 poultry outbreak occurred in November of 2008, affecting native chickens in two provinces (World Organisation for Animal Health, 2009). X-ray campaigns are now conducted twice each year but only in provinces considered to be at highest risk (Mana Keawyai, personal communication 2012).

Outbreaks of H5N1 in Thailand have followed a fairly seasonal pattern (Figure 1.3). All of the outbreaks recorded thus far have had their onset at the start of the rainy season (July) or during the winter months (October through January) (Gilbert et al., 2006; Chaichoune et al., 2009). None of the outbreaks have commenced or continued through the hot summer months of April through June. Despite this pattern of inconstant persistence, molecular analysis of Thai H5N1 virus isolates indicates that in most cases, the viruses isolated in new outbreaks share the same clade 1 lineage and display characteristics of genetic drift commonly associated with influenza A virus circulation, as opposed to characteristics of reassortment or novel strain introduction (Amonsin et al., 2006; Buranathai et al., 2007; Chaichoune et al., 2009; Amonsin et al., 2010). This evidence indicates that there may be an animal or environmental reservoir maintaining H5N1 viruses during the summer months prior to re-emergence in the rainy season.

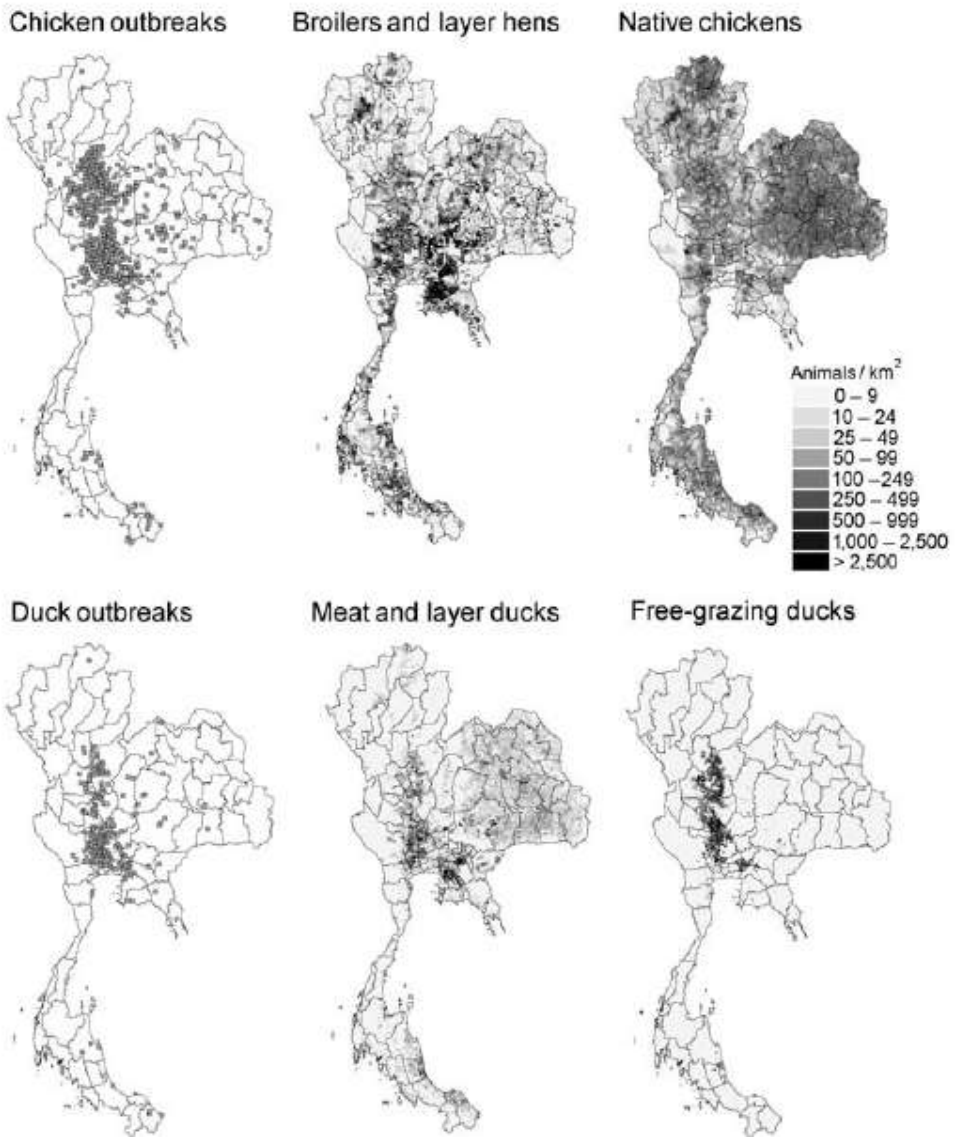

Figure 1. 4 Distribution of highly pathogenic avian influenza (HPAI) outbreaks in chickens and ducks, Thailand, July 3, 2004–May 5, 2005, and respective distribution of broilers and layers hens, native chicken, meat and layer ducks, and free-grazing duck populations, highlighting the correlation between HPAI outbreak distribution and free-grazing duck populations. The divisions are Thailand provinces. Reproduced from public domain article (Gilbert et al., 2006).

Free-Grazing Ducks and HPAI H5N1

Duck Agriculture in Thailand

Prior to the first outbreaks of H5N1, approximately 23 million ducks were raised each year in Thailand using four types of production systems: high-biosecurity closed houses, moderate biosecurity open houses, rice fields after harvest, and backyards (Songserm et al., 2006). At the beginning of 2004, 10-11 million ducks were raised in a free-grazing system, where ducks are transported among rice fields post-harvest to feed on residual rice, insects and snails (Songserm et al., 2006). Hatching occurs inside of a brooding house, and the hatchlings are kept in an enclosed area to grow until they are able to graze on rice fields (often three weeks), after which they are moved to the fields. Flocks are brought to post-harvest fields in numbers as high as 4000 birds/ hectare. Ducks are moved, often by truck, to a new rice field when the rice supply has been exhausted (Gilbert et al., 2006; Gilbert et al., 2007).

While most rice fields produce only one crop per year, areas like Central Thailand, where there is irrigation available outside of the monsoon season, can produce two or three crops per year, making this an ideal location for free-grazing ducks. This type of duck production is mutually beneficial to duck and rice field owners, with the ducks providing a chemical-free form of pest control as well as manure fertilizer and owners having low overhead for duck feed. Duck owners identify fields for grazing though networks, often built over many years of experience, which include friends, relatives and other field-owning connections (unpublished data, Chapter 6). If there are no fields available in the home village, flocks are moved among neighboring subdistricts and districts within the home province. Depending on the owner's rice field network, a flock may be moved inter-provincially, over hundreds of kilometers.

FGD farming in Thailand had its beginnings in the late 1980s, when it was promoted by the Thai Department of Livestock Development (DLD) as a low-overhead, extensive method of duck production (Safman, 2010). The advent of mechanized harvesting facilitated the emergence of this duck production type, as when compared to hand-harvesting techniques, mechanized harvesting results in the loss of large amounts of rice grains back into the field (Nitipong Homwong, personal communication 2012; (Safman, 2010). Irrigation systems in Central

Thailand facilitates rice production year-round (three harvests), which in turn facilitates year-round grazing on post-harvest rice fields in some areas. Without a fallow period, fields generate a build-up of pests such as snails, insects and slugs, further adding to the suitability of these fields for duck grazing. While many Thais have direct ownership of FGD flocks, in some circumstances the flocks are owner by foreign (e.g. Chinese) financiers who provide funds for duck purchase and feeding until they are old enough to move to the field (Safman, 2010).

Association of Free-grazing Duck Farming and HPAI H5N1

In a study conducted from February through September of 2004, samples were taken from ducks in both open and closed systems to test for H5N1 infection (Songserm et al., 2006). None of the ducks from closed systems were infected, although infection was identified in 28 of 61 free-grazing duck flocks (45.9%). In addition, when 10 flocks were followed from February through July of 2004, there was no virologic or serologic evidence of infection until they were moved to the rice fields, after which H5N1 was detected in all 10 flocks, with most ducks showing no signs of clinical disease. Interestingly, the clinical and non-clinical ducks had similar virus titers. Ducks with clinical signs showed depression, lethargy, cloudy cornea, blindness, high fever, dyspnea, diarrhea, ataxia, incoordination, convulsions, ocular and nasal discharge and conjunctivitis.

In October of 2005, after nearly one year without an outbreak of H5N1, Kanchanaburi Province reported a human case of avian influenza (Songserm et al., 2006). Though circumstantial evidence, this case was preceded by the illegal grazing of 3000-5000 free-grazing ducks in nearby rice fields. Despite no contact between the ducks and village chickens, chickens began dying, leading to human contact and illness. There was no sequencing of human and avian isolates conducted to confirm the link between the duck, chicken and human viruses. The potential source of virus in duck flocks on rice fields is unclear and controversial (Songserm et al., 2006).

Multiple spatial analyses have identified an association between H5N1 outbreaks and free-grazing duck and rice field density (Gilbert et al., 2006; Gilbert et al., 2007; Gilbert et al., 2008; Paul et al., 2009; Tiensin et al., 2009). Because of this association and their ability to be infected with and shed H5N1 virus with no clinical disease signs, domestic ducks have been identified as a population with the potential for sustaining low-level H5N1 virus transmission

during periods with no overt poultry outbreaks (Chen et al., 2004; Sturm-Ramirez et al., 2005; Songserm et al., 2006; Chaichoune et al., 2009), and thus presenting a source of infection for new outbreaks in nearby poultry.

Free-grazing Duck Regulations and the Societal Implications

Since the first emergence of H5N1 in Thailand, the Department of Livestock Development (DLD), under the Ministry of Agriculture and Cooperatives, has implemented various regulations on the production of free-grazing ducks. These regulations have ranged from country-wide registration and pre-movement testing, to nation-wide prohibition, to establishment of different rulings for different regions of the country.

In October of 2004, stringent regulations on raising ducks in the free-grazing system were put into place, with producers given three months to move their flocks into closed systems, without contact with other poultry or open waterways (The Nation, 2004). The Thai government initiated a program to assist duck farmers both logistically and financially (through low-interest loans) to move their ducks inside, however, a plan to buy out all duck flocks was stopped short in February of 2005 with a veto by Prime Minister Thaksin Shinawat (Safman, 2010). It is thought that the Prime Minister's reluctance to embrace the transition fully was a result of pressure from many wealthy financiers of FGD flocks, who recognized the decrease in profitability that would accompany such changes. Contributing to what could be considered a vacillating national policy, in February of 2005 the DLD discontinued the ban on open-field grazing and considered the use of vaccination (The Nation, 2005a; Safman, 2010). A vaccination plan never materialized, and the government used control of poultry movement in known affected areas as the primary control measure (The Nation, 2005b).

By June of 2005, the number of grazing ducks being raised in Thailand had decreased from 12 to 5 million (WHO, 2005c), and by 2006, 60% of flocks were thought to have switched to a non-grazing production system (The Nation, 2006). While some of this decrease in grazing duck numbers was due to farmers switching to closed systems, many simply could not afford to do so, subsequently losing their flocks. Though farmers have protested at multiple times throughout the years, various adjustment to the bans, such as establishing zones of movement, have not been maintained, and the prohibition has repeatedly been reinstated (WHO, 2005a; WHO, 2005b; WHO, 2005c; The Nation, 2006). Imposed regulations, have had a significant

effect on the livelihood of those who raise them, and vocal discontent has centered around the implementation of movement bans and stamping out instead of vaccination as the primary control measure (Safman, 2010).

A decrease in the number of outbreaks of highly pathogenic avian influenza H5N1 virus occurred in close timing to these restrictions, but by the time that the transition was planned to reach completion (February of 2005), a decline in outbreaks had already begun (Figure 1.3). The role of FGD in this dynamic is still poorly understood. In research conducted after the regulation change, the decrease in the number of FGD flocks caused ducks and rice-cropping intensity to be poorer predictors of poultry outbreaks, thereby strengthening the argument for grazing duck regulations. (Gilbert et al., 2008).

Wild Birds and Rice Field Grazing

The same irrigation characteristics that make rice fields desirable to duck farmers also are attractive to migratory waterfowl and other bird species (Gilbert et al., 2006; Gilbert et al., 2008), leading to side-by-side grazing of wild and domestic birds. The species of wild waterfowl present on rice fields depend on geographic location of the fields as well as harvest season (Rachod Tantilertcharoen, personal communication 2009). On rice fields in Central Thailand, it is common to see the Eurasian tree sparrow (*Passer montanus*), plain-backed sparrow (*Passer flaveolus*), white-rumped munia (*Lonchura striata*), scaly-breasted munia (*Lonchura punctulata*), common myna (*Acridotheres tristis*), white-vented myna (*Acridotheres grandis*) during all seasons. During the harvest and post-harvest seasons, egrets, herons, and Asian open-bill storks (*Anastomas oscitans*) are commonly seen. The most common egret species on the rice fields include the little egret (*Egretta garzetta*) and cattle egret (*Bubulcus ibis*). Areas with nearby large pig farms or poultry farms near the rice field commonly host species of swifts that include Asian palm swift (*Cypsiurus balasiensis*) and house swift (*Apus affinis*). Doves are also commonly found in Central Thailand.

Of these species that are known to feed at Thai rice fields, the little egret, Eurasian tree sparrow, scaly-breasted munia, common myna, white-vented myna, Asian open-bill stork, heron and dove have been identified with confirmed HPAI H5N1 infection (Dierauf et al., 2006; Siengsanan et al., 2009). Of these positive species, the scaly-breasted munia, Asian open-bill stork and dove were identified in Thailand. In China, one surveillance study conducted from 2004-2007

identified H5N1 in 1% of the tree sparrows sampled (Kou et al., 2009). In addition, sparrows have been found to be capable of infection with HPAI H5N1, and subsequent shedding, after consumption of infected chickens' drinking water (Forrest et al., 2010). One Thai study sampling wild birds between 2004 and 2007 found no difference between the prevalence of H5N1 in waterfowl and nonwaterfowl, and wild birds were only found to be positive for HPAI H5N1 in areas of reported outbreaks in domestic poultry, indicating potential spillover from domestic poultry to wild birds (Siengsanan et al. 2009). The overall prevalence was found to be 1%, and the frequency of infection in wild birds increased significantly during winter. Investigators found that almost 1% of the healthy-looking birds tested positive for H5N1, as did 4% of the birds found dead. Of the 50 provinces included in the sampling area, 12 had positive specimens, including Suphanburi Province and five of the nine provinces surrounding Suphanburi (Phra Nakhon Si Ayutthaya, Kanchanaburi, Nakhon Pathom, Ratchaburi and Ang Thong). The shared rice field habitat provides ample justification for continued avian influenza surveillance in the wild waterfowl populations of Thailand.

Summary of the Importance of Free-grazing Ducks in HPAI H5N1 Surveillance

FGD flocks are on the HPAI H5N1 risk factor radar for three major reasons: 1) Ducks are the natural reservoir for flu and (especially in large flocks) can sustain virus replication without clinical signs; 2) FGD flocks travel farther and more frequently than most other poultry populations; 3) FGD are grazed in locations where they may come into contact with infected wild birds. FGD spend their days on water-covered rice fields, which may provide an environmental source of indirect transmission within infected FGD flocks. Transmission of avian influenza within a large flock will display dynamics that are different from infection in an individual bird or a small flock. As is seen in large chicken farms (Swayne, 2008), it is likely that infection within the flock may persist for extended periods of time due to a large number of susceptible birds and the presence of environmental contamination, both in the rice field water and in the pen environment during the evenings (Brown et al., 2007b). This contaminated water may also present a source of infection for wild birds feeding nearby in the post-harvest rice fields. Since FGD flocks are often transported over long road distances, the knowledge that long distance transport of poultry can lead to stress and prolonged shedding of influenza A viruses (Ziegler et

al., 1999), and that roadway junctions and highways are spatially associated with poultry HPAI H5N1 outbreaks (Paul et al., 2009), may play a role in influenza dynamics within this population.

Avian Influenza Antibody Detection Methods

Prior exposure and/ or infection with influenza viruses can be detected by multiple serologic methods. Antibodies may be detected within 7 days of infection (Swayne and Halvorson, 2008; Spackman et al., 2009). In poultry, serology is often used prior to export of poultry or poultry products from an influenza-endemic country, or as an adjunct to influenza outbreak surveillance, where it is useful in identifying birds with subclinical infection. In humans, serologic methods are used to generate diagnoses of influenza infection (by a four-fold rise in serial titers) and to assess populations for subclinical infection (e.g. for epidemiologic and immunologic studies). The laboratory methods used for animal and human influenza serology are analogous. Methods do vary, within both human and animal diagnostic classifications, by individual laboratory protocol, assay reagents and antigenic test components.

The available serologic assays are hemagglutination inhibition (HI), microneutralization (MN), enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID). Serologic assays for influenza antibodies include both universal influenza A antibody detection (e.g. detection of antibodies to NP or matrix proteins) and subtype-specific detection (e.g. detection of antibodies to H1, H3, H5, H7). Assays that can detect all subtypes are often used as initial screening tests and are followed by assays for individual subtypes. With subtype-specific serologic assays, if the subtype of exposure is not known or if a testing for all subtypes antibodies is being conducted, a major constraint is the need for a panel of antigenic reagents that is representative with regard to virus HA subtype (at least 16 representative viruses needed) (Pedersen, 2008; Spackman et al., 2008; Swayne and Halvorson, 2008). This significantly limits the ability of these tests to be used for rapid detection of antibodies to novel influenza A viruses. Another limitation of subtype-specific serologic tests is non-specific cross-reactivity due to previous infection with other influenza A viruses.

While serology is a useful tool in understanding influenza transmission, it is not perfectly sensitive. For example, it has been shown that, even after infection with human influenza viruses, not all persons with confirmed infections develop an antibody response (Fox et al., 1982; Beare

and Webster, 1991). The details behind this phenomenon are yet undetermined, although, as in poultry (Figure 1.2), exposure to influenza viruses does not always lead to immune-stimulating infection. In fact, one intensive study of seasonal influenza over a four-year period showed that 13-27% of virus-positive individuals had no detectable antibody response (Fox et al., 1982). One piece of this puzzle may be that serum diagnostics can be affected by the choice of test antigen, as will be discussed below.

The following sections describe blocking ELISA, HI and MN, the serologic methods which are most commonly used in influenza research and surveillance, and which are utilized and discussed in the following chapters. ELISA is often used as a screening test, and after serum samples are evaluated for non-specific influenza A seropositivity, HI and MN can be used to determine the subtype(s) of exposure (Swayne and Halvorson, 2008).

Blocking Enzyme-Linked Immunosorbent Assay

ELISA techniques are inexpensive, many samples can be processed at once, and they are faster and may be more sensitive than AGID (Spackman et al., 2008; Spackman et al., 2009). The cutoff value of diagnostic tests that have a quantitative outcome, such as blocking ELISA, can be adjusted based upon the research question at hand. This makes them useful as screening tests, allowing the user to determine the importance of maximizing either sensitivity or specificity, based on the cost of false negative and false positive outcomes, respectively (Dohoo, 2009).

Blocking ELISA methods detect influenza virus antibodies through the use of antigen-coated 96-well plates and reagent antibodies specific for those antigens (Figure 1.5). The reagent antibodies used in the assay are conjugated to an enzyme that allows their detection using a spectrophotometer. During preparation of the assay, the plate wells can be coated with whole virus or individual virus proteins (e.g. HA, NP, matrix protein). When the diagnostic serum sample is added to the antigen-coated wells, antibodies specific for the antigen (if present) bind. After incubation and washing, reagent antibodies (with conjugated enzyme) are added to the wells. The amount of the reagent antigen that binds to the plate is inversely proportional to the amount of diagnostic serum antibodies that have bound. If there is antigen-specific antibody in the serum sample, there will be little binding of the reagent antibody due to saturation (blocking)

of the receptor sites. The amount of reagent antibody that has bound to the plates is determined through quantification of the optical density after activation of the conjugated enzyme. The optical density is low if there is antibody present in the diagnostic serum sample and high if there is no antibody present in the diagnostic serum sample. The test result is determined by comparing the optical density of each sample well (S) to the optical density of a known negative well (N), yielding an S/N ratio. For blocking ELISAs, when the S/N ratio is higher than a determined cutoff, the test is negative, and when it is lower than that cutoff, the test is positive.

Blocking ELISA testing can be conducted using individual laboratory protocols and reagents or by using a manufactured commercial kit that includes all of the necessary test components. There are such kits available for general detection of antibodies to influenza A viruses (screening tests which use a conserved influenza protein such as NP or matrix) and for detection of HA subtype-specific antibodies (such as H5 and H7). A major commercial screening blocking ELISA kit (MultiS-Screen ELISA (FlockCheck), Idexx™, Westbrook, ME) has been assessed for use in multiple poultry types, and has been found to have an 82% sensitivity (95% CI 76%, 87%) and 100% specificity (95% CI 97%, 100%) at the manufacturer's cutoff (S/N = 0.5) and was more sensitive than AGID for detecting antibodies to both LPAI and HPAI viruses (Brown et al., 2009). The test showed a higher sensitivity for detection of antibodies to HPAI viruses (96%; 95% CI 88%, 100%) than LPAI viruses (74%; 95% CI 67%, 83%). In the Brown et al. study, ROC curve analysis indicated that the number of correctly classified samples could be maximized with a S/N cutoff of 0.7, increasing the sensitivity to 93%, decreasing the specificity to 92%. Other commercial ELISA kits exist, and they have been compared to the MultiS-Screen ELISA and found to be similar in diagnostic efficacy (Lebarbenchon et al., 2012).

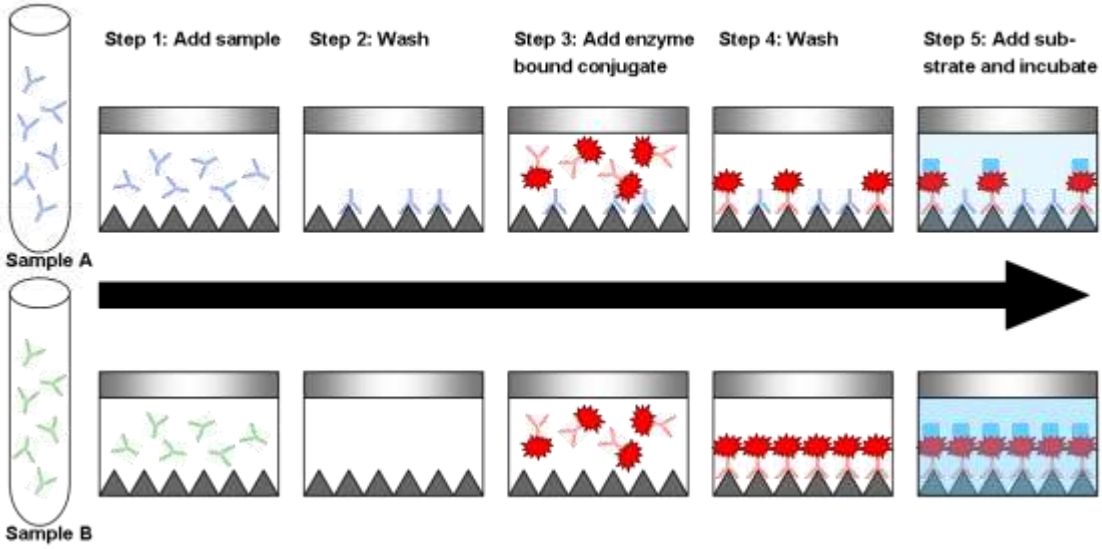

Figure 1. 5 Steps in a blocking ELISA test. The optical density of sample A is lower than sample B because the influenza A antibody in sample A bound to the antigen coated on the bottom of the well, partially blocking the binding of the enzyme bound conjugate. The antibodies in sample B did not bind to the antigen and were therefore washed out in step 2. Figure adapted from http://www.idexx.com/pubwebresources/pdf/en_us/livestock-poultry/0965846.pdf. Figure and description reproduced with permission from (Detmer, 2011).

Hemagglutination Inhibition

In the presence of the influenza HA protein, erythrocytes undergo hemagglutination (clumping) (Hirst, 1941). Antibodies to the infecting influenza virus can inhibit this hemagglutination by binding to a viral antigenic site, thus preventing viral attachment to erythrocyte sialic acids. Such inhibition can be observed in the laboratory and provides the basis for the HI assay (Pedersen, 2008). The laboratory test allows the quantification of serum antibodies based on the titer at which the serum loses its ability to inhibit the agglutination of erythrocytes exposed to influenza antigen (Figure 1.6). This test is standard for assessment of human, avian, swine and other species exposure to influenza viruses. The optimal type of erythrocytes to utilize as the hemagglutination indicator in the HI assay (e.g. chicken, turkey or horse erythrocytes) depends primarily on the type of exposure antigen (i.e. avian or human influenza virus), and prior to conducting HI assays in some species, methods may be needed to

remove non-specific inhibitors of hemagglutination, a step which varies with the species of the serum to be tested (Pedersen, 2008; World Organization of Animal Health, 2011).

While HI assays are dependable and widely-used tests, HI titers are dependent on the antigenic relatedness of the serum antibodies being detected and the HA viral antigen used in the assay (Pedersen, 2008; Spackman et al., 2008). As a result, significant antigenic drift can lead to a reduced sensitivity of HI over time unless the isolate used in the assay is updated (Spackman et al., 2008). Another complicating factor in the laboratory is the presence of nonspecific inhibitors (Swayne and Halvorson, 2008). These serum components interfere with the specificity of the HI and other tests through their activity against certain viruses, and sera should be treated to remove these or reduce their activity. The importance of nonspecific inhibitors to the outcome of diagnostic serology tests differs with the species being tested. Another complicating laboratory factor is nonspecific agglutination of chicken erythrocytes used in HI testing by the serum of some avian species, such as turkeys and geese (Swayne and Halvorson, 2008). This problem, too, can be reduced with pretesting steps to reduce this activity.

One advantage of HI is that the test can be performed without live virus, thereby decreasing the risk for laboratory exposures and allowing HPAI-work to proceed without a biosafety level 3 (BSL 3) facility. It is also relatively quick and is easy to perform (Spackman et al., 2008).

In animal diagnostics, HI using chicken erythrocytes is the OIE and WHO-preferred method for serologic diagnosis, with seropositivity at a titer of $\geq$ 1:16 after a starting dilution of 1:8 (World Health Organization, 2002; World Organization of Animal Health, 2011). Antibodies can be detected by HI 7 days after poultry infection (Swayne and Halvorson, 2008). Chicken erythrocytes are widely available and are used with success in poultry HI assays, however it is unknown if they are the optimal choice (Erica Spackman, personal communication 2012). In ducks, HI with chicken erythrocytes has been shown to be more sensitive than both AGID and ELISA (Spackman et al., 2009) and can detect antibodies for a longer period (Spackman et al., 2008; Spackman et al., 2009). It must be noted, however, that the sensitivity of HI will vary with the closeness of the field strain of exposure and laboratory strain used for the assay (Spackman et al., 2009).

While HI is widely used as a serological technique for identifying human exposure to human influenza viruses (Blanton et al., 2011), HI has been found in some cases to be insensitive for detecting antibodies to avian influenza viruses in human sera (Hinshaw et al., 1981; Profeta and Palladino, 1986; Beare and Webster, 1991; Rowe et al., 1999; Stephenson et al., 2003). Human influenza viruses will agglutinate turkey, human and guinea pig erythrocytes due to their expression of α2,6Gal and α2,3Gal linkages, but they will not agglutinate horse erythrocytes because they express only α2,3Gal linkages (Stephenson et al., 2004). For this reason, HI using horse erythrocytes has been proposed as a good way to assess human serum for antibodies to avian influenza viruses, which (as discussed above) target α2,3Gal linkages (Stephenson et al., 2004; Gill et al., 2006; Meijer et al., 2006) and has been found in one case to return results comparable to MN and have a greater sensitivity for some avian influenza antibody subtypes (Kayali et al., 2008). With specific respect to HPAI H5N1 antibodies in humans, it has been found that HI lacks specificity in persons over 60 years of age (Rowe et al., 1999), potentially because of cross-reactivity with antibodies from other influenza virus exposures.

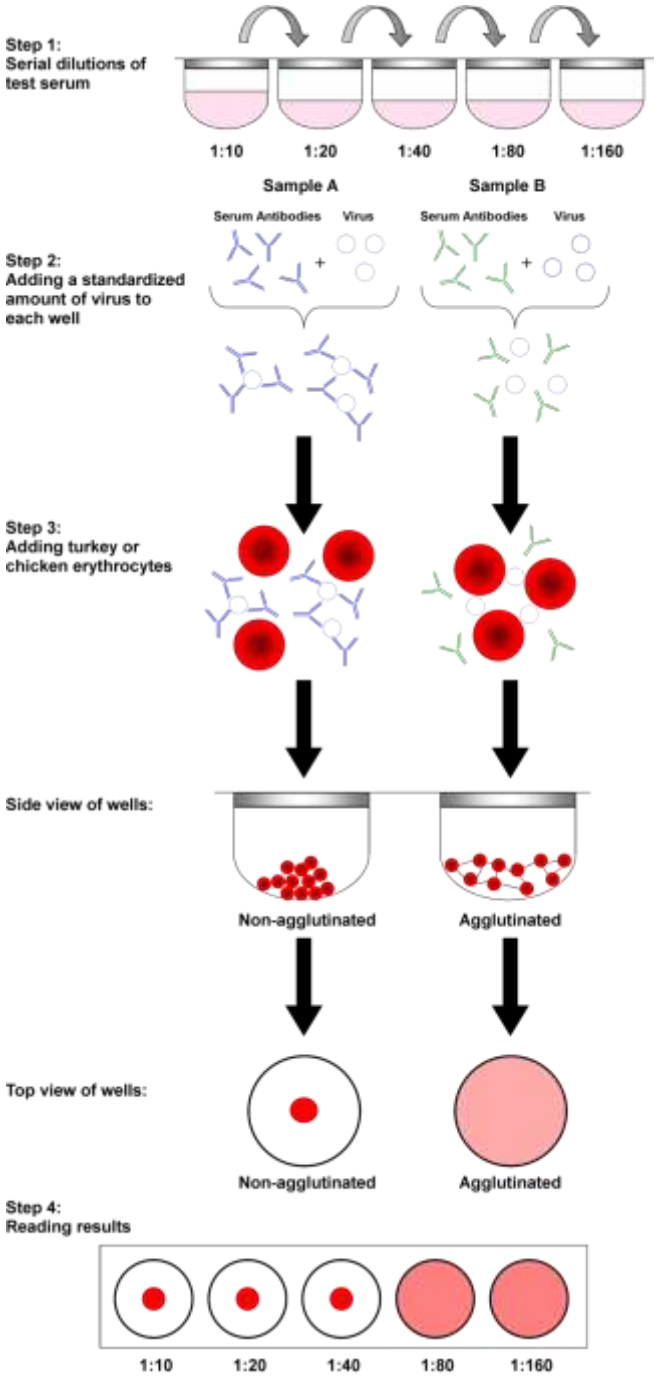

Figure 1. 6 Steps in a hemagglutination inhibition reaction. The antibodies on the left in sample A prevent the virus from agglutinating the erythrocytes. Whereas the antibodies on the right in sample B do not bind to the virus in step 2, which agglutinate the erythrocytes in step 3. The antibody titer shown in step 4 is read out as 1:40. Figure and description used with permission from (Detmer, 2011).

Microneutralization

MN is a very sensitive and specific assay that is used to detect neutralizing antibodies in animal and human serum. This assay is more precise in detecting antibodies to specific viruses (including antibodies to avian influenza viruses in human serum) than is HI because it measures the ability of the antibody to neutralize the activity of a specific live virus (World Health Organization, 2010a). In other words, MN detects a functional anti-HA antibody which is highly specific for the subtype in question. The method includes three major steps (Figure 1.7): 1) Combining the virus isolate of choice with the serum (animal or human) to be tested; 2) Inoculating a layer of cells (often MDCK cells) with the mixture; and 3) Assessing the cells for viral infection (Detmer, 2011). There are two potential methods for identifying infected cells: direct estimation of cytopathic effects (depicted in Figure 1.6) and use of ELISA to identify infected cells (World Health Organization, 2010a; Detmer, 2011). If the serum contains antibodies specific for the laboratory virus isolate, those antibodies neutralize the virus, resulting in no cytopathic effects and an ELISA negative for influenza nucleoprotein in infected cells. If the serum has no antibodies specific for the viral isolate, the virus will infect the cells, resulting in cytopathic effects and a positive ELISA test.

MN has been found to be more sensitive than HI for human infection with HPAI H5N1 and was able to better detect low titers in serum samples (Rowe et al., 1999). In that work, HI (conducted using turkey erythrocytes) could detect H5-specific antibody in samples with high neutralizing titers. One other benefit of MN is that it utilizes live virus, allowing it to be used quickly after identification of a novel influenza virus. MN is the WHO-recommended test for measurement of human antibodies to H5N1, although, because it requires greater technical skill than does HI, is more expensive, requires the use of live virus (necessitating the use of appropriate biosafety level facilities) and is less rapid, it is not always utilized for routine diagnostic testing (World Health Organization, 2007a).

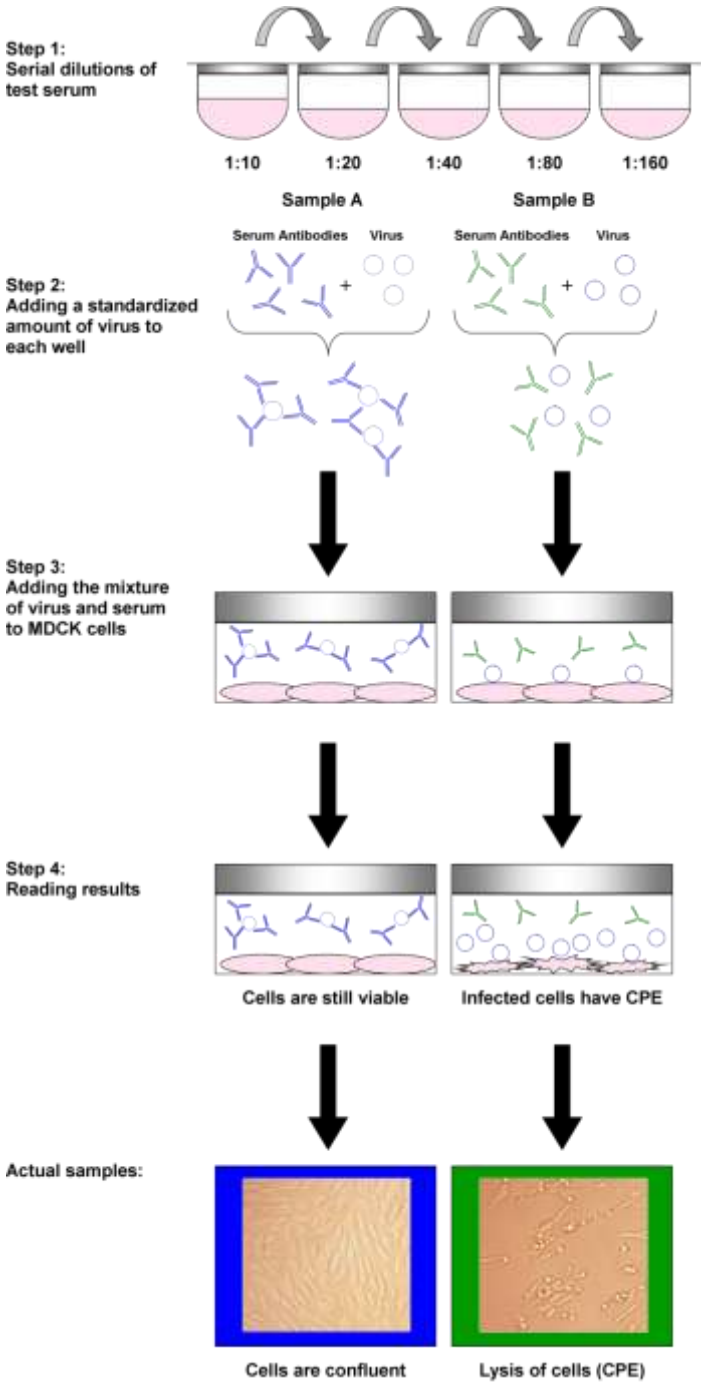

Figure 1. 7 Steps in a serum neutralization reaction. The antibodies in sample A on the left neutralized the virus in step 2. This resulted in no cytopathic effects (CPE) in step 4. Whereas the antibodies in sample B on the right did not neutralize the virus in step 2, resulting in infection of the MDCK cells and CPE in step 4. Figure and description reproduced with permission from (Detmer, 2011).

Protective Measures for Humans Working with Avian Influenza-infected Poultry

Persons exposed to poultry or other animals with influenza viruses are encouraged to use protective behaviors and equipment by the Centers for Disease Control and Prevention and the WHO. In general, recommendations are written for those persons with occupational exposure.

Hand Hygiene

Frequent hand washing with soap and water is recommended to all those in contact with avian influenza-infected or contaminated poultry, human patients, surfaces and specimens, either suspected or confirmed (World Health Organization, 2005e; United States Department of Agriculture, 2006; Centers for Disease Control and Prevention, 2008b; World Health Organization, 2008b). Hand washing should be conducted immediately after removal of gloves and prior to eating, drinking, smoking, or using the restroom. The proper hand washing procedure is well-outlined in multiple sources: wet hands and apply clean soap, placing bar soap in a location where it can drain effectively between washings. Rub hands together for 15-20 seconds, scrubbing all surfaces, including the tops and palms of hands, between fingers and around nails. Rinse and dry hands with a clean disposable towel. Use the towel to turn off the water supply. If soap and water are not available, alcohol-based hand sanitizers may be used.

Personal Protective Equipment

Personal protective equipment is defined by the United States Food and Drug Administration (FDA) as "any type of face mask, glove, or clothing that acts as a barrier between infectious materials and the skin, mouth, nose, or eyes (mucous membranes)" (Food and Drug Administration, 2010b). The principle components of the personal protective equipment protocol for protection against avian influenza include gloves, respirators, protective clothing, protective footwear and protective eyewear. Each of these items makes an essential contribution to overall PPE efficacy. Selection of PPE should be based upon several factors: 1) the type of work activities associated with the exposure, 2) health effects that may result from exposure, 3) properties of the pathogen, and 4) host factors such as immunological and immunization status (Occupational Safety and Health Administration, 2004).

AI can be transmitted by inhalation of contaminated droplets and particles, including dust and respiratory aerosols. Particulate respirators filter small droplets and airborne particles and are

recommended as the minimum respiratory protection for use in exposure to AI when used as part of a comprehensive respiratory protection program (Occupational Safety and Health Administration, 2006). The N95 respirator is the minimum respiratory protection recommended in cases of exposure to avian influenza. The term "particulate" means that the respirator is an air-purifying respirator that filters particles out of the air as it is inhaled (Food and Drug Administration, 2010a). The use of surgical masks and respirators is recommended when the wearer may be splashed by or exposed to body fluids. These are key concerns for people working with animals and human patients either confirmed or suspected of being infected with avian influenza.

Surgical masks are designed to fit loosely over the mouth and nose and are not sized to perfectly fit each individual (Food and Drug Administration, 2010a). These masks help protect the wearer against large particles and body fluids. In addition, they help protect others against exposure to the wearer's saliva and respiratory secretions. Regular surgical masks are not respiratory protection. More protective, surgical N95 respirators are designed to guard against small droplets of respiratory fluids and other airborne particles, in addition to providing the same droplet and splash protections as the surgical mask. N95 respirators require fit-testing for each individual in order to form a tight seal over the mouth and nose.

Contamination of the ocular mucous membranes (conjunctiva) with avian influenza virus is a possible route of transmission from animals to humans. Eye protection reduces direct exposure to contaminated dust and aerosols. Protective eyewear also provides a physical barrier to prevent the wearer from touching the eyes with contaminated hands. To function as an effective barrier to aerosols and contaminated dust, goggles should be non-vented, or, at a minimum, indirectly vented (Occupational Safety and Health Administration, 2004).

Medical gloves are disposable gloves that help prevent contamination among caregivers, patients and contaminated objects (Food and Drug Administration, 2010c). This class of glove includes examination, surgical and chemotherapy gloves. It is important to remember that disposable gloves can be punctured or torn, and they should always be changed if their integrity is compromised. Those exposed to potentially contaminated animals, human body fluids or surfaces should always wash their hands after removing medical gloves. These gloves should never be reused.

Protective clothing, such as coveralls (reusable or disposable), surgical gowns and impermeable aprons, prevents direct skin contact with contaminated materials (Occupational Safety and Health Administration, 2004). In addition, protective clothing serves to prevent carriage of contaminated materials outside of the contaminated site. Protective clothing should be chosen based on both the job requirements and the temperature conditions. In general, chemical-resistant coveralls create a higher heat-stress risk than lightweight cotton coveralls, but their protection against penetration is greater. Any type of protective clothing should be removed promptly if soiled, especially if permeated.

Guidance specifically for persons in countries with poultry outbreaks of HPAI H5N1 has been provided by WHO (World Health Organization, 2005d; World Health Organization, 2008b). The recommendations include protective clothing, heavy-duty rubber work gloves, standard, well-fitted surgical masks if N95 respirators are not available, goggles and washable boots.

PPE Compliance

Compliance with PPE recommendations by those dealing with poultry potentially or confirmed to be infected with AI is a concern for public health advocates. Investigations of PPE-use by those involved in avian influenza outbreak situations show that noncompliance is not unique to certain influenza outbreaks, or locations (Skowronski et al., 2007; Cai et al., 2009). A study of those involved in outbreak control during the 2004 H7N3 outbreak in British Columbia, Canada revealed that compliance with PPE recommendations was inconsistent, and though the outbreak lasted only three months, approximately 15% of the participants reported not wearing gloves and masks at all times when entering a barn with infected birds (Skowronski et al., 2007). In the 2003 H7N7 outbreak in the Netherlands, there was a low reporting of consistent mask-use (6%) and goggle-use (1%) by farmers involved in controlling the outbreak (Bosman et al., 2005). In the same outbreak, those involved in the culling of infected animals displayed a slightly better compliance, at 25% and 13% for masks and goggles, respectively. There was, however, no protective effect from the use of PPE that could be shown in the population conducting outbreak control (Bosman et al., 2004).

Chapter 2: Risk factors for exposure to influenza A viruses, including subtype H5 and H7 viruses, in Thai free-grazing ducks

Introduction

Highly pathogenic avian influenza (HPAI) H5N1 has caused high mortality of wild birds and domestic poultry throughout Asia and in some parts of Africa, Europe and the Middle East (Webster et al., 2006; Emerging Centre for Transboundary Animal Diseases, 2011). Since HPAI H5N1 was first reported in Thailand in January 2004, there have been seven outbreaks, most recently in October and November of 2008 (Amonsin et al., 2006; Tiensin et al., 2007; Chaichoune et al., 2009; World Organization for Animal Health, 2011c). Recent outbreaks occurred largely in Central and Northern Thailand (World Organization for Animal Health, 2011c). Response measures have included stamping out, poultry movement control, biosecurity improvements and door-to-door surveillance (Buranathai et al., 2007; World Organization for Animal Health, 2011c). Stamping out is a widely-used strategy for quickly eliminating a newly introduced disease and involves a combination of zoning, intense surveillance, quarantine and movement restrictions, culling of susceptible animals, safe carcass disposal and disinfection (Geering et al., 2001). Influenza vaccination of poultry is prohibited in Thailand (Buranathai et al., 2007).

Thai HPAI H5N1 outbreaks have a seasonal pattern, starting in the rainy season (July-September) or winter (October- January) (Chaichoune et al., 2009; Tiensin et al., 2009). No outbreaks began in or continued through the hot summer season (April- June). Despite this intermittent pattern, 2008 Central Thailand HPAI H5N1 isolates share the same clade 1 lineage as earlier Thai isolates, with genetic drift rates typically associated with sustained virus circulation (Amonsin et al., 2006; Buranathai et al., 2007; Chaichoune et al., 2009; Amonsin et al., 2010). This suggests an animal or environmental reservoir maintaining H5N1 viruses during summer prior to rainy season re-emergence. Because ducks can shed influenza viruses asymptomatically, domestic populations may sustain low-level H5N1 virus transmission during summer (Chen et al., 2004; Sturm-Ramirez et al., 2005; Songserm et al., 2006; Chaichoune et al., 2009). Free-grazing duck (FGD) flocks in Central Thailand were identified as a risk factor for HPAI H5N1 outbreaks through spatial analyses (Tiensin et al., 2005; Gilbert et al., 2006; Gilbert et al., 2007; Gilbert et

al., 2008; Tiensin et al., 2009). In this management system, FGD farmers graze their flocks on post-harvest rice fields, where they eat residual rice, insects and snails, frequently sharing fields with resident and migratory wild waterfowl (Gilbert et al., 2006). Owners identify grazing fields through social networks, built over years of experience, and if no nearby fields are available, flocks are moved out of the area, sometimes to other provinces (Beaudoin, unpublished data).

FGD influenza research in Thailand has been largely conducted in Suphanburi Province, where irrigation facilitates two to three rice crops yearly with year-round duck grazing, and where almost 50% of all Thailand's duck outbreaks occurred during the second wave of HPAI H5N1 outbreaks in 2004 (Gilbert et al., 2006; Songserm et al., 2006). Despite the identification of FGD as a risk factor for HPAI H5N1 outbreaks, little is known about transmission within and among FGD flocks or the prevalence, incidence and viral subtypes of infection (Emerging Centre for Transboundary Animal Diseases, 2011). Our study goals were to improve the understanding of FGD flock management and movement in Suphanburi Province and estimate the seroprevalence of influenza A antibodies, including those to H5 and H7 subtype viruses, in this population. H5 and H7are the only two influenza subtypes that have the potential to possess characteristics of high pathogenicity. In addition, both subtypes have zoonotic potential and have been associated with human morbidity and mortality.

Methods

Study Design

We conducted a cross-sectional study of FGD flocks within Suphanburi Province in July and August of 2010. Suphanburi last experienced HPAI H5N1 poultry outbreaks in November of 2005. In June 2010, the Thai Department of Livestock Development (DLD) completed a province-wide census and registration of FGD flocks. Flocks were identified through existing registration lists, communication with local livestock officers and registration announcements. The DLD considers flocks "free-grazing" if they move away from home to graze. The census identified 340 flocks, registered based on owner residence in 9 of the 10 municipal districts of Suphanburi Province (Don Chang District had no FGD flocks). All were invited by local livestock officers to participate. Flocks located outside the province at the time of sample collection were excluded due to collection constraints. There were 139 flocks outside the

province or unable to be contacted. All other owners agreed to participate, yielding a 201-flock study cohort (59% of registered flocks).

A total of 6254 samples were taken from ducks in the 201 flocks. All samples were tested with a screening test (NP ELISA) for influenza A antibodies. Due to resource constraints, only a subset of sera (1423 samples) was tested for H5 and H7 antibodies. For statistical analyses, flocks not currently grazing or with unknown grazing history (n=41), or with incomplete survey responses (n=32) were excluded. For the analysis of influenza A seropositivity, the final dataset consisted of 3978 ducks in 128 flocks, and for the H5 and H7 seropositivity analyses, the final dataset consisted of 962 ducks in 122 flocks.

Serum Collection

Blood was collected at grazing locations during July and August, 2010. With a test sensitivity of 96%, a sample size of 30 ducks per flock provided > 95% chance of detecting a single seropositive duck if the true flock seroprevalence was ≥10%. With this sample size, assuming a conservative flock-level seroprevalence of 50%, we have 95% confidence that the precision of the within-flock seroprevalence estimates is ≤18%. Convenience samples of ducks were selected from each flock for venipuncture.

NP ELISA

Sera were analysed by commercial enzyme-linked immunosorbent assay (ELISA) according to manufacturer instructions (FlockCheck Avian Influenza MultiS-Screen Ab Test Kit, IDEXX Laboratories, Westbrook ME; referred to as NP ELISA). This screening test identifies avian antibodies to the highly-conserved influenza A virus nucleoprotein (NP), allowing non-subtype-specific detection of antibodies against all subtypes. To obtain our positive cutoff value, we referred to one published study (Brown et al., 2009) and used unpublished duck-specific experimental data from that work to determine the optimal cutoff for duck serum. This cutoff has an expected 96% test sensitivity and 88% specificity. Sera were initially positive with a sample/negative (S/N) value of < 0.6, and suspect-positive with 0.6< S/N <0.7. Suspect samples were assayed in triplicate and the mean S/N value was determined. To obtain a dichotomous outcome of duck-level seropositivity, samples with S/N (or mean S/N) of <0.7 were considered positive.

H5 and H7 ELISA

A subset of NP ELISA-positive samples was assessed using ELISA kits that identify avian serum antibodies to H5 subtype influenza A viruses (ID Screen Influenza H5 Antibody Competition and ID Screen Influenza H7 Antibody Competition, ID Vet, Montpellier, France; referred to as H5 ELISA). The H5 kit was manufactured using a LPAI H5N2 virus isolated in Italy between 2005 and 2006. Samples were processed using manufacturer instructions and cutoffs (positive if S/N ≤0.35, suspect if > 0.35 and ≤0.39). Serum from ducks vaccinated with inactivated H5N1 vaccine was included as an additional positive control. The H7 kit was manufactured using a LPAI H7N3 virus isolated in Italy. Samples were processed using manufacturer instructions and cutoffs (positive if S/N ≤0.50, suspect if > 0.51 and ≤0.59). Suspect-positive samples were considered positive for analysis. No sensitivity or specificity information could be obtained from the manufacturer for these kits.

H5 and H7 ELISAs were used on a 1423-serum subset. Samples were selected from every flock with positive ducks. If there were less than 10 positive samples in one flock, all samples were tested (45 samples from 13 flocks). All samples from 18 flocks with 100% seroprevalence (562 samples), including the youngest and oldest flocks from the 9 districts were tested to estimate flock H5 seroprevalence. Five positive samples were randomly selected from all other flocks with positive ducks (1005 samples from 163 flocks). Thirty NP ELISA-negative samples were randomly chosen for assay.

Hemagglutination Inhibition

H5 and H7-positive samples were further assessed using hemagglutination inhibition (HI) as published previously (World Organization of Animal Health, 2011). Pre-treatment of sera to reduce the effect of non-specific inhibitors was conducted by incubation of 100 µl serum with 400 µl of 20% kaolin, centrifugation, and adsorption of the supernatant with 10 µl of 50% chicken RBC. The resulting supernatant yielded a stating dilution of 1:5 for the HI assay. HI was conducted with inactivated clade 1 influenza virus A/chicken/Thailand/CU-K2/2004/H5N1 and H7N4, and samples were considered positive with a titer ≥1:20.

Flock Owner Interviews

A 53-question survey regarding flock characteristics, management, grazing practices, movement history and experiences with HPAI H5N1 in 2004-05 was developed. Questions were written in English, translated into Thai and back-translated into English to reduce loss of meaning through translation. Phone surveys were conducted with verbal consent after the blood collection. Seventeen flock owners could not be contacted, and data for those flocks was limited to age and seropositivity status. Interviews were conducted in Thai by native speakers, trained on the background and intentions of the study. Responses were recorded in Thai and translated into English. FGD owners with whom researchers had prior contact piloted the survey.

Analysis, NP ELISA

Descriptive statistics calculated with the full dataset (n=201 flocks, 6254 ducks) characterized seroprevalence and age of flocks. Statistical analyses were conducted using the dataset with complete survey information and known grazing history (n=128 flocks, 3978 ducks). For all analyses, duck-level serostatus was included as the dichotomous dependent variable (seropositive or seronegative). Univariate analyses of duck and management parameters included t-tests, Chi-square, Fisher's exact test and Wilcoxon rank-sum test. Multivariable analysis was conducted using generalized estimating equation (GEE) methods, with flock as a repeated measures variable. GEE methods account for similarities within clusters and may better model clustered discrete data than generalized mixed models (Dohoo, 2009). We assumed an exchangeable working correlation matrix, where every duck within a flock is equally correlated with every other duck in the flock. The full multivariable model included independent parameters with p-values of ≤ 0.25 on univariate analysis. Manual model backward and forward building techniques were used, and independent variables (or groups of indicator variables) with p-values of > 0.05 were removed. Model fit was guided by the quasi-likelihood under the independence model criterion (QIC), where QIC value decreases with improved model fit (Pan, 2001). Statistics were conducted with SAS 9.2 software (SAS Institute, Inc., Cary, NC).

Analysis, H5 and H7 Diagnostics

Descriptive statistics were calculated for all samples tested for H5 and H7 seropositivity (n=1423 ducks in 194 flocks). Statistical analyses were conducted using the dataset with

complete survey information and known grazing history (n=122 flocks, 962 ducks). Univariate and multivariable analyses were conducted as described above for NP ELISA data.

Ethical Considerations

This work was approved by the University of Minnesota Institutional Review Board **(**#0701M01041) and Institutional Animal Care and Use Committee (#1003A78795).

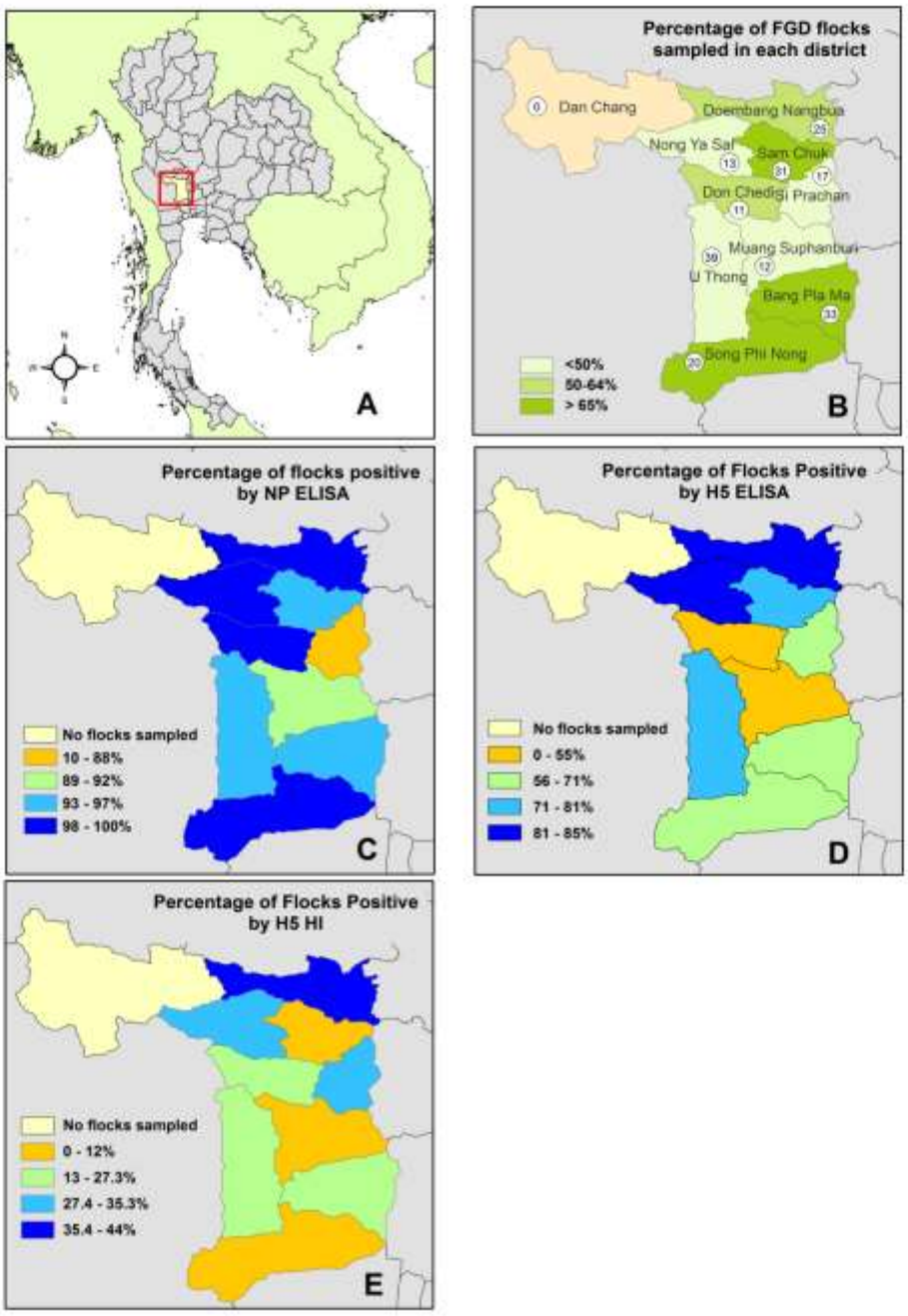

Figure 2. 1 Sampled flocks and seropositivity in Suphanburi Province. Location of Suphanburi Province within Thailand (A); Percentage of registered flocks in each district of Suphanburi that were included in the study, with number of flocks included in the circle (B); Percentage of NP ELISA-positive flocks (with at least one NP-seropositive duck) in each district (C); Percentage of H5 ELISA-positive flocks (with at least one H5-seropositive duck) in each district (D); and Percentage of H5 HI-positive flocks (with at least one HI-seropositive duck) in each district (E). Jenks natural breaks classification was used to generate the choropleth map data classes.

Table 2. 1 Results of univariate analyses of flock and duck factors for association with duck seropositivity on NP ELISA (n=3978)

Parameter	*Median for positive ducks (IQR)*	*Median for negative ducks (IQR)*	*p-value**
Flock size	2000 (1200, 2800)	2000 (1500, 3000)	<0.01
Age (mo)	8.0 (7.0, 10)	4.0 (3.0, 5.0)	<0.01
Age when first grazed (days)	30.0 (25, 60)	30.0 (20, 30)	<0.01
Time spent grazing over lifetime (mo)	4.0 (2.5, 7.0)	3.0 (1.5, 3.5)	<0.01

Parameter	*Category*	*Number of positive ducks (n=3268)*	*Number of negative ducks (n=710)*	*OR*	*CI*	*p-value* †
Duck use	Meat	82	13	ref		0.28
	Eggs	3186	697	0.72	0.40, 1.3	
Grazed on rice fields for entire life to-date	No	1988	336	ref		<0.01
	Yes	1280	374	0.58	0.49, 0.68	
Egg collection modality	Bring to market	588	99	7.4	5.4, 10.2	<0.01
	Picked up	2496	449	7.0	5.4, 9.0	<0.01
	Combination	58	4	18.1	6.4, 51.4	<0.01
	No eggs	126	158	ref		
Egg collections per week	≤ 1	494	254	ref		
	2-4	2381	415	3.0	2.5, 3.5	<0.01
	≥ 5	393	41	5.0	3.5, 7.0	<0.01
Owner has other poultry	No	1709	415	ref		
	Yes	1559	295	1.3	1.1, 1.5	<0.01
Duck contact with other poultry	No	2389	648	ref		
	Yes	879	62	3.8	2.9, 5.0	<0.01
Visitors come to flock	No	1486	563	ref		
	Yes	1782	147	4.6	3.8, 5.6	<0.01
Ducks graze year-round	No	893	94	ref		
	Yes	2375	616	0.41	0.32, 0.51	<0.01
Ducks have moved out of province	No	3031	697	ref		
	Yes	237	13	4.2	2.4, 7.4	<0.01
Ducks have moved out of district	No	2760	589	ref		
	Yes	508	121	0.9	0.72, 1.1	0.32
Ducks have moved out of village	No	1353	193	ref		
	Yes	1915	517	0.53	0.44, 0.63	<0.01

(Continues…)

Duck purchase age	Raise from eggs	39	24	0.40	0.24, 0.68	<0.01
	Buy baby ducks	2684	666	ref		
	Buy adult ducks	516	17	7.5	4.6, 12.3	<0.01
	Buy both ducks	29	3	2.4	0.73, 7.9	0.15
Nightly pen location	Near field	672	173	0.80	0.67, 0.97	0.03
	Back to barn	2520	521	ref		
	Either	76	16	0.98	0.57, 1.7	0.95
Transport modality	Truck	2788	592	ref		
	Walk	130	59	0.47	0.34, 0.64	<0.01
	Walk and truck	350	59	1.3	0.94, 1.7	0.12

*Nonparametric Wilcoxon test †Chi square analysis for equality of proportions

Results

Suphanburi Flock Influenza A Seroprevalence

The proportion of FGD flocks in each district that was included in this study can be seen in Figure 5.1. Positive flocks were located in all nine districts with FGD flocks (Figure 1). Of the 201 flocks sampled, 194 (97%) had at least one duck with influenza A antibodies identified by NP ELISA. The within-flock seropositivity distribution (Figure 2.2) is left-skewed, with a median value of 97% (interquartile range (IQR) 90%-100%). Fifty-six flocks (28%) had 100% seropositivity, and seven flocks (3.4%) were seronegative. Among all ducks sampled, (n=6254 ducks in 201 flocks), 85% (5305 ducks) were seropositive on NP ELISA. Seropositive ducks were older than seronegative ducks, with mean (± standard deviation) ages of 9.1 (±3.8) and 5.1 (±3.2) months ($p<0.01$), and median of 8 and 4 months, respectively (Table 2.1).

Flock Characteristics and Management

Survey results can be seen in Table 2.1. In the 128-flock dataset used for analysis, flock size ranged from 300-11000 ducks (mean 2296, median 2000). Most flocks (99.2%) were of homogenous age, ranging from 1.5 to 24 months. One flock (0.8%) was of multiple ages. The most common breed was the egg-producing Khaki Campbell or a mix thereof (97.6%), although some owners raise Beijing (0.8%) or Cherry Valley (1.6%) meat ducks. Marketing of eggs occurs on average 2.4 days per week (median 2 days). In most cases, the eggs are picked up for sale

(79.8%), rather than delivered to market by the owner (18.5%) or a combination of the two methods (1.7%).

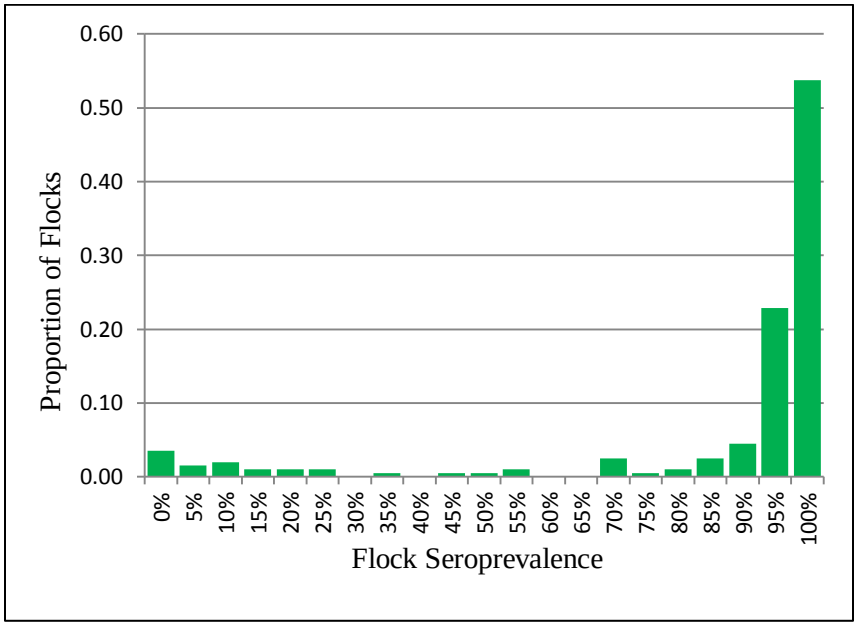

Figure 2. 2 Distribution of flock seroprevalence for avian influenza antibodies by NP ELISA (n=201)

After grazing on fields near home, FGD flocks are usually brought home to a barn or partially covered pen at night. Ducks are transported by truck (93.3%), by foot (5.6%) or by a combination of the two (1.1%). The average daily maximum distance to the grazing location is 5 km, though, rarely, flocks undergo daily movement up to 30 km. Ducks are penned together overnight for 12 hours on average (range 10-13) and are kept in an open-sided roofed barn (79.5%) or an open uncovered area (20.5%). Flocks not brought back to a barn are penned in a dry location near the rice field.

Most owners (65%) move to the same locations for grazing each year, while others (35%) use variable grazing locations. Flock movement across administrative boundaries decreases with increased distance. Thirteen per cent of FGD flocks leave Suphanburi Province during a typical year. While in Suphanburi, 20% move outside their district, and while in the home district, 54%

will move outside their subdistrict. Eighty-four per cent of FGD flocks move to other villages in their subdistrict to graze.

Nearly all flock owners reported purchasing their flocks as a group, with most ducks purchased at less than 4 months old (86%). Median purchase age was 1 day. Ducks bought at < 4 months were from duck farms (95%) or from markets, dealers or neighbors (5%). Older ducks were obtained from farms (67%), or from a dealers or markets (33%). The mean and median sale age of egg ducks is 24 months, the age at which production declines. Meat breeds are sold to slaughter at 3 to 5 months old. Ducks are sold to traders (95%), neighbors (1%) and slaughterhouses (4%). Flocks are usually sold as one group (80%), though small group sales (20%) may occur if purchased by another duck farmer or a small-scale trader. Market sales of live poultry are uncommon in Central Thailand, and 95% of owners reported never visiting live bird markets. Additionally, 95% of respondents never go to slaughterhouses, relying on traders as middle-men.

Nine per cent of respondents that owned ducks at that time (n=103), reported having an infected flock during the 2004-05 HPAI H5N1 outbreaks, and 19% had a flock culled. During the outbreaks, 65% raised ducks but did not graze, 28% stopped raising ducks and 7% continued grazing.

Univariate Analysis, NP ELISA

Seropositive ducks had spent more time grazing (Table 2.1). Only some of the effect of grazing time was explained by duck age (data not shown), and both of these parameters were included in the multivariable analysis. Ducks from flocks having contact with other poultry had a higher odds of seropositivity, as did ducks with flock visitors and egg pickup, and ducks that had left the province. Other significant parameters include movement out of the village when within the home subdistrict, duck purchase age, night pen location and transport modality. Seventeen significant parameters were included in the multivariable analysis.

Multivariable Analysis, NP ELISA

The final GEE multivariable model included duck age at sampling, contact with other poultry, visitors to the flock and duck purchase age (Table 2.2), all which were positively associated with seropositivity. The final model's estimated working correlation was 0.004,

indicating that, after controlling for independent parameters, 0.4% of the outcome variance of influenza A seropositivity occurs at the flock level. In other words, belonging to a certain flock has little to do with exposure to influenza A viruses, because seropositivity is ubiquitous.

Table 2. 2 Results of multivariable analysis of flock and duck factors for association with duck seropositivity on NP ELISA (n=3978)†

Independent variable	*Category*	*OR**	*95% CI*	*p-value*	*Confidence limit ratio*
Age	2 month difference	2.3	1.7, 3.2	<0.001	1.9
Contact with other poultry	No	ref			
	Yes	3.9	2.1, 7.1	<0.001	3.4
Visitors come to flock	No	ref			
	Yes	4.5	2.1, 9.3	<0.001	4.4
Duck purchase age	Eggs	1.2	0.75, 1.9	0.44	
	Ducks < 4 months old	ref			
	Ducks ≥ 4 months old	5.7	2.3, 13.8	<0.001	6.0
	Ducks of varying age	0.50	0.19, 1.3	0.15	

*Measures of association are adjusted for flock membership by inclusion of flock as a cluster variable.
†The working correlation estimate for this model was 0.004

H5 Seropositivity

H5-seropositive ducks were located in all 9 districts with registered flocks (Figure 5.1). Of the 1423 NP ELISA-positive specimens assayed with H5 ELISA, 553 (39%) were positive for H5 antibodies, 57 (4%) were suspect, and 813 (57%) were seronegative. Two (6.7%) of 30 NP ELISA-negative samples were positive on H5 ELISA. Manufacturer negative and positive controls and the H5-positive serum controls consistently returned the expected result. The 1423 samples were from 194 different flocks. Of these, 151 flocks (78%) had at least one H5 ELISA-positive or suspect specimen, with the youngest flock being 2 months old.

The 610 positive and suspect samples were assayed using HI, and 74 (12%) had a positive titer (≥1:20; range 1:20-1:160), 90 (15%) had a negative but detectable titer (<1:20), and 446 (73%) had no detectable titer. The 74 specimens with titers ≥1:20 belonged to 49 flocks, located all 9 study districts (Figure 5.1).

All samples from 18 flocks with 100% seropositivity on NP ELISA (n=564) were tested using the H5 ELISA in order to estimate flock-level H5 seroprevalence. Two of 18 flocks were seronegative for H5. The median within-flock seroprevalence was 44% (interquartile range 16-71), and maximum seroprevalence was 87%. Flock seroprevalence was not correlated with age (data not shown).

Analysis, H5 ELISA

Some of the potential risk factors for NP ELISA seropositivity (Table 2.1) were not significant, or were even protective, with regard to H5 ELISA seropositivity (Table 2.3). This is likely a result of selecting only NP ELISA-positive samples for assessment with the H5 ELISA. After multivariable analysis (GEE modeling) controlled for flock, duck H5 seropositivity by ELISA is explained by duck age alone (Table 2.4). The estimated working correlation for the final model was 0.32, indicating that 32% of the outcome variance of duck H5 seropositivity is at the flock level.

Table 2. 3 Results of univariate analyses of flock and duck factors for association with duck seropositivity on H5 ELISA (n=962)

Parameter	***Median for positive ducks (IQR)***	***Median for negative ducks (IQR)***	***p-value****
Flock size	2000 (1200, 3000)	1550 (1300, 2800)	0.89
Age (mo)	9.0 (8.0, 14)	7.0 (4.5, 10)	< 0.01
Age when first grazed (days)	30 (30, 60)	30 (25, 60)	0.22
Time spent grazing over lifetime (mo)	4.0 (2.0, 7.0)	3.0 (2.0, 5.0)	< 0.01

Parameter	***Category***	***Number of positive ducks (n=405)***	***Number of negative ducks (n=557)***	***OR***	***CI***	***p-value*** †
Duck use	Meat	0	15			
	Eggs	405	542	-		< 0.01
Grazed on rice fields for entire life to-date	No	252	314	ref		
	Yes	153	243	0.78	0.60, 1.0	0.07
Egg collection modality	Bring to market	67	86	3.1	1.6, 6.0	0.01
	Picked up	324	416	3.1	1.7, 5.6	< 0.01
	Both/ no eggs	14	55	ref		

(Continues…)

Egg collections per week	≤ 1	38	148	ref		
	2-4	276	331	3.2	2.2, 4.8	< 0.01
	≥ 5	91	78	4.5	2.8, 7.3	< 0.01
Owner has other poultry	No	220	305	ref		
	Yes	185	252	1.0	0.79, 1.3	0.89
Duck contact with other poultry	No	347	437	ref		
	Yes	58	120	0.61	0.43, 0.86	< 0.01
Visitors come to flock	No	206	211	ref		
	Yes	199	346	0.59	0.45, 0.76	< 0.01
Ducks graze year-round	No	66	170	ref		
	Yes	339	387	2.3	1.6, 3.1	< 0.01
Ducks have moved out of province	No	368	529	ref		
	Yes	37	28	1.9	1.4, 3.2	0.01
Ducks have moved out of district	No	342	479	ref		
	Yes	63	78	1.1	0.79, 1.6	0.50
Ducks have moved out of village	No	139	285	ref		
	Yes	266	272	2.0	1.5, 2.6	< 0.01
Duck purchase age	Raise from eggs	2	8	ref		
	Buy baby ducks	331	481	ref		
	Buy adult ducks	67	68	1.4	0.99, 2.1	0.06
	Buy both ducks	5	0	ref		
Nightly pen location	Near field	77	67	1.7	1.2, 2.5	< 0.01
	Back to barn	321	482	ref		
	Either	7	8	1.3	0.47, 3.7	0.60
Transport modality	Truck	369	482	ref		
	Walk	10	41	0.32	0.16, 0.64	< 0.01
	Walk and truck	26	34		0.60, 1.7	0.99

*Nonparametric Wilcoxon test †Chi square analysis for equality of proportions

Table 2. 4 Results of multivariable analysis of flock and duck factors for association with duck seropositivity on H5 ELISA (n=962) †

Independent variable	***Category***	***OR****	***95% CI***	***p-value***	***Confidence limit ratio***
Age	2 month difference	1.3	1.1, 1.5	< 0.01	1.3

*Odds ratio is adjusted for flock membership by inclusion of flock as a cluster variable.
† The working correlation estimate for this model was 0.37.

H7 Seropositivity

H7-seropositive ducks were located in all nine districts with registered flocks. Of the 1423 influenza antibody-positive specimens tested with the H7 ELISA, 117 (8.2%) were positive for H7 antibodies, 70 (4.9%) were suspect, and 1236 (87%) were seronegative. These H7 ELISA-positive and suspect ducks were from 65 (34%) of the 194 flocks tested. The 187 positive and suspect samples were assayed using HI, and none of the ducks had positive titers (≥1:20).

Flock H7 Flock Seroprevalence and Analysis of Potential Risk Factors for H7 Seropositivity

H7 flock seroprevalence was calculated for the above mentioned eighteen flocks. The median seroprevalence was 6% (interquartile range 0, 18), and the maximum seroprevalence was 37%. Five of these flocks had no antibodies to H7 influenza. The factors associated with H7 seropositivity can be seen in Table 2.5. GEE analysis, controlled for flock membership, indicates that after selection for NP seropositivity and its affiliated characteristics (Table 2.1), duck H7 seropositivity by ELISA is associated with visitors coming to the flock and penning ducks next to the field overnight (Table 2.6). The estimated working correlation for the final GEE model was 0.11, indicating that in this model 11% of the variance in the outcome of H7 seropositivity occurs at the flock level.

Discussion

Influenza Viruses

Our results indicate that FGD flocks in Suphanburi are widely exposed to influenza A viruses, including subtypes H5 and H7, and these viruses have circulated in flocks as recently as May of 2010, as the youngest seropositive flock was 2 months old. While influenza A seropositivity in waterfowl is a normal finding (World Organization for Animal Health, 2011a), of the influenza-seropositive ducks tested, 39% were positive on H5 ELISA, and 27% of these were positive by H5 HI, the international serological standard (World Organization of Animal Health, 2011). In South East Asia, where HPAI H5N1 circulates in poultry populations, this is an important finding.

For even the oldest ducks, those with positive H5 titers were exposed when there were no known H5N1 outbreaks in Suphanburi Province (after November 2005). The assays used in this

study do not distinguish between exposure to HPAI H5N1 and other H5 viruses (LPAI or HPAI), nor do they allow us to determine if the FGD flocks are maintaining virus circulation or were seropositive due to spillover virus from free-living birds. Nonetheless, FGD are a potential reservoir for influenza viruses, including subtype H5N1 between identifiable outbreaks, and these findings underscore the importance of year-round surveillance, including during summer months.

Recently, a small number of H4, H6 and H10 viruses were isolated from healthy Muscovy ducks at a central Bangkok live bird market (Wisedchanwet et al., 2011). Additionally, H12N1 viruses were isolated from wild ducks and a watercock in Central Thailand, both species which may frequent flooded rice fields (Wongphatcharachai et al., 2012). Surveillance elsewhere in Asia indicates that a variety of LPAI subtypes has been present in wild or domestic avian populations since 2000, including H5N2 viruses in poultry of Japan and Taiwan, and H5N3 in domestic and wild species of Southern China (Alexander, 2007; Duan et al., 2007). Also identified in ducks of Southern China are LPAI H3, H4, H6, H8, H10 and H11 viruses (Duan et al., 2007), and H9N2 subtype influenza has been endemic in Asian poultry since the 1990s (Swayne and Halvorson, 2008). These and other unclassified LPAI virus subtypes may have contributed to the nearly 100% flock influenza A seropositivity found here.

The large proportion of H5 ELISA-positive, H5 HI-negative samples (88%) may indicate exposure to a LPAI H5 virus, particularly as a whole-virus LPAI H5N2 was used in the ELISA. Additionally, while recent molecular studies show minimal virus evolution of the predominant clade 1 viruses beyond genetic drift in Central Thailand, there is some evidence of reassortment (Amonsin et al., 2010), and incursions of non-clade 1 HPAI H5N1 cannot be ruled-out. Studies have shown limited cross-reactivity and low neutralizing titers between clade 1 antigens and clade 2.3.4 antiserum (Chen et al., 2004; Chen et al., 2006; Balish et al., 2010). Low titers could also result from waning antibody titers or poor seroconversion. Experimental studies report low influenza HI titers in ducks after experimental infection (Saito et al., 2008). In one study, ducks inoculated with a Thai HPAI H5N1 duck isolate showed 50-75% mortality but no seroconversion in the survivors (Saito et al., 2008). The same study also showed that infection with other isolates leads to a range of geometric mean HI titers, from 0 to 128 at 14 days post infection, with cross-bred ducks showing lower titers. Other work has suggested that previous infection with homologous LPAI viruses may be important to the humoral response and viral replication

resulting from subsequent infection with HPAI H5N1 (Costa et al., 2011). Regardless of etiology, low titers could allow persistence of virus in ducks during the hot summer months as suggested elsewhere (Chaichoune et al., 2009; Amonsin et al., 2010; Magor, 2011). Additionally, minimal duck seroconversion may help limit virus evolution in Central Thailand, particularly in an unvaccinated population, as host immunity plays a role in influenza virus evolution (Webster et al., 1992).

Risk Factors for Influenza Seropositivity

It is not surprising that age was associated with seropositivity, as older ducks accrued more time for exposure. Ducks that are grazed year-round and have grazed for their entire lifetime were less likely to be NP ELISA-positive on univariate analysis, perhaps because they were kept at a lower flock density for longer periods than ducks that were penned or kept in a barn. When grazing, ducks move around the field in a loose group, resulting in a lower contact rate than would be experienced in a closed location. Ducks in confined pens or barns consume water from buckets or small ponds, rather than from water-covered rice fields, facilitating waterborne transmission. Duck distancing as a protective factor is further supported by increased odds of seropositivity for ducks undergoing vehicular transport and inter-provincial movement (Table 2.1). Transport is a high-density, stressful experience, often with two thousand ducks confined in one multilevel truck. High road density and short distance to highway junctions have been identified as spatial risk factors for poultry H5N1 outbreaks, supporting the association between transport and influenza infection (Ward et al., 2008; Paul et al., 2009; Rivas et al., 2009; Loth et al., 2010; Yupiana et al., 2010).

Table 2. 5 Results of univariate analyses of flock and duck factors for association with duck seropositivity on H7 ELISA (n=962)

Parameter	*Median for positive ducks (IQR)*	*Median for negative ducks (IQR)*	*p-value**
Flock size	2,250 (1,300, 3,300)	1,500 (1,200, 3,000)	< 0.01
Age (mo)	9 (4.5, 12)	8 (6, 12)	0.27
Age when first grazed (days)	30 (30, 60)	30 (25, 60)	0.84
Time spent grazing over lifetime (mo)	4.0 (2.3, 8.0)	3.5 (2.0, 6.0)	0.05

Parameter	*Category*	*Number of positive ducks (n=116)*	*Number of negative ducks (n=846)*	*OR*	*CI*	*p-value*[†]
Duck use	Meat	0	15	ref		0.15
	Eggs	116	831	-	-	
Grazed on rice fields for entire life to-date	No	58	508	ref		0.04
	Yes	58	338	1.5	1.0, 2.2	
Egg collection modality	Bring to market	12	141	0.30	0.13, 0.71	< 0.01
	Picked up	87	653	0.47	0.25, 0.91	0.02
	Combination	4	6	2.4	0.58, 9.6	0.23
	No eggs	13	46	ref		
Egg collections per week	≤ 1	24	162	ref		
	2-4	54	553	0.66	0.40, 1.1	0.11
	≥ 5	38	131	2.0	1.1, 3.4	0.02
Owner has other poultry	No	59	466	ref		
	Yes	57	380	1.2	0.90, 1.7	0.40
Duck contact with other poultry	No	95	689	ref		
	Yes	21	157	0.97	0.59, 1.6	0.91
Visitors come to flock	No	36	381	ref		
	Yes	80	465	1.8	1.2, 2.8	< 0.01
Ducks graze year-round	No	15	221	ref		
	Yes	101	625	2.4	1.4, 4.2	< 0.01
Ducks have moved out of province	No	99	798	ref		
	Yes	17	48	2.9	1.5, 5.2	< 0.01
Ducks have moved out of district	No	97	724	ref		
	Yes	19	122	1.2	0.7, 2.0	0.58
Ducks have moved out of village	No	53	371	ref		
	Yes	63	475	0.93	0.63, 1.4	0.71
Duck purchase age	Raise from eggs	0	10	ref		
	Buy baby ducks	94	718	ref		
	Buy adult ducks	21	114	1.4	0.85, 2.4	0.18
	Buy both ducks	1	4	ref		

(Continues…)

Nightly pen location	Near field	33	111	2.6	1.7, 4.1	< 0.01
	Back to barn	83	720	ref		
	Either	0	15	ref		
Transport modality	Truck	108	743	ref		
	Walk	3	48	0.43	0.13, 1.4	0.16
	Walk and truck	5	55	0.63	0.25, 1.6	0.33

*Nonparametric Wilcoxon test †Chi square analysis for equality of proportions

Table 2. 6 Results of multivariable analysis of flock and duck factors for association with duck seropositivity on H7 ELISA (n=962) †

Independent variable	***Category***	***OR****	***95% CI***	***p-value***	***Confidence limit ratio***
Visitors come to flock	No	ref			
	Yes	2.3	1.1, 5.0	0.03	4.6
Nightly pen location	Near field	3.3	1.5, 7.2	< 0.01	4.9
	Back to barn/ either	ref			

*Odds ratio is adjusted for flock membership by inclusion of flock as a cluster variable.
† The working correlation estimate for this model was 0.37.

In Thai smallholder flocks, biosecurity is often not practiced, and farm visitors are often other poultry owners or poultry-related traders. Such visitors have been identified as a risk factor for H5N1 outbreaks in Vietnamese smallholder poultry farms and in other countries (Henning et al., 2009b; Fasina et al., 2011). Facilitated by people moving among flocks, movement of fecal material and virus-containing debris is a known risk factor for influenza transmission (Webster et al., 2006; Halvorson, 2008). In our univariate analysis, flocks that sold eggs had a higher odds of seropositivity for influenza A (Table 2.1). Poultry traders onsite and the exchange of egg trays have been identified as risk factors for HPAI H5N1 infection (Biswas et al., 2009a; Desvaux et al., 2011). Contact between ducks and other poultry, which was associated on univariate analysis with influenza A seropositivity, has been previously identified as a risk factor for backyard chicken HPAI H5N1 outbreaks (Biswas et al., 2009b).

The final H5 multivariable GEE model indicates that 37% of the variance in duck H5 seropositivity is at the flock level (i.e. flocks must be in the right place at the right time for exposure). This differs from the flock-level variance identified in the NP ELISA analysis (0.4%). This finding may be due to a limited geographic distribution of H5 subtype viruses as compared

to influenza A viruses generally; however, we cannot ignore the potential role of transmission variation. HPAI H5N1 viruses are preferentially shed via the respiratory tract in ducks, while other influenza A viruses replicate primarily in gastrointestinal mucosa (Perkins and Swayne, 2002; Sturm-Ramirez et al., 2004).

Study Limitations

It is possible that there were unregistered FGD flocks in Suphanburi (and therefore not included in the study) who intentionally or unintentionally missed the registration call. In addition, flocks from other provinces could have been grazing in Suphanburi Province at the time of the study. These flocks would not have been registered in the province and thus not included in our study. The sensitivity and specificity of the H5 and H7 ELISAs could not be obtained from the manufacturer, and it is unknown how these attributes change with virus pathogenicity, subtype and time after infection. Several antigenic site sequences of the hemagglutinin (HA) gene, including that of the HA receptor binding site, differ between low and high pathogenicity isolates (Bosch et al., 1981; Saito et al., 1994; Senne et al., 1996). A controlled study of both the H5 and H7 ELISAs using serum obtained from experimentally-infected avian species is necessary to address these test characteristics. Two samples negative on the NP ELISA were positive on H5 ELISA, a finding perhaps due to imperfect sensitivity and specificity of the NP and H5 tests, respectively, or a longer persistence of antibodies to H5 antigens than NP antigens. This study is limited by the inability to conduct the H5 subtype assays on all serum samples. In addition, the subset of samples for H5 analysis was not selected randomly (see Methods), because the authors were interested in identifying the flock seroprevalence of H5 where possible.

FGD Surveillance

The large proportion of NP ELISA-positive samples that were H5 and H7-negative (52%) highlights how little we know about the many other LPAI subtypes that infect ducks in Central Thailand. Current regulations require that FGD owners register in the owner's home province and, after testing negative for HPAI H5N1 by cloacal swabbing, are given a flock passport and can graze within the province (Personal communication, Department of Livestock Development 2011). Pre-movement swabbing is required before leaving the province, but as flock movement often depends on rapidly fluctuating factors, including

field availability, feed supply, and flooding, FGD owners often leave their province on short notice and without testing (Beaudoin, unpublished data).

One approach for improved surveillance of these flocks could be cloacal and oropharyngeal sampling of FGD flocks *after* transport out of the province. If transport creates opportunities for within-flock influenza transmission and viral shedding, sample collection after 3 days (the approximate latent period) may provide a means to detect virus (Sturm-Ramirez et al., 2004; Tian et al., 2005; van der Goot et al., 2005; Bouma et al., 2009). Such a strategy may be accepted by flock owners for whom changing location when feed has been exhausted is imperative. This would also improve the knowledge of how many visiting FGD flocks are in a province at any time. Interviews with livestock officers indicate that because movements are rarely reported, the number of flocks in an area is often unknown (Beaudoin, unpublished data). While not ideal, identification of an HPAI H5N1-positive flock after transport is preferable to no diagnosis. Another method that would provide an opportunity to monitor flocks for HPAI H5N1, as well as generate data regarding LPAI viruses, would be sampling water from troughs that are used by potentially thousands of penned ducks each night. These containers present an environment for virus collection and persistence (Sturm-Ramirez et al., 2004; VanDalen et al., 2010). Additionally, as co-penning of ducks and chickens has been found to be a risk factor for backyard chicken outbreaks (Biswas et al., 2009b), one or more caged sentinel chickens in the night pen of FGD flocks may present a noninvasive and inexpensive way to monitor for subclinical virus shedding.

We have shown that some FGD in Central Thailand were exposed to H5 influenza viruses as recently as May 2010. This supports the hypothesis that H5 influenza viruses may circulate at low levels within FGD populations when there are no overt poultry outbreaks. Ongoing surveillance of FGD flocks, a population representing the intersection of wild birds and domestic poultry, is crucial to improving the knowledge of influenza viruses circulating in Thailand. Intensive antigen-based surveillance will also facilitate

the generation of comprehensive panels of viruses for use in diagnostic tests and improving laboratory capabilities.

Chapter 3: Seroprevalence of H5N1 antibodies among poultry-exposed persons in Central Thailand, 2008

Background

Thailand's first identified outbreak of highly pathogenic avian influenza (HPAI) H5N1 was in January of 2004 (World Health Organization, 2011d), followed by intermittent poultry outbreaks in 2005, 2006, 2007 and 2008. There have been 25 human cases of HPAI H5N1 reported in Thailand, including 17 fatalities (World Health Organization, 2011c). Direct contact with or recent exposure to sick or dead poultry have been identified as important risk factors for human infection (Areechokchai et al., 2006; Dinh et al., 2006; Zhou et al., 2009). While seroprevalence studies conducted in various affected countries have indicated that subclinical HPAI H5N1 infection is uncommon (for example, (Vong et al., 2006; Wang et al., 2006; Ortiz et al., 2007; Hinjoy et al., 2008; Dejpichai et al., 2009; Vong et al., 2009; Cavailler et al., 2010) , there is little seroprevalence data from Suphanburi Province, which had the highest cumulative number of outbreaks during Thailand's large second outbreak wave in 2004 and was home to nearly 50% of all infected duck flocks (Gilbert et al., 2006). The goal of this work was to investigate the seroprevalence of antibodies to HPAI H5N1 in Suphanburi poultry owners, assess these findings in light of demographic data, and poultry production, hand hygiene, and PPE practices, and follow up with seropositive persons to assess antibody persistence.

Methods

Study Location and Household Selection

Suphanburi Province was the location of the first HPAI H5N1 outbreak in 2004 and experienced recurrent poultry outbreaks and documented human cases throughout 2004 and 2005. The over 850,000 people in Suphanburi Province raised just under 10 million chickens and ducks in over 36,500 individual households or farms in 2010 (Thailand National Statistical Office, 2000; Department of Livestock Development Thailand, 2010). The two districts that experienced HPAI poultry outbreaks in 2005, Muang Suphanburi and U Thong, were selected for this study (Figure 3.1). These two districts had the highest number of outbreaks reported to the Department of Livestock Development in 2005, with Muang Suphanburi District reporting nine separate

outbreaks during the year and U Thong reporting ten outbreaks (Department of Livestock Development Thailand, 2007). Within each district, one subdistrict that had experienced two or more poultry outbreaks in 2005 and one subdistrict that had experienced only one H5N1 poultry outbreak were selected. In each district, the two subdistricts have a similar number of villages, and the populations are similar in size, density and agricultural occupations. Selection of the four subdistricts was dependent upon the cooperation of local health officers. The study subdistricts were as follows: Ban Pho (three reported outbreaks) and Sala Khao (one reported outbreak) Muang Suphanburi District, and Ban Don (two reported outbreaks) and Sa Yai Som (one reported outbreak) in U Thong District. Interviews and blood collection commenced in villages that experienced poultry outbreaks in 2005, and then nearby villages were visited until 1000 households were visited. In each village, all households with poultry were invited to participate.

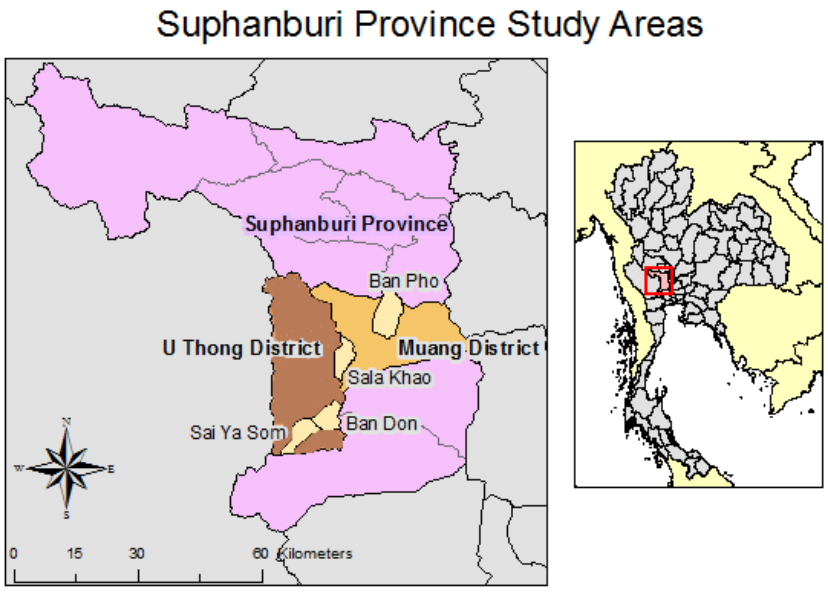

Figure 3. 1 Map showing the study area which includes two districts in Suphanburi Province, Thailand: Muang Suphanburi and U Thong. Within the two districts the study was conducted in four subdistricts: Ban Pho and Sala Khao in Muang Suphanburi District, and Ban Don and Sa Yai Som in U Thong District.

This work was part of a larger avian influenza study, for which surveys regarding poultry-raising and protective behaviors were conducted for 968 households in May-June, 2008. The surveys included retrospective questioning regarding the time of highest H5N1 activity (July-November, 2005) as well practices in 2008. In December of 2008, each participating household was revisited for blood collection from one household member. Where possible, the same person who answered the survey questions was identified for blood collection. Results from persons over 60 years of age were excluded from the analysis, as hemagglutination inhibition has been shown to lack specificity in this age group (Rowe et al., 1999). Blood was collected from 506 persons under 60 years of age, and 363 samples could be linked to surveys and were utilized for the statistical analyses presented here. An age and gender summary is provided for the full set of samples. A second blood sample was taken from the seropositive participants and an equivalent number of negative controls 28 months later (April, 2011). This study was approved by the Ethics Review Committee of the Thai Ministry of Public Health, the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Thailand, and the Institutional Review Board of the University of Minnesota.

Serologic Testing

All serum samples were processed at the Center of Excellence in Clinical Laboratory, Faculty of Medicine, Chulalongkorn University Faculty in Bangkok, Thailand. Testing was done in duplicate using hemagglutination inhibition (HI) to look for antibodies specific to HPAI H5N1. Serum specimens were treated with RDE (receptor destroying enzyme) produced by *Vibrio cholerae* Ogawa type 558 (Denka Seiken, Co. Ltd., Tokyo, Japan) to destroy nonspecific protein receptor, following the specifications of the manufacturer. Serum and RDE were mixed in a ratio of 1:3 and incubated at 37 °C for 18-20 hours, then incubated at 56 °C for 30 minutes to inactivate the RDE and complement system, followed by 10-fold dilution with phosphate-buffered saline (PBS). Two-fold serial dilutions of RDE-treated sera (25 µL) were incubated with eight HA units of avian influenza virus A/Thailand/NK165/2005 (H5N1) (25 µL) in each well of a V-shaped 96-well plate (Greiner Bio-One GmbH, Kremsmuenster, Austria) for 30 minutes, followed by addition of 50 µL of 0.5% chicken erythrocyte suspension and incubation at room temperature for 30 minutes. HI titers were reported as the reciprocal of the highest dilution of serum that inhibited virus-induced hemagglutination of the erythrocyte solution, and specimens

with duplicate HI titers of ≥1:160 were considered to be positive, as recommended by the World Health Organization (World Health Organization, 2007a).

Table 3. 1 Village-level results for H5N1 seropositivity

Village	Blood Samples	Number Seropositive
1	30	5 (16.7)
2	16	1 (6.3)
3	55	13 (23.6)
4	12	0
5	27	0
6	14	0
7	35	10 (28.6)
8	30	0
9	38	0
10	31	0
11	41	0
12	37	1 (2.7)
13	11	0
14	14	0
15	20	0
16	24	0
17	32	0
18	9	0
19	7	0
20	23	0
Total	506	30 (5.9)

Data Analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute, Inc.; Cary, NC). The survey responses from seropositive and seronegative participants were compared using chi-square analysis, Fisher's exact test (for expected cell values < 5) and Student's t-test. For all analyses, a p-value <0.05 was considered statistically significant. Multivariable analysis of seropositive and seronegative participants was conducted by generalized estimating equation (GEE) modeling, assuming an exchangeable working correlation matrix. Village was included as the clustering variable, and the working correlation matrix was estimated using compound

symmetry. The model was built by successively adding parameters with $p<0.25$ upon univariate analysis. Interaction between all significant main effect variables was evaluated. Model selection was based upon the significance of predictors ($p<0.05$).

Results

Demographic Characteristics and Serology Results

We obtained age and gender information with all blood samples, regardless of the ability to link the sample with a full survey. The mean age of the 506 participants was 44.4 years, with men (n=207) younger than women (n=299), at 42.1 and 46.0 years, respectively. This group may under-represent men, as the male:female ratio in Suphanburi reported in 2000 was 0.94:1, and is older than the population as a whole, which has a median age 32 years (Thailand National Statistical Office, 2000). Occupations of respondents included rice or other farming, livestock production, non-agricultural employment, housewife or currently unemployed. The major types of poultry owned by the interviewed participants were backyard chickens, fighting cocks, ducks, farmed chickens and grazing ducks. A small number of respondents reported owning doves, quail, pigeons and other bird types.

Thirty of 506 individuals (5.9%) were seropositive with an HI titer of ≥1:160. Of these positive samples, 20 had a titer of 1:160, 5 had a titer of 1:320 and 5 had a titer of 1:640. In addition, 219 individuals (43.3%) had a detectable but negative titer (≥1:10 and <1:160). The 30 seropositive individuals were located in five (25%) of the 20 study villages (Table 3.1).

Use of Hand Hygiene and PPE by Poultry Producers

Detailed information about the use of hand hygiene and PPE for this study population has been published elsewhere (Somrongthong et al., 2012), and can also be found in Chapter 4. Respondents reported a limited use of PPE (gloves and masks) and inconsistent hand washing practices after carrying poultry and gathering eggs. Hand washing was practiced by nearly 100% of respondents after cooking or slaughter activities. The use of PPE was infrequent by persons for daily poultry production tasks (i.e. carrying, egg gathering, slaughtering or cooking).

Univariate Analysis of H5N1 Seropositivity

Univariate analysis of demographic and poultry-related factors was conducted for individuals that had completed the survey themselves and provided a blood sample (n=363). The results can be seen in Table 3.2. Twenty three (6.3%) of the 363 individuals were seropositive. The mean age of the seropositive and seronegative participants was similar, at 43.3 years and 46.3 years, respectively (p=0.15, data not shown). Seropositive persons were more likely to be female, single, raise farmed chickens and own pigs. Although the number of respondents who raised farmed chickens was quite low (n=3), 66.7% of those individuals were seropositive for H5N1. While the odds of seropositivity was higher for single women than for single men, the Breslow Day test indicated that there was not effect modification by gender on the marital status-seropositivity association (p=0.71). Education, dog or cat ownership, having dead poultry in 2005, carrying dead poultry with bare hands, and use of hand hygiene, gloves and masks (data not shown) were factors not associated with seropositivity. Distance from water and the nearest market were also not associated with seropositivity (data not shown).

Multivariable Analysis of H5N1 Seropositivity

The final GEE model indicates that marital status and raising farmed chickens may be predictors of seropositivity in Thai poultry-owning households after controlling for similarities within villages (Table 3.3). Pig ownership was no longer significant after controlling for the other variables. While gender is not significant in the final model, exclusion of this parameter led to insignificance of marital status, so it was included on the basis that it may be a confounder of the marital status-seropositivity relationship. Raising farmed chickens is a predictor of seropositivity, and despite the small number of chicken farmers, the estimate of its effect is the most precise parameter in the model, with a confidence level ratio of 4.5 (Poole, 2001).

Follow-up Serology

Blood samples taken from the 30 seropositive individuals after a 28-month period yielded no antibody titers ≥1:160. One of the 30 individuals (3.3%), with an initial titer of 1:320, had a detectable but negative HI titer (1:40), and the rest of the samples were seronegative. Within the same villages, blood was also taken from age and gender-matched controls that had been

seronegative in 2008. All controls had undetectable titers except two individuals with titers of 1:80 (both were 1:40 in 2008).

Discussion

In this study we identified a low level of seropositivity (5.9%) in poultry owners with no known history of H5N1 infection living in HPAI H5N1 outbreak areas. There have been 16 studies since the emergence of HPAI H5N1 that have aimed to determine the frequency of seropositivity of persons exposed to poultry in outbreak situations (Katz et al., 1999; Bridges et al., 2002; Vong et al., 2006; Ortiz et al., 2007; Hinjoy et al., 2008; Lu et al., 2008; Cai et al., 2009; Dejpichai et al., 2009; Santhia et al., 2009; Schultsz et al., 2009; Vong et al., 2009; Wang et al., 2009a; Wang et al., 2009b; Cavailler et al., 2010; Robert et al., 2010; Khuntirat et al., 2011). These studies have been conducted in Hong Kong, mainland China, Cambodia, Nigeria, Thailand, Vietnam, Germany and Indonesia. A table summarizing these studies is provided in Appendix A. This body of literature indicates that the subclinical transmission of HPAI H5N1 to those working with poultry is infrequent. The highest measures of subclinical seroprevalence were both identified during the 1997-98 Hong Kong outbreak, with 10% seropositivity in poultry workers (Bridges et al., 2002) and 12% in household contacts of confirmed human cases (Katz et al., 1999). The seroprevalence that we present here is higher than most estimates from later seroprevalence studies, which range from 0% (for example, (Vong et al., 2006) to 5.6% (Khuntirat et al., 2011), using varied cutoff values for positivity (see more references above).

We identified single persons and those working with farmed chickens to be at an increased risk for seropositivity. Unmarried persons may perform more poultry-related tasks and at a higher frequently than those with a spouse (and perhaps children) with whom to share the work. Single persons in our study had a significantly lower household size than those married or divorced (3.8 and 4.7 persons, respectively, $p=0.01$), however we do not have data regarding household members' roles in poultry-related tasks. Farmed chickens are kept in larger numbers and at higher densities in closed spaces than backyard birds or grazing ducks. This may put those working with such birds at a greater risk for exposure to large amounts of virus in the air, on birds or on farm equipment. These serological findings indicate that persons working in farm settings should be given specific information regarding biosecurity, hand hygiene and PPE-use during public health education campaigns. While pig ownership did not remain significant in the

multivariable analysis, pigs are known to have receptors for avian influenza viruses and could be a source of infection during outbreaks (Nidom et al., 2010).

Table 3. 2 Results of univariate analysis of factors potentially associated with H5N1 seropositivity in poultry Thai owners, 2008 (n=363). Comparisons are made using chi-square, unless otherwise noted.

	Number seropositive (%)	Number seronegative (%)	Odds Ratio	95% CI	p-value
Number of questionnaire-linked serum samples	23 (6.3)	340 (93.7)			
Age					
< 20 years	1 (25)	3 (75)	ref		
20-29 years	2 (9.5)	19 (90.5)	0.32	0.02, 4.7	0.89
30-39 years	3 (5.4)	53 (94.6)	0.17	0.01, 2.2	0.34
40-49 years	9 (7.3)	115 (92.7)	0.24	0.02, 2.5	0.61
50-59 years	8 (5.1)	150 (94.9)	0.16	0.02, 1.7	0.16
Gender					
Male	5 (4.1)	117 (95.9)	ref		
Female	18 (7.5)	223 (92.5)	1.8	0.68, 5.2	0.21
Marital status					
Married/ divorced	17 (5.2)	311 (94.8)	ref		
Single	6 (17.1)	29 (82.9)	3.8	1.4, 10.3	0.02[£]
Education					
None	1 (6.7)	14 (93.3)	ref		
Grades 1-6	19 (6.3)	283 (93.7)	0.94	0.12, 7.5	0.62
Grades 7-12	2 (5.0)	38 (95.0)	0.74	0.06, 8.8	0.48
Bachelor's degree or beyond	1 (16.7)	5 (83.3)	2.8	0.15, 53.7	0.32
Type of poultry owned					
Backyard poultry	19 (6.7)	264 (93.3)	1.4	0.45, 4.1	0.18
Fighting cocks	1 (1.6)	62 (98.4)	0.2	0.03, 1.5	0.13[£]
Farmed chickens	2 (66.7)	1 (33.3)	32.3	2.8, 370.6	0.01[£]
Ducks	3 (5.8)	49 (94.2)	0.89	0.26, 3.1	1.0[£]
Grazing ducks	0	3 (100)			1.0
Pig ownership	8 (13.6)	51 (86.4)	3.0	1.2, 7.5	0.02[£]
Dog ownership	17 (7.0)	226 (93.0)	1.4	0.55, 3.7	0.46
Cat ownership	6 (4.6)	124 (95.4)	0.61	0.24, 1.6	0.31
Carry dead poultry with bare hands	7 (9.2)	69 (90.8)	1.7	0.68, 4.3	0.28[£]
Dead poultry in 2005	11 (5.3)	198 (94.7)	0.66	0.28, 1.5	0.33

Table 3. 3 Results of final generalized estimating equation multivariable analysis of factors potentially associated with H5N1 seropositivity of Thai poultry owners, 2008 (n=363). The final model's estimated working correlation was 0.18, indicating that, after controlling for independent parameters, 18% of the outcome variance of seropositivity occurs at the village level.

Independent parameter	Response	Number seropositive (%)	Number seronegative (%)	Estimate	Odds ratio	95% CI	Confidence level ratio	p-value
Intercept				-3.9				
Gender	Male	5 (4.1)	117 (95.9)		ref			
	Female	18 (7.5)	223 (92.5)	0.86	2.4	0.57, 9.9	17.3	0.24
Marital status	Married/ divorced	17 (5.2)	311 (94.8)		ref			
	Single	6 (17.1)	29 (82.9)	1.05	2.9	1.1, 7.34	6.7	0.03
Own farmed chickens	No	21 (5.8)	339 (94.2)		ref			
	Yes	2 (66.7)	1 (33.3)	2.5	12.7	6.0, 26.7	4.5	<0.001

It is thought that the human antibody response to HPAI H5N1 is similar to the response to human influenza A viruses; after the onset of clinical symptoms, neutralizing antibodies in humans infected with HPAI H5N1 have been detected at 14 days and are found to increase after 20 days (Katz et al., 1999). A study of confirmed human cases found that antibody titers may last up to 5 years (Kitphati et al., 2009). As there have been no reported cases of HPAI H5N1 in Suphanburi Province since November, 2005, titers of the participants may have waned over the 3 years before blood collection, contributing to the large proportion of detectable but negative titers (43%). Cross-reactivity among H5-specific antibodies and heterologous H5 antigens is also know to occur, yielding titers of varying intensity (Rowe et al., 1999; Ducatez et al., 2011). While there has been little variation in the genetic makeup of viruses isolated from Thai poultry (Amonsin et al., 2010), it is unknown how these genetic differences might affect human antibody response. Additionally, Khuntirat et al. identified an association between seropositivity to both H5N1 and a seasonal H1N1 (Khuntirat et al., 2011), and in vaccine studies in the United States, up to 3% of naïve persons had neutralizing antibodies to H5N1 on baseline evaluation (Treanor et al., 2006; Goji et al., 2008). These scenarios may have contributed to the large number of titers < 1:160.

This study is limited by two time lags. The first is the three-year period between the last recorded poultry infections in Suphanburi Province and the human serum collection, which could have led to recall bias regarding hygiene practices and poultry death in 2005. Also, the time between survey completion and serum collection inhibited our ability to obtain serum samples from the same household members who completed the survey and decreased the sample size for statistical analysis. In 2007, over 50% of cases were in persons younger than 40 years of age (World Health Organization, 2007c). While we did not find an age association with seropositivity, our study cohort consisted of only three individuals under 20 years of age.

The result of an exposure to avian influenza or any influenza virus is dependent upon multiple factors, including virus inoculum and antigenicity, exposure route, host adaptation of the virus and host immunity (Swayne, 2008). Our work has identified a higher seroprevalence that have most other studies. Based on the negativity of the 2008 follow-up titers for the 30 seropositive persons and the known ability of HPAI H5N1 antibodies to persist for up to five years (Kitphati et al., 2009), participants in this study likely became seropositive during the three large waves of outbreaks that affected Thailand in 2004 and 2005 (Tiensin et al., 2007). The first seroprevalence study in Thailand was conducted in May of 2004, just after the smaller first wave of 2004 (Hinjoy et al., 2008). This study did not identify any seropositive poultry farmers (n=322) in 5 provinces, including in Suphanburi Province, perhaps due to the short exposure period in 2004 (5 months). A second study (n=901) conducted in late 2005 also identified no evidence of antibodies to H5N1 (Dejpichai et al., 2009), and a more recent study in North Central Thailand found seroprevalence of up to 5.6% using a liberal ≥1:10 cutoff (Khuntirat et al., 2011).

The relatively high seroprevalence in our study may have to with differences in poultry population or environmental persistence of virus. In addition to having the most outbreaks in the second 2004 outbreak wave, Suphanburi Province was home to almost 50% of all duck outbreaks in the country (Gilbert et al., 2006). Central Thailand is home to many domestic duck flocks, including free-grazing ducks that are grazed on open rice fields and, as ducks can be infected without showing clinical signs, have been speculated to be potential reservoirs for HPAI H5N1 (Sturm-Ramirez et al., 2004; Sturm-Ramirez et al., 2005; Gilbert et al., 2006; Songserm et al., 2006; Chaichoune et al., 2009). Additionally, Suphanburi Province is well-irrigated to allow rice cropping year-round, supplementing the significant monsoon rains. In Vietnam, HPAI H5N1

seropositivity has been associated with swimming and bathing in community ponds (Vong et al., 2006; Vong et al., 2009). While these are not common activities in Suphanburi, these findings indicate that the presence of water may play a role in virus persistence and human exposure.

Another factor that may have contributed to the high seroprevalence in this study is the chicken erythrocyte method used for the HI assay. This method has been found to be less sensitive than HI with horse or turkey erythrocytes and is less sensitive and specific than microneutralization (Hinshaw et al., 1981; Profeta and Palladino, 1986; Beare and Webster, 1991; Rowe et al., 1999; Stephenson et al., 2003; Kayali et al., 2008). At the time that this paper is being written, all of the samples with an HI titer of $\geq$ 1:160 as well as a random sample of negative samples are being analyzed using microneutralization at the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University Faculty in Bangkok, Thailand. Laboratory results will be compared and risk factors for seropositivity reassessed when those results are available.

Continued serological monitoring of both humans and animals is necessary to identify subclinical poultry cases and human exposures. More frequent and broad seroepidemiologic investigations of non-affected humans in countries with poultry outbreaks are important to ensure that the generally low frequency of bird-to-human transmission does not increase with continued circulation and endemnicity of HPAI H5N1.

Chapter 4: Use of personal protective measures by Thai households in areas with avian influenza outbreaks

Introduction

Historically, influenza A viruses, including highly pathogenic viruses, have not been of veterinary or human health concern in Thailand. This changed significantly with the emergence of highly pathogenic avian influenza (HPAI) H5N1 in Asia. Thailand's earliest experience with HPAI H5N1 was in January of 2004 (World Health Organization, 2011d). Between January of 2004 and November of 2005, there were three large distinct waves of poultry outbreaks affecting 60 of the country's 76 provinces (Tiensin et al., 2005). Since that time, additional poultry outbreaks occurred in 2006, 2007 and 2008 (World Health Organization, 2011d). Twenty-five human cases of HPAI H5N1 avian influenza have been reported in Thailand, including 17 fatalities (World Health Organization, 2011c). Epidemiologic studies have documented that direct contact with or recent exposure to sick or dead poultry are important risk factors for human infection (Areechokchai et al., 2006; Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus, 2008).

Most of the country's poultry production is located in the Central and Eastern regions of Thailand (Tiensin et al., 2005). Poultry are raised in four production sectors: high-biosecurity closed systems, low biosecurity open houses, free-grazing and backyard, where poultry roam freely in human domestic settings (Tiensin et al., 2005; Songserm et al., 2006). Ninety percent of the country's poultry are produced in the most biosecure sectors, but these sectors include only 5% of flocks. The remaining flocks are raised in backyard conditions and have regular contact with people and other animals (Tiensin et al., 2007).

When H5N1 first emerged in Thailand, the Thai government initiated programs aimed at educating the general population about the risks of avian influenza. The village health volunteer system was vital to this endeavor as an early warning and surveillance system, with volunteers reporting unusual poultry deaths as well as human influenza-like illness (World Health Organization, Regional Office for South-East Asia, 2006; World Health Organization, 2007b). Several educational campaigns launched by ministries of the Thai government, including the

Ministries of Education and Public Health, were conducted in an effort to teach Thai children and their families about ways to protect themselves against avian influenza infection (UNICEF, 2006). These educational and risk communication efforts used by rural peoples have been evaluated (Olsen et al., 2005; Takeuchi MT, 2006; Maton et al., 2007). Maton *et al.* observed that those who received avian influenza educational materials had more knowledge on the disease and Olsen *et al*. noted that there was a barrier between imparting knowledge and changing high-risk behavior.

Our goal was to assess the poultry contact activities of households in Suphanburi Province and evaluate the use of protective measures by these households. Respondents were asked to recall practices used during the third wave of HPAI in 2005 (July-November), as well as to describe current (May- June, 2008) practices. This provided a mechanism to better understand the protective behaviors of poultry-owning households as well as to identify how these behaviors vary in areas with differing H5N1 experiences.

Methods

Study Location and Household Selection

Suphanburi Province, located in Central Thailand, was chosen for this study because of recurrent outbreaks of HPAI H5N1 in poultry and documented human cases in 2004-2005. According to the 2000 Thai Census, Suphanburi has a population of 855,900, who, in 2010, raised 9.5 million chickens and ducks in over 36,500 individual households or farms (Thailand National Statistical Office, 2000; Department of Livestock Development Thailand, 2010). Within Suphanburi, participating districts were chosen based upon their outbreak experience during 2005 (Department of Livestock Development Thailand, 2007). Of the eight districts that experienced poultry outbreaks, the two most affected districts, Muang Suphanburi and U Thong, were selected for this work (Figure 3.1). These two districts had the highest number of outbreaks reported to the Department of Livestock Development in 2005. Muang Suphanburi District reported nine outbreaks during the year, with over 282,000 total poultry deaths (died and culled), and U Thong reported ten outbreaks with 2,100 total poultry deaths. The Muang Suphanburi district outbreaks affected several large poultry farms, yielding the high numbers of poultry affected. Within each district, one subdistrict was identified that had two or more poultry outbreaks in 2005. A second

subdistrict with similar population size and density, number of villages and agricultural occupations that had experienced only one H5N1 poultry outbreak was also selected in each district. Selection of the four subdistricts was dependent upon the cooperation of local health officers. In Muang Suphanburi District, the two subdistricts included were Ban Pho (three reported outbreaks), and Sala Khao (one reported outbreak). In U Thong District, the subdistricts were Ban Don (two reported outbreaks) and Sa Yai Som (one reported outbreak). Household interviews began in the villages where the 2005 outbreaks were reported, and adjacent villages were visited with a goal of surveying 1000 households (in accordance with study budget). All poultry-owning households in each village were asked to participate in the study.

Household Selection and Survey Completion

From May through June 2008, a cross-sectional study of poultry-owning households in the selected villages was conducted. Eligible households owned poultry (backyard and/ or farmed) at the time of the 2005 H5N1 outbreaks in Suphanburi or at the time of interview, or held an occupation specifically involving poultry transport, slaughter or cooking. In each village, these households were identified with assistance from a local public health officer or village health volunteer. Survey questions were answered by the household member with the most frequent poultry contact, and participants were asked to answer questions regarding poultry production, hygiene and personal protective practices. Questions focused on practices utilized both in 2005 and at the time of the interview in 2008. Laboratory results to validate owner reports of poultry death from H5N1 were not available. Ten data collectors were trained to administer the surveys, which were conducted in person and lasted approximately 30 minutes. Verbal consent was obtained from each respondent. The study was approved by the Ethics Review Committee of the Thai Ministry of Public Health, the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Thailand, and the Institutional Review Board of the University of Minnesota.

Data Analysis

Data analysis was performed using SAS version 9.2 (SAS Institute, Inc.; Cary, NC). Comparisons of survey responses for different demographic groups and for 2005 and 2008 were made using chi-square analysis or Fisher's exact test, with a p-value <0.05 considered statistically

significant. Information regarding hand hygiene and PPE-use was obtained from participants in five-responses (never, hardly ever, sometimes, usually and always). For analysis of PPE-use, these responses were consolidated into two categories (never or hardly ever and sometimes, usually or always), and for analysis of hand washing, these responses were consolidated into three categories (never or hardly ever, sometimes, and usually or always). Poultry mortality in 2005 was calculated from the survey responses for flock size and number of poultry dead.

Results

Demographic Characteristics of the Study Population

A total of 968 households participated in the survey, representing 84% of poultry-raising households in the study area. Study subjects were from 20 villages within the four subdistricts. Household demographics are displayed in Table 4.1. Respondents had a median age of 55 years, and 44% were men. Suphanburi Province has an overall median age of 32 years and 48% men, based upon data from the most recent census, indicating that our study population was older than the general population in the area (Thailand National Statistical Office, 2000). The occupation most frequently reported was farmer, with 84% of farmers working in rice fields (data not shown).

Poultry Ownership and Activities

The most common type of poultry owned by the respondents in this study was backyard chickens (81%), followed by fighting cocks (16%) and backyard ducks (11%). A small number of respondents owned free-grazing ducks, quail or farmed chickens (Table 4.1). The majority of backyard chicken owners used an open housing system, where birds were allowed to roam and feed. Fifty percent of those respondents raising farmed chickens utilized closed housing systems, where the birds are penned at all times. Backyard duck owners used both open and closed types of housing, and fighting cocks were typically kept in individual cages, with or without netting around the cage. Between 2005 and 2008, the median flock size decreased by eight birds (Table 4.2).

For households owning chickens and ducks, there was no difference in the husbandry tasks performed by male and female participants. Both genders fed, carried, collected eggs,

slaughtered, and cooked. However, 53 of 108 men (49%), compared to 8 of 35 women (23%), reported cleaning the mucous secretions of their fighting cocks in 2008 ($p < 0.01$).

Most respondents reported current use of measures to prevent transmission to humans or other poultry when birds are found dead. In 70% of households, sick or dead poultry are separated from the rest of the flock, and in nearly 60% of households, people are kept from the area containing sick birds until inspected by livestock officials. Bare-handed transport of dead poultry was reported by 28%, while cooking and selling birds found dead are actions reported by only a small number of respondents (< 2%).

Poultry Deaths in 2005

Of the 836 households owning poultry in 2005, 694 (67%) reported increased mortality. Of the 695 households raising backyard chickens in 2005, 68% reported abnormal deaths during July through November of 2005, and this finding was similar in all subdistricts ($p = 0.06$). Among those backyard flocks, the median mortality was 100% (interquartile range, IQR, 48-100). The median mortality rate for larger chicken farms was 41% (IQR, 8.0-100).

Table 4. 1 Participant demographic and poultry production information. Categorical data are given by N (%), and continuous data by median (interquartile range).

Number of participants	968
Median age, years	55 (44, 67)
Age range (years)	10-93
Gender, Male	427 (44%)
Mean persons in household	3.98
Education level	
None	107 (11%)
Grades 1-6	752 (78%)
Grades 7-12	97 (10%)
Bachelor's degree or beyond	12 (1.2%)
Accommodation type	
Home	958 (99%)
Farm/ other	10 (1%)
Occupation	
Unemployed	170 (18%)
Farmer	416 (43%)
Livestock producer	21 (2.1%)
Housewife	11 (1.1%)
Employee	276 (29%)
Other	74 (7.6%)
Median monthly income, baht	5,000 (3,000, 8,333)
Poultry activities	
Production	
Backyard chickens	781 (81%)
Farmed chickens	9 (0.9%)
Grazing ducks	7 (0.7%)
Quail	1 (0.1%)
Fighting cocks	159 (16%)
Backyard ducks	111 (11%)
Transport, purchase, sale/ slaughter	5 (0.5%)
Cooking outside the home	6 (0.6%)

Table 4. 2 Summary of households owning poultry in 2005 and 2008. SEM is standard error of the mean.

Households with poultry, 2005	836 (86%)
Flock size of poultry owners, 2005	
Mean (SEM)	233 (102)
Median	40
Interquartile range	20-70
Households with poultry, 2008	861 (89%)
Flock size of poultry owners, 2008	
Mean (SEM)	210 (140)
Median	22
Interquartile range	1-40
Change in flock size, 2005 to 2008	
Mean (SEM)	-22.34 (49)
Median	-8

Use of Hand Hygiene and PPE by Poultry Producers

The reported hand hygiene practices for the 2005 and 2008 outbreak periods are presented in Table 4.3. This table shows hand washing frequency for backyard chicken and fighting cock owners after activities involving poultry contact (i.e. carrying, gathering eggs, slaughtering, cooking or cleaning mucous secretions). Hand washing was practiced "usually" or "always" in nearly all households participating in slaughter and cooking activities in 2005 and 2008. Hand washing was reported to be less frequent in both time periods after carrying poultry and gathering eggs, despite the direct contact with poultry or contact with poultry feces that is associated with these activities. There was no statistical difference between the 2005 and 2008 hand washing practices of backyard chicken owners or fighting cock owners for any contact activity (Table 4.3). When analyzed on a individual respondent basis, 17% of people owning chickens, ducks or fighting cocks in both 2005 and 2008 reported an increase in hand washing frequency from 2005 to 2008 when carrying birds, 14% when gathering eggs and cleaning mucous discharge and 3% when slaughtering and cooking (data not shown). The positive change associated with slaughtering and cooking was small because such a large proportion of

respondents already had reported good hand hygiene habits during these activities. There were a small number of respondents who decreased their frequency of hand-washing from 2005 to 2008.

Table 4. 3 Hand washing by households with backyard chickens, ducks and fighting cocks. In 2005 there were 695 households with backyard chickens, and 131 with fighting cocks. The sample sizes given in each column indicate the number of households practicing that poultry-related activity. In 2008, there were 691 households with backyard chickens and 143 with fighting cocks.

Hand Washing Frequency	Households with backyard chickens				Households with fighting cocks		
	Carrying	Gathering eggs	Slaughtering	Cooking	Carrying	Slaughtering	Cleaning mucous secretions
2005	n= 182	n=30	n=153	n=187	n=93	n=30	n=55
Never/ hardly ever	29 (16%)	11 (37%)	2 (1.3%)	5 (2.7%)	7 (7.5%)	0	2 (3.6%)
Sometimes	47 (26%)	7 (23%)	5 (3.3%)	8 (4.3%)	35 (38%)	1 (3.3%)	5 (9.1%)
Usually/ always	106 (58%)	12 (40%)	146 (95%)	174 (93%)	51 (55%)	29 (97%)	48 (87%)
2008	n=161	n=10	n=135	n=177	n=102	n=32	n=61
Never/ hardly ever	26 (16%)	3 (30%)	1 (0.7%)	4 (2.3%)	8 (7.8%)	0	1 (1.6%)
Sometimes	36 (22%)	0	0	6 (3.4%)	27 (27%)	0	3 (4.9%)
Usually/ always	99 (62%)	7 (70%)	134 (99%)	167 (94%)	67 (66%)	32 (100%)	57 (93%)
Comparison 2005-08*	p = 0.77	p = 0.18	p = 0.08	p = 0.89	p = 0.23	p = 0.48	p = 0.52

*Fisher's exact test

The use of masks and gloves at any time or frequency was low, with reported use appearing to decline in 2008 (Table 4.4). Due to the small number of respondents who used the protective measures, glove and mask-use is presented as a two-level frequency over all poultry activities. The percentages in the table indicate the use of gloves and masks "sometimes, usually

or always" by respondents raising backyard chickens, ducks and fighting cocks. There was no statistical difference between the 2005 and 2008 glove and mask-use, except for a decrease in frequency of mask use among duck owners (p=0.03). It must be noted that the number of respondents using masks and gloves is so small that these comparisons are imprecise and of questionably utility. Neither hand washing nor PPE practices differed between respondents who did and did not report high poultry mortality in 2005 (data not shown).

Table 4. 4 PPE-use by households. Values indicate percentage of respondents reporting use "sometimes, usually or always". Households with backyard chickens in 2005, n=695 and in 2008, n= 691. Households with backyard ducks in 2005, n=56 and in 2008, n= 97. Households with fighting cocks in 2005, n=131 and in 2008, n= 143.

Protective measure	Households with backyard chickens	Households with backyard ducks	Households with fighting cocks
Glove-use			
2005	15 (2.2%)	5 (8.9%)	5 (3.1%)
2008	6 (0.87%)	1 (1.0%)	2 (1.3%)
2005 vs. 2008*	p = 0.08	p = 0.03	p = 0.67
Mask-use			
2005	14 (2.0%)	7 (13%)	3 (1.9%)
2008	12 (1.7%)	4 (4.1%)	2 (1.3%)
2005 vs. 2008*	p = 0.84	p = 0.10	p = 0.67

*Fisher's exact test

Other Poultry Occupations

The household survey included ten respondents (1.0%) who were currently involved in poultry purchase, transport or slaughter (five respondents) or poultry cooking activities outside of the household (six respondents). Respondents did not report purchasing birds from farms with abnormal poultry deaths, although one individual reported selling birds with signs of avian influenza. Only two of the individuals involved in poultry transport reported cleaning the cages and transport equipment daily or after each job. Two respondents clean the slaughter area daily, and two change their clothes after working. Of the six individuals who cook outside of the home, three clean eggs before use, four individuals utilize separate cutting boards for raw and cooked

meat, and five always wash their hands after cooking. The other individual reported "hardly ever" washing her hands.

Discussion

Major findings from this survey include: 1) a limited use of PPE (gloves and masks); and 2) inconsistent hand washing practices after carrying poultry and gathering eggs. The use of PPE was infrequent by persons for daily poultry production tasks (i.e. carrying, egg gathering, slaughtering or cooking). Respondents routinely reported high-contact activities such as slaughtering and cooking poultry and cleaning the mucous discharge of fighting cocks, as well as other activities that entail exposure to potentially contaminated materials, such as gathering eggs and moving fecal material. Some participants reported other types of contact, such as carrying dead birds with bare hands and cooking or selling dead birds. Follow-up focus groups by the authors revealed that unfamiliarity, discomfort, apathy and poor availability were the major barriers to glove and mask-use by backyard poultry owners (Somrongthong et al., 2010). Barriers to use should be assessed for individual areas affected with HPAI, and public health initiatives should focus on potential alternatives to gloves and masks as well as acknowledge the most common reasons that people have for noncompliance. One alternative to gloves mentioned frequently during the follow-up focus groups in Suphanburi Province was the use of plastic bags, which, when selected properly for integrity, could provide an available, inexpensive and effective way to pick up dead poultry and keep it contained. The self-perceived risk to persons in this population is incompletely understood, but some focus group participants considered their risk in 2008 to be low and related to the frequency of poultry contact (unpublished data). In addition, because outbreaks in Thailand have been seasonal, some participants thought that their risk varied with season (unpublished data; (Chaichoune et al., 2009).

The findings of minimal PPE-use by those potentially exposed to HPAI H5N1 are not unique to this study, though this is the first time that such use has been quantified for a large study group. Other efforts have been made to assess the use of gloves and masks in Thailand and other countries where HPAI H5N1 has circulated, with analysis indicating minimal use of PPE by poultry owners (Maton et al., 2007; Fatiregun and Saani, 2008; Van Kerkhove et al., 2008). Similar investigations of PPE-use by those involved in avian influenza outbreak situations show that noncompliance is not unique to H5N1 outbreaks, Asia or rural persons (Skowronski et al.,

2007; Cai et al., 2009). A study of those involved in outbreak control during the 2004 H7N3 outbreak in British Columbia, Canada revealed that compliance with PPE recommendations was inconsistent, and though the outbreak lasted only three months (as compared to years of H5N1 circulation in some Asian countries), approximately 15% of the participants reported not wearing gloves and masks at all times when entering a barn with infected birds (Skowronski et al., 2007).

It would perhaps be easier to encourage poultry owners, production employees and outbreak respondents to utilize equipment such as masks, gloves and protective eyewear if there was a better understanding of the efficacy of these products. Unfortunately, the efficacy of these items is difficult to assess even in a controlled environment such as a healthcare facility. One recent randomized trial of surgical masks and N95 respirators found no difference between the two in protection against human seasonal influenza; however, the respiratory equipment was worn only in the workplace (Loeb et al., 2009). The participants were potentially exposed to influenza viruses outside of the healthcare facility, making true interpretation of the study results difficult (Finkelstein et al., 2010). This concept of part-time protection against a potentially full-time exposure is relevant to those with household poultry in H5N1-affected areas.

While this study focused on eliciting information about poultry husbandry activities, those living in close proximity with poultry are exposed on a daily basis to materials and objects through indirect contact. This may include exposure to fecal material, feathers in the yard and home, as well as blood and gastrointestinal residues in slaughter areas. A recent investigation of reported cases of human HPAI H5N1 infection indicates that, in addition to direct poultry contact, indirect exposure to influenza virus is a potential risk factor (Rabinowitz et al., 2010). Poultry owners should be made aware that certain species (ducks and geese most commonly) are known to shed virus subclinically and could contaminate the environment without visible illness (Shortridge et al., 1998; Perkins and Swayne, 2002). Specific recommendations on the use of PPE need to be practical, accepted by the at-risk groups, and economical.

In a limited number of situations, a decrease in the frequency of protective behaviors was evident from 2005 to 2008. This could be a result of a decreased perception of risk, an increase in apathy or a decreased availability of soap, masks and gloves. Message fatigue is an ongoing problem for public health officials. Hence it is important to identify key interventions to protect farmers and re-emphasize those messages. This likely includes providing ongoing reminders of

the importance of hand washing. It is more difficult to encourage the use of masks and gloves for routine tasks which may be considered neither economical nor practical.

This study may be limited by recall bias resulting from asking participants to remember practices used three years prior to survey completion. Although respondents were asked about 2005, the number of poultry deaths reported may be more generalized and encompass experiences since the first emergence of HPAI in 2004. This is supported by the similarity of poultry death reports by respondents in all four subdistricts, despite of the number of 2005 outbreaks reported by the Department of Livestock Development (as seen in the Methods section). For our purposes of assessing protective measures, this differentiation is likely of little importance. In addition, only the person with the most poultry contact from each household was interviewed. This provides little insight into the poultry-associated roles of other household members, especially children. This research has, however, identified specific issues for rural Thai people with regard to poultry practices and PPE, setting the stage for targeted educational efforts. The hygiene practices and poultry-associated activities of rural households described in this study help to identify trends in protective behaviors. However, the actual risk to these individuals and families is still incompletely understood. These data would be enhanced by comparing work activities to individual serologic data. In summary, we were able to document the use of personal protective equipment and hand hygiene by rural poultry owners while conducting activities involving poultry contact and the change in these practices over time. These data are important for ongoing characterization of risk and crafting of educational messages.

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Chapter 5: Using agent-based modeling of the local poultry sector in Thailand to describe and simulate inter-flock contacts and transmission of HPAI H5N1

Background

Highly Pathogenic Avian Influenza H5N1

Since 2003, highly pathogenic avian influenza virus H5N1 infection has adversely affected poultry production and both human and avian health in many Asian, European and African nations. HPAI H5N1 has caused the death or culling of hundreds of millions of birds, and, as of April 12, 2012, there have been 602 human cases of H5N1 with 355 deaths (59% case fatality) (World Health Organization, 2012). Thailand confirmed its first outbreaks of highly pathogenic avian influenza H5N1 in early 2004 and last reported cases in 2008 (World Organisation for Animal Health, 2009).

While it is well understood that wild birds, particularly ducks and shorebirds, act as a natural reservoir for influenza A viruses, outbreaks of influenza in and transmission among domestic poultry holdings are thought to be largely facilitated by the movement of poultry and humans as well as the transport of equipment, vehicles and other vectors (Swayne, 2008). The production of poultry in domestic environments has led to sustained transmission and adaptation of avian influenza viruses in non-reservoir host species. Further, raising poultry in facilities with poor or no biosecurity measures (e.g. open and outdoor systems) drives the introduction, adaptation and maintenance of avian influenza viruses in domestic populations and presents opportunity for spreading of viruses among poultry establishments (Swayne, 2007; Swayne, 2008). Accordingly, an understanding of poultry-related contacts and situations that may lead to virus exposure is important in the estimation of risk for individual farms and the broader poultry sector.

Poultry Production in Thailand

As discussed in Chapter 1, poultry in Thailand are raised in four types of production systems (Table 1.2): Sector 1, where poultry live in high-biosecurity closed systems, with all aspects of production and transport conducted internally (vertical integration); Sector 2, contract

farms, in which poultry are in closed facilities with basic physical barriers; Sector 3, characterized by low biosecurity open houses with or without netting where poultry may be allowed outside of the housing (includes free-grazing duck flocks); and Sector 4, the backyard or village setting, where poultry roam freely in human domestic settings (Tiensin et al., 2005; Songserm et al., 2006; Food and Agriculture Organization, 2011a). Each of these sector types, as well as individual poultry sites within each, utilizes different contacts and frequencies for animal purchase and sale, feed purchase, and animal product distribution. As a result, the number of human and animal contacts experienced within each system varies.

In Thailand, households typically have backyard flocks of up to 50 native chickens, most with hens as well as roosters that may be used for cock fighting. These backyard flocks are free to roam around the yard and frequently will roam amongst backyard poultry from other households. While low-biosecurity open houses (Sector 3) also have minimal biosecurity, the birds are partially protected from contact with wild birds and outside persons by cages inside open barns (chickens) or open barns and adjacent pen around swimming area (ducks). These Thai poultry production systems may differ in their day-to-day business routines, however it is likely that they share similar poultry sources, export locations, traders and transport vehicles. In addition, in situations with limited biosecurity, human interactions connect all aspects of the local poultry sector.

At the start of 2004, 10-11 million free-grazing ducks (FGD) were raised in Thailand, where ducks are transported daily among post-harvest rice fields to feed freely on residual rice, insects and snails (Gilbert et al., 2006; Songserm et al., 2006; Gilbert et al., 2007). The FGD system provides a logical focal point for investigation of local poultry sector dynamics in rural Thailand. In this system, a major influenza reservoir species is managed using frequent movements with no incorporated biosecurity. In addition to being a natural reservoir for influenza A viruses, ducks have been identified as a biologic “bridging species” for influenza virus transmission between wild waterfowl and gallinaceous poultry (e.g. chickens) (Swayne and Slemons, 2008). Domestic ducks have been shown to be capable of shedding HPAI H5N1 asymptomatically (Chen et al., 2004; Li et al., 2004; Hulse-Post et al., 2005) and studies have found a strong spatial association between highly pathogenic avian influenza outbreaks and the density of free-grazing ducks (Gilbert et al., 2006). Despite these findings, the details of the

relationship between FGD flocks and local poultry outbreaks are unclear, and their role in initiating and maintaining transmission has yet to be determined.

Agent-Based Modeling of Infectious Disease Transmission

Agent-based models (ABM) combine theory and computation to produce a computerized artificial society (Gilbert, 2008), providing a dynamic platform for exploring the role of contacts in the transmission of infectious disease. Because ABM are simulation models, agents can represent anything the model designer desires, from individual people or animals, households or farms to cells or molecules. These individual agents are specifically represented in the model and interact with each other and the model environment. Castle notes that there is not a single way to define the "agent" component of an ABM, but the traits of autonomy, heterogeneity, activity, mobility and learning are shared by most agents (Castle and Crooks, 2006). The ability of agents to interact sets agent-based modeling apart from other types of computational modeling (Gilbert, 2008). The fate of each agent can be tracked and observed, as can any interactions that they have. In addition to observing the specific fate of individual model components, agent-based modeling facilitates the observation of population-level (macro) patterns that emerge from the occurrence of agent-level (micro) activities (Castle and Crooks, 2006). Software used for agent-based modeling allows for the linkage of geospatial data with a simulation modeling system so that, when appropriate data is in-hand, the ABM can be specific in a spatial context. Agent-based modeling has been utilized in ecology, economics, social science and the biological sciences. This method has been used to investigate transmission of human seasonal and pandemic influenza (e.g. (Lee et al., 2008; Carpenter and Sattenspiel, 2009; Lee et al., 2010; Yang et al., 2011).

The objective of the work presented in this chapter was to build an ABM of the local smallholder poultry sector in Central Thailand, use the model to assess the types and frequencies of contacts among sector members, and simulate the transmission of HPAI H5N1 throughout the poultry sector after introduction of the virus by one initially-infected FGD flock.

Methods

Data collection

Poultry-sector contact data was obtained through 61 in-depth interviews and 12 shorter phone interviews with FGD owners, backyard poultry owners, poultry traders, slaughterhouse

workers and livestock officers. The majority (62%) of these interviews were conducted with residents of Don Kam Yan Subdistrict (DKY), Muang District in Suphanburi Province, Thailand. The rest of the interviews were conducted with people in neighboring subdistricts of Muang District (34%) or in an adjacent district (4.1%) in Suphanburi Province. Suphanburi was the first Thai province affected by HPAI in early 2004, and its poultry experienced multiple isolated outbreaks throughout 2004-2005 (World Organisation for Animal Health, 2004). DKY was chosen as the primary study location because of the varied poultry types and farm sizes as well as the willingness of the subdistrict livestock officer to facilitate flock identification and enrollment. The flock census for DKY at the time of data collection was provided by the subdistrict livestock officer and can be seen in Appendix C. The proportion of the flocks in the subdistrict that were interviewed is shown in Table 5.1. While we only interviewed 5% of the backyard flock population in the subdistrict, we interviewed more FGD flock owners (n=9) than the six that were on the recent livestock census. Outside of DKY, we interviewed 15 FGD owners and one layer chicken farm owner. We conducted seven interviews with people that trade live poultry, seven with people that trade duck and/or chicken eggs, one with the manager of a duck egg cooperative and five with people running small slaughterhouses.

Table 5. 1 Summary of poultry owner interviews in Don Kam Yan Subdistrict (DKY) used to inform model parameters

Flock Type	No. in DKY, Summer 2011 (flock census)	Number Interviewed, DKY (% of census)
Free-grazing duck flocks	6	9 (150%)
Backyard chicken flocks	260	13 (5%)
Fighting cocks	300*	7 (2.3%)
Non-grazing duck flocks	81	10 (12%)
Layer chicken flocks	5	4 (80%)
Quail farms	3	1 (33%)

* Estimate, not a count, of the number of households that own fighting cocks in DKY, from the subdistrict livestock officer

Seven general categories of data were collected from the participants (Table 5.2). Respondents were asked to provide numerical estimates of frequency for each type of contact that they reported. Where possible, the average, minimum and maximum contact frequency was obtained, as well as the number of potential sources of each type of contact. In addition to providing quantitative data, the participants were encouraged to discuss at length their poultry management, transport, sale and purchase practices. Summaries of the in-depth interview results, grouped into poultry flock type or trader type can be found in Appendix D.

Table 5. 2 Poultry sector data collected during in-depth interviews

Parameter	Description
Role in poultry sector	Flock owners: free-grazing ducks, farmed chickens, farmed ducks, farmed quail, backyard poultry, fighting cocks Traders: live bird traders, egg traders
Contact types and number	Flock owner contacts with egg and poultry traders Flock owner feed purchase contacts Cock fighting contacts Social contacts (visits) with poultry-owning neighbors, friends, relatives
Contact frequencies	Frequency of interactions between participants and their contacts
Contact location	On or off-site (e.g. market, neighbor's home)
Movement locations	Destinations of flocks, flock-owners, traders
Movement frequency	Frequency of flock, flock-owner, trader movement
On-farm characteristics	Holding type: Closed barn, open barn, open pen, field-grazing, free-roaming

Model-Building and Data Analysis Software

We modeled the local poultry sector using NetLogo 4.1.2 (Wilensky, 1999), organized data generated from model simulations using Microsoft Excel and analyzed model output using SAS 9.2 (SAS Corporation, Cary, NC). NetLogo is free, open access software for developing agent-based models. It is written in Logo language and is fully programmable. In NetLogo, the model world (in our case, a geographic background representing a Thai subdistrict) is a grid made up of individual patches, each which can not only be the physical location of model agent(s) but

can also hold its own attributes. In our model, such attributes include a level of contamination (i.e. by influenza-infected poultry) and state of rice production (e.g. growing or harvested). Patches are unable to move and are located in the model world with permanent (x, y) coordinates. Agents are able to move over this patch background, carrying their own sets of attributes, rules for action and response to events in the model world, and autonomy with regard to all other agents in the model. The NetLogo programming for the baseline model and screenshots of the model world and user interface page can be seen in Appendix G.

Creation of the Model World

The agent-based model was created so as to be representative of DKY with regard to land area represented, road structure, and flock number and types. The model world (background) is 60 x 60 patches and was informed by GIS land-cover obtained from the Royal Thai Survey Department (RTSD) in Bangkok, Thailand. The proportions of rice field land area and non-rice field land area in Don Kam Yan Subdistrict were determined to be 73% and 27%, respectively. These proportions were similar over the area of Muang District, as the region is characterized by intensive rice production. For this model, 70% of the model space was designated as rice field area (referred to as field patches) and 30% as non-field area, approximating the GIS estimate. The road structure for the model was based on the road structure observed in the DKY GIS data. A GIS layer was made that included all roads (excluding excluding railroads), and the number of intersections in the subdistrict and the number of roads of each type were recorded. The road types were defined by the RTSD as hard surface two lane with median (road type A), hard surface two lane (road type B), loose surface one lane (road type C), and nonspecific road (road type D). The intersect tool in Arc Toolbox was used to identify all intersections, and an intersection type was attributed based type of intersecting roads. The road system used in the model world was informed by this data, and represents the type and frequency of intersections and roads in DKY.

In the model world, households, fighting cock practice arenas, a fighting cock match arena, markets and an egg cooperative (collectively referred to as non-field patches) are distributed around the road structure at the start of each simulation. The approximate proportion of residences and commercial spaces located on each road type was estimated subjectively by examination of Google Earth photos of DKY Subdistrict. Before each simulation, 60% of non-field patches are randomly placed on patches around road type B, 30% around road type C, and

10% around road type D. No non-field patches are placed around the model's type A road, as it is a highway.

Because the model was established using interview, census and geographic data in large part from one subdistrict in Central Thailand, it is not generalizable to all poultry sectors affected by HPAI H5N1, including other areas of Thailand that may have different poultry populations, FGD grazing practices, poultry marketing practices and social interactions. One major difference between the modeled sector and those in other South East Asian countries is the lack of live bird markets in the poultry market system. Such markets have been well-established as a risk factor for HPAI H5N1 infection and persistence in poultry (Chen et al., 2006; Sims, 2007). Additionally, the model presented here does not include any poultry holdings that are in Sectors 1 or 2, as DKY has few such establishments, and they, by design, have minimal interaction with the local smallholder sector.

Poultry Sector Agents

The model agent groups are as follows: FGD flocks (n=9), FGD owners (n=9), backyard chicken flocks (n=253), backyard chicken owners (n=253), fighting cock owners (n=200, subset of backyard chicken owners), backyard duck flocks (n=80), backyard duck owners (n=80), large layer chicken farms (n=9), layer chicken farm owners (n=9), large layer duck farms (n=3), layer duck farm owners (n=3), live poultry traders (n=3) and egg traders (model-generated count).

The number of agents included in the model is based upon the census of poultry flocks in DKY Subdistrict conducted by the subdistrict livestock officer in the summer of 2011. There were more FGD flocks owned by persons living in the subdistrict at the time of our interviews than were thought to be present by the flock counts. As mentioned in Chapter 2, livestock officers at the district level estimated that there may be up to 29% more flocks in the district than are registered. We interviewed all known flocks in the subdistrict at the time of the study (nine), and included as many flocks in the model.

Closed broiler flocks or meat duck flocks (Sectors 1 and 2) were not included in this model, because according to the subdistrict livestock officer, there is only one large meat chicken flock in DKY, and upon meeting with the owner of this flock, we found that he is a contract farmer, and the young chickens, feed, medical support are provided directly by the overhead

company, the barn is a moderately-high biosecurity building with no other employees, and at 45 days of age, the chickens are picked up by the overhead company and transported directly to slaughter at their own facility. Most large broiler farms in Thailand are operated in a similar fashion, and, if proper biosecurity is maintained, the interaction of the flock with other aspects of the poultry sector may be infrequent.

Model Overview

This model simulates 1) human interactions that occur in the Thai local poultry sector using stochastic agent-based modeling techniques; and 2) transmission of avian influenza throughout the simulated poultry sector model occurs by susceptible, latent, infectious, recovered (SEIR) state transition. Initiation of the SEIR transition for any agent in the simulation occurs in a probabilistic framework. The model proceeds in time steps equal to one day.

Figure 5.1 (A) displays the model components and relationships among them. Eggs are collected by traders from FGD flocks, large layer flocks and small duck flocks. The eggs are brought by the traders to egg markets and, in some scenarios, an egg cooperative. It is uncommon for backyard chicken owners to sell chicken eggs (Appendix D), so these agents are not included in the egg trade aspect of the model. Live poultry is purchased and picked up by traders from small duck flocks and backyard chicken flocks in their vicinity, and the live poultry are brought back to the home of the trader for slaughter. This is consistent with the activity of local poultry slaughterhouses in DKY. FGD flocks are brought to the fields for grazing, and fighting cocks are brought to practice and match arenas. Roads traveled by FGD flocks provide an indirect contact between FGD and flocks along the road route. Flock owners of all types conduct visits at the homes of nearby agents.

Figure 5.1 (B) shows the SEIR influenza transmission framework. When contacts occur that have infectious potential (more on this below), the probability of infection ($\Pr(\text{inf})_e$) is determined by sampling from a probability distribution specific for that contact exposure type *e*. Thus, for each individual agent in the model, the risk of becoming infected via one type of infectious contact in a given time step *t* is given by (Vynnycky and White, 2010),

$$\text{Risk}(t) = 1 - (1 - \Pr(\text{inf})_e)^{\text{M}_e(t)} \quad (1)$$

where $M_e(t)$ is the number of contacts of type *e* experienced in time *t*. If there are multiple contact types (e.g. contact type *x* and contact type *y*) that can lead to infection, the agent's risk of becoming infected in time step *t* is given by:

$$\text{Risk}(t) = 1 - (1 - \Pr(\text{inf})_x)^{M_x(t)} (1 - \Pr(\text{inf})_y)^{M_y(t)} \quad (2)$$

Thus, the more contacts an agent makes in the simulation with potentially infectious agents, the more difficult it will be to avoid infection.

Provision of rules to the agents in an ABM is a key component of programming. In this model, the rules dictate agent movement as well as the type, number and frequency of interactions among agents. Rule sets are specific for each agent type, although some of the rules included in the sets are the same for multiple agents (Appendix E). Rules that are similar among agent types often are parameterized differently. During each time step agents carry out actions in the following order: egg traders collect eggs and bring them to market, FGD flocks move to field, poultry owners deliver eggs to market, live poultry traders pick up poultry and bring to their homes for slaughter, fighting cocks go to practice or match arena (depending on the day of the week), FGD move back home and poultry owners make visits to neighbors. After these actions are carried out, exposed poultry become infected based on the probabilities determined at their exposure, and the SEIR model transitions through one time step, changing the disease state of infected poultry accordingly. At the end of the day, the supply of feed in each rice field is adjusted based on the amount consumed by the flock during the day, and the contamination state of roads and traders is reassessed based on the parameterized duration of contamination.

Although FGD may be walked, trucked or both in the course of daily movement to and from the fields, in this model we make the assumption that the ducks are walked, and the same probability distribution dictating the risk of road contamination is used for all flocks the model.

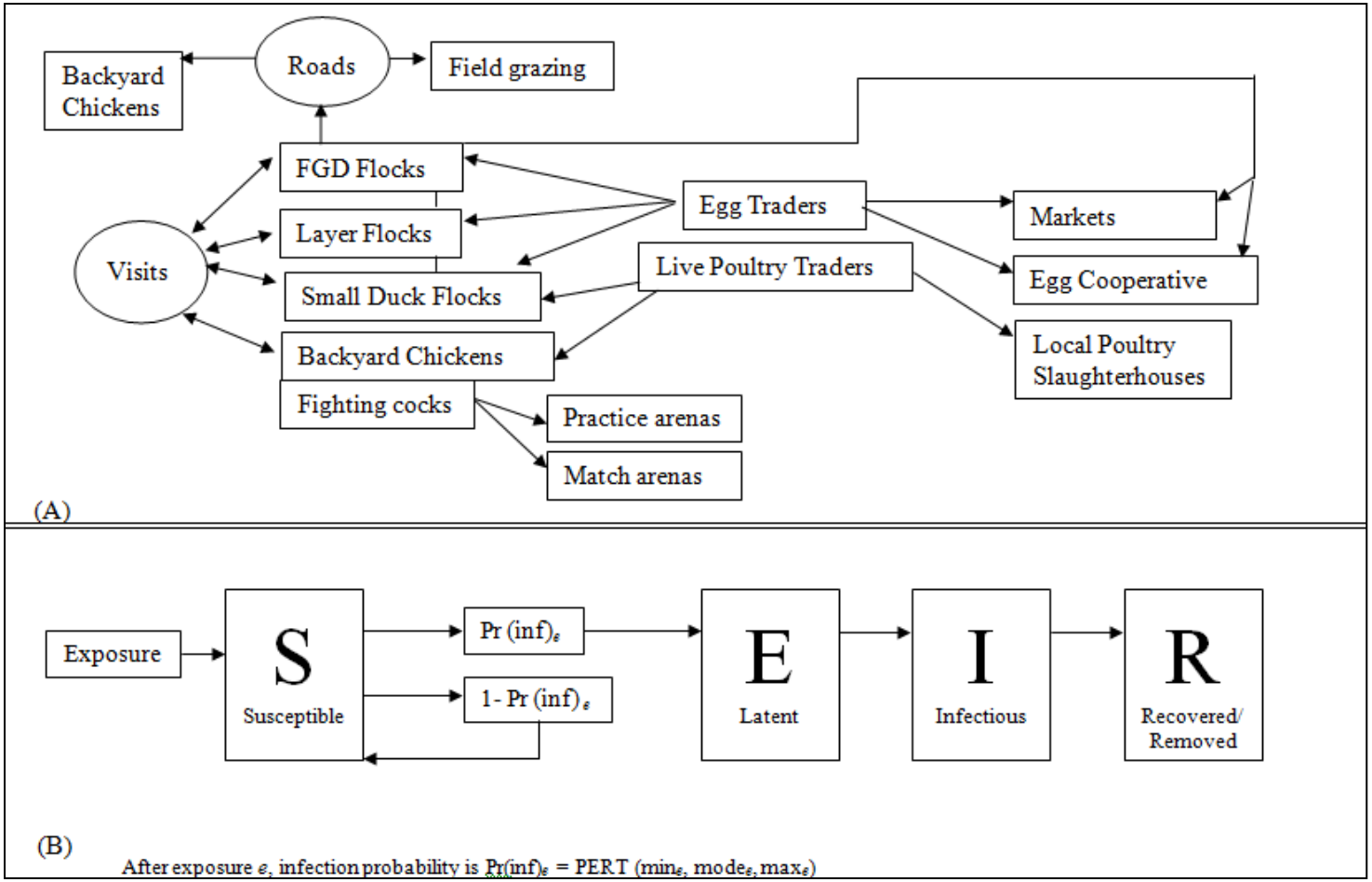


Figure 5. 1 (A) Diagram displaying the connections among members of the local Thai poultry sector. (B) SEIR influenza transmission framework, through which individual flocks in the ABM transition. Flocks moved from the susceptible (S) to latent (E) pool after a contact exposure (*e*) that leads to influenza transmission with an exposure-specific probability ($Pr(inf)_e$). Once latently infected, flocks transition into the infectious and recovered pools based on the incubation and infectious periods, respectively.

Model Parameters

Model parameters were developed from the interview responses, expert opinions and existing literature (Table 5.3). Contact frequencies for each agent type were determined by generating summary responses from the in-depth and phone interviews. Where the data were limited, or when possible responses included a small number of integers, discrete distributions were made to describe the data specifically. For other parameters, PERT distributions were generated from the minimum, maximum and most likely interview responses. In one case, a binomial distribution was used.

Discrete and PERT distributions are empirical non-parametric distributions, where the mathematics of the distribution are defined by the required shape (Van Hauwermeiren and Vose, 2009). The PERT distribution is a version of the Beta distribution and is a continuous univariate distribution that can take any value within the range defined by the minimum (min), maximum (max) and most likely (mode) values. PERT distributions are defined by the following probability density function, seen in equation set (3). They are commonly used for modeling estimates provided through expert opinion (Van Hauwermeiren and Vose, 2009).

$$f(x) = \frac{(x-\min)^{\alpha_1-1}(\max-x)^{\alpha_2-1}}{B(\alpha_1,\alpha_2)(\max-\min)^{\alpha_1+\alpha_2-1}} \qquad (3)$$

where $\alpha_1 = 6\left[\frac{\mu-\min}{\max-\min}\right]$ $\alpha_2 = 6\left[\frac{\max-\mu}{\max-\min}\right]$

with $\mu(=\text{mean}) = \frac{\min+4\text{mode}+\max}{6}$

and $B(\alpha_1,\alpha_2)$ is a Beta function

Discrete distributions are defined by the possible parameter values and the probability weights for each possible value, as seen in equation (4) (Van Hauwermeiren and Vose, 2009). The probability mass function for discrete distributions is shown in equation (5).

$$\text{Discrete}\ (\{x_i\}\{p_i\}) \qquad (4)$$

$$f(x_i) = p_i \quad (5)$$

The binomial distribution, discrete and parametric, is defined by the number of trials and the probability of a success, as in equation (6) (Van Hauwermeiren and Vose, 2009). The probability mass function is described in equation (7).

$$B(n, p) \quad (6)$$

$$f(x) = \left(\frac{n}{x}\right) p^x (1-p)^{n-x} \quad (7)$$

An average rice field holding in Central Thailand is 3 hectares, or approximately 20 rai. People in the interviews reported staying in one place for 4-60 days, sometimes more, but those staying for longer periods tend to graze just around their homes, allowing the ducks to wander year-round (data not shown). This model includes a discrete distribution to inform rice field size. Single patches represent fields of various sizes, serving as the access point for what, in real life, would be many coalescing fields used as a single grazing site over time. This distribution has a most likely value of 150 rai and ranges from a minimum of 20 rai (the average size of one holding) to a maximum of 610 rai, which could have the potential to feed one 2,000-duck flock for two months. Based on the 70% of land cover that is rice cultivation in the subdistrict modeled, the total amount of rice field-covered area represented in the model space is approximately 6552 rai. We have represented all of the rice field grazing areas using 40 patches, a number determined by the mean of the probability distribution function for patch size. If a model simulation continues so long that the FGD flocks have consumed all of the rice in the model space, thereby giving them no new target to move to, the flocks "move out" of the model subdistrict as if to graze at rice fields in other subdistricts. There is no source of influenza infection for these flocks when they are outside of the model space.

Elicitation of Expert Opinion

To inform the probabilities for agent contamination with virus and transmission of influenza to other agents, opinions were elicited from three experts in the field of avian influenza. The experts consulted have different experiences and interests with regard to the field of avian

influenza. David Castellan, DVM, MPVM, DACPV, DACVPM is a Senior Veterinary Epidemiologist at the Emergency Center for Transboundary Animal Diseases at the Food and Agriculture Organization Regional Office for Asia and the Pacific in Bangkok, Thailand. Dr. Castellan has experience with poultry systems, biosecurity and disease in the United States and South East Asia. David Halvorson, DVM is a Professor at the University of Minnesota College of Veterinary Medicine in Saint Paul, MN. In the 1980s, Dr. Halvorson played a key role in establishing an understanding of avian influenza in free-living duck populations and transmission of influenza between free-living and domestic avian species. During his career, Dr. Halvorson has worked with domestic poultry facilities to improve biosecurity and has conducted research to better understand the movement of influenza virus among poultry holdings. David Swayne, DVM, PhD, DACPV, DACVP is Laboratory Director at the Southeast Poultry Research Laboratory at the Agricultural Research Service of the United States Department of Agriculture in Athens, GA. Dr. Swayne's research interests include transmission of avian influenza viruses among poultry and their molecular characterization. He is the editor of the comprehensive text, Avian Influenza (Swayne, 2008).

Table 5. 3 Model parameters used in baseline model scenario

Agent Type	Parameter	Value	Distribution (if no value)	Distribution Characteristics*	Data Source	NetLogo Parameter Name†
FGD Flocks	Egg pickup days/ week	-	Discrete	({1, 2, 3, 7}, {0.07, 0.64, 0.2, 0.1})	Interview data	pickup-days
	Number of onsite traders	-	Discrete	({1, 2, 3, 4}, {0.51, 0.38, 0.1, 0.01})	Interview data	onsite-trader-num
	Rice field area consumed/ duck/ day (rai)	-	PERT	0.005	FGD surveys (Chapter 2)	rai-per-duck
	Proportion moving between home and field	0.75	-	-	FGD surveys (Chapter 2)	prop-fgd-barntofield
	Number initially infected	1	-	-	Model assumption	num-fgd-inf
FGD Owners	Egg delivery days/ week	-	Discrete	({1, 2, 3, 7}, {0.09, 0.52, 0.17 ,0.22})	Interview data	delivery-days
Penned ducks	Egg pickup days/ week	-	Discrete	({1, 2, 3, 7}, {0.07, 0.64, 0.2, 0.1})	Interview data	pickup-days
	Number of onsite traders	1	-		Interview data	onsite-trader-num
	Probability of infection by contaminated road, Pr(inf)	-	PERT	(0, 25, 40)	Expert opinion, modeler selection	road-infect-pen-prob
Penned duck owners	Egg delivery days/ week	-	Discrete	({1, 2, 3, 7}, {0.09, 0.52, 0.17, 0.22})	Interview data	delivery-days

* Discrete distributions are described by [values] [probability weight for each value]; PERT distributions are described by (minimum, most likely, maximum) values; binomial distribution described by (number of trials, probability of success)

†In NetLogo, parameters may have the same name among multiple agent types, but the parameter values are specific for each

Table 5. 3 (cont.) Model parameters used in baseline model scenario

Agent Type	Parameter	Value	Distribution (if no value)	Distribution Characteristics*	Data Source	NetLogo Parameter Name†
Egg farms	Egg pickup days/ week	-	Discrete	({2, 3, 7}, {0.33, 0.33, 0.33})	Interview data	pickup-days
	Number of onsite traders	-	Discrete	({1, 2, 3, 4, 5, 6, 7, 8}, {0.22, 0.36, 0.22, 0.12, 0.05, 0.02, 0.01, 0.001})	Interview data	onsite-trader-num
	Probability of infection by contaminated road, Pr(inf)	-	PERT	(0, 25, 40)	Expert opinion, modeler selection	road-infect-pen-prob
Egg farm owners	Egg delivery days/ week	-	Discrete	({2, 3, 7}, {0.33, 0.33, 0.33})	Interview data	delivery-days
Backyard chickens	Probability of infection by contaminated road, Pr(inf)	-	PERT	(29,55,71)	Expert opinion	road-infect-prob
	Probability of infection by infectious cock returning home, Pr(inf)	-	PERT	(67, 83, 100)	Expert opinion	fc-bkyd-infect-prob
Backyard chicken owners	Probability of fighting cock becoming infectious at arena with infectious cock, Pr(inf)	-	PERT	(38, 48, 57)	Expert opinion	fc-infect-prob
All poultry owners	Visits made/ day	-	PERT	(0, 1, 10)	Interview data	random-visit-int
	Visit days/ week	-	Binomial	(7, 0.42857)	Interview data	n-visit-days-per-week

* Discrete distributions are described by [values] [probability weight for each value]; PERT distributions are described by (minimum, most likely, maximum) values; binomial distribution described by (number of trials, probability of success)

†In NetLogo, parameters may have the same name among multiple agent types, but the parameter values are specific for each

Table 5. 3 (cont.) Model parameters used in baseline model scenario

Agent Type	Parameter	Value	Distribution (if no value)	Distribution Characteristics*	Data Source	NetLogo Parameter Name†
All poultry owners	Probability of being contaminated during visit to infected premises, then infect own flock	-	PERT	(5, 15, 30)	Expert opinion (2 of 3 estimates)	from-visit-infect-prob
	Probability of being contaminated by infectious own flock, then infecting another during visit	-	PERT	(10, 20, 40)	Expert opinion	to-visit-infect-prob
Egg traders	Number of egg traders in model, median (range)	25 (14-40)	-		Model-generated	-
	Number of suppliers for pickup	-	PERT	(1, 1, 10)	Interview data	eggtrader-links
	Probability of contamination at infectious location, Pr(contam)	-	PERT	(53, 70, 88)	Expert opinion	flock-eggtrader-contam-prob
	Probability of infecting new flock while contaminated, Pr(inf)	-	PERT	(55, 70, 88)	Expert opinion	eggtrader-flock-infect-prob
Live poultry traders	Number of pickup days/ week	-	Discrete	({1, 2, 3}, {0.33, 0.33, 0.33})	Interview data	n-bird-pickup-days
	Number flocks/ pickup day	4	-		Interview data	-
	Probability of contamination at infectious location, Pr(contam)	-	PERT	(68, 83, 95)	Expert opinion	live-trader-contam-prob

* Discrete distributions are described by [values] [probability weight for each value]; PERT distributions are described by (minimum, most likely, maximum) values; binomial distribution described by (number of trials, probability of success)

†In NetLogo, parameters may have the same name among multiple agent types, but the parameter values are specific for each

Table 5. 3 (cont.) Model parameters used in baseline model scenario

Agent Type	Parameter	Value	Distribution (if no value)	Distribution Characteristics*	Data Source	NetLogo Parameter Name†
Live poultry traders	Probability of infecting new flock while contaminated, Pr(inf)	-	PERT	(68, 82, 92)	Expert opinion	live-trader-infect-prob
All flock agents	Latent period (days)	1	-		Expert opinion, (Das et al., 2008)	latent-period
All chicken agents	Infectious period (days)	4	-		Expert opinion, (Li et al., 2008; Bouma et al., 2009; Jeong et al., 2009; Forrest et al., 2010; Kwon and Swayne, 2010)	shedding-period-chicken
All duck agents	Infectious period (days)	7	-		Expert opinion, (Shortridge et al., 1998; Perkins and Swayne, 2002; Sturm-Ramirez et al., 2004; Hulse-Post et al., 2005; Li et al., 2008; Kwon and Swayne, 2010)	shedding-period-duck
Road patches	Contamination period (days)	1	-		(Beard et al., 1984)	road-contam-period
Rice field patches	Contamination period (days)	30	-		Unpublished data, (Brown et al., 2007b)	field-contam-period
All traders	Contamination period (days)	1	-		(Beard et al., 1984)	trader-contam-period

* Discrete distributions are described by [values] [probability weight for each value]; PERT distributions are described by (minimum, most likely, maximum) values; binomial distribution described by (number of trials, probability of success)

†In NetLogo, parameters may have the same name among multiple agent types, but the parameter values are specific for each

The three expert opinion interviews were conducted separately and without disclosure of the other participants' responses. Table F.1 in Appendix F shows the eight contamination or transmission categories for which opinions were elicited. Where appropriate, the scenarios were disaggregated into direct and indirect transmission components. This practice is known to lead to better accuracy of estimates by obtaining estimates for several comprehensible contributory scenarios, thereby decreasing the overall uncertainty associated with making one more complex estimate (Vose, 2000; Clemen and Winkler, 2007). Disaggregation also helps the expert identify dependencies in the components (Vose Software, 2007). For example, the experts were requested to give estimates for the probability of infection of a flock after direct contact with a virus-contaminated poultry trader and after indirect contact with the trader (i.e. resulting from deposition of contaminated material from the trader's clothes or vehicle into the yard or barn of the flock). The experts were asked to provide a maximum, minimum and most likely (MMML) probability estimate, in that order. Identification of the maximum and a minimum first helps to prevent anchoring of the estimate, which may occur if the most likely value is provided first (Vose, 2000; Vose Software, 2007). In this type of bias, the expert remains "anchored" to their most likely value, and the adjustments to that value to generate the maximum and minimum may not be sufficient to incorporate all the values that should be in the range.

Once the MMML estimates were obtained for each transmission or contamination scenario, a PERT probability distribution was generated for each. Each expert was sent a graphical display of the probability distributions and with a summary of his MMML estimates for review. The experts were given the option of altering their estimates after seeing the distributions graphically, although none wished to do so.

The final estimates can be seen in Table 5.3. One expert made the suggestion that in considering the probability of transmission from an infectious FGD flock moving on the roads to backyard chickens in adjacent yards, consideration of whether the ducks are walking or being trucked would make a big difference in his estimate. As mentioned above, we have made the assumption that the mode of transport will be walking in the model at this time. We recorded the expert's additional estimate for future model scenarios. Additionally, one expert thought it pertinent to provide separate estimates for the probability of transmission from an infectious fighting cock to a susceptible fighting cock when in direct and in indirect contact situations. Our

model assumes direct contact between the cocks, so this additional estimate, too, was recorded for potential future use.

Aggregation of Expert Opinions

There are many different ways to combine opinions from multiple experts, and they are generally classified into mathematical and behavioral approaches (Clemen and Winkler, 2007). The method that is used may depend on the desired "quality" of the response (e.g. high calibration on a large scale vs. diagnosticity of individual events) (Rantilla and Budescu, 1999). Mathematical modalities include simple averaging, weighted linear combination, and complicated modeling techniques, including those with Bayesian frameworks. Behavioral approaches are based on interaction among the experts and arrival at an agreed upon aggregate. Comparison studies show that complicated mathematical models do not outperform simple models, and it is generally understood that simpler aggregation methods, such as averaging, perform as well or better than complex methods (Rantilla and Budescu, 1999; Clemen and Winkler, 2007). The use of multiple opinions can yield a more reliable estimate, and the distribution generated from an averaging of multiple opinions counteracts the overconfidence of experts by yielding a distribution that is broader than any of the component distributions (Troffaes, 2006; Clemen and Winkler, 2007). Because basic combination does perform well, it is intuitive that the inclusion of multiple expert opinions or information sources will improve the estimation, but the effect has been found to taper off at approximately three experts for some types of estimations (Rantilla and Budescu, 1999). Sensitivity analysis can be conducted to assess variation in the outcome distribution based on the method of aggregation (Clemen and Winkler, 2007).

The direct and indirect estimates for each expert were aggregated to get one estimate that includes the possibility of both direct and indirect contact for each of the eight scenarios. The three expert opinions were then aggregated into one probability distribution for each contamination or transmission scenario (Table 5.3). The resulting aggregate distributions were used in the model to inform the probability of contamination or infection upon the corresponding agent interaction. In two cases, one expert's estimates were very different from the other two experts (both visit-associated transmission probabilities) (Table F.1). In these cases, the average estimate from the two experts in close agreement was used (Table 5.3).

Model Verification

Verification, or checking that the model behaves as expected, was done by writing the code for the model in small increments (e.g. for one interaction type at a time) and testing the outcome over multiple parameter values before moving on to the next piece of code. The NetLogo Command Center was used to print out specific values during each of these tests, and in some cases monitors were generated on the user interface page to provide a direct understanding of the effect of the new code on individual agents or agent types. Multiple simulations were run after each code addition to ensure the absence of errors. In addition, code debugging was carried out throughout the model-building process.

Simulation

For each scenario, data were obtained from 100-500 simulations. Median outbreak duration, day of peak poultry infection, number of flocks infected, and proportion of each transmission type (i.e. road-to-flock, visitor-to-flock, flock-to-visitor, egg trader-to-flock and poultry trader-to-flock) were determined for each set of simulations. The average day of peak infection was determined by extracting the maximum poultry infection day from each simulation and averaging over all 500 simulations. Results are given in most cases as the median and the 95% probability interval (PI), which is a range that contains 95% of the outcome values.

The baseline scenario with the deterministic and stochastic components seen in Table 5.3 was simulated 500 times to inform the analytic technique for which to assess other model scenarios. The results of the simulations were averaged and the variation over the simulations assessed to determine whether running each subsequent scenario over 500 simulations would achieve any benefit over running 100 simulations. The variation over 500 simulations was not less than that over 100 simulations (data not shown), and thus, the smaller number of 100 replicates was used for the subsequent simulations. The model variation among simulations was large, as will be seen below. In the baseline scenario, it was assumed that the only source of influenza infection is the initially infected flock.

Sensitivity analysis is a way to establish the influence of model components and to assess the conceptual validity of the model (Reeves et al., 2011). Individually, selected stochastic parameters were set to their minimum or maximum values, and selected deterministic parameters were altered from their baseline value while the rest of the parameters were held at their baseline values to determine the effect of the parameter on the model outcome. Comparisons were made

between the baseline scenario and each scenario by qualitative assessment of the median and 95% probability intervals (PI) of the major output values (Vynnycky and White, 2010). Conceptual validity, or verifying that the model behaves as it would be expected to in the real world, was also described using these model outcomes (Reeves et al., 2011). Where conducted, distribution fitting of model output was conducted with @Risk (Palisade Corp.; Ithaca, NY).

Hypothesis Testing

Several scenarios with policy implications were evaluated, also changing one model parameter at a time. The hypotheses tested were as follows: 1) If all FGD flocks are kept on the rice field where they are grazing both day and night (eliminating daily road travel), the duration and number of flocks involved in the outbreak will be lower than in the baseline model; 2) If all eggs from FGD flocks are delivered by the owners to a centralized egg cooperative, the duration and extent of the outbreak will be mitigated; and 3) The number of egg pickup locations serviced by egg traders in one day is directly related to the extent of the outbreak, and limiting traders to egg pickup from only one location per day will alleviate the outbreak duration and spread.

Estimation of the Reproduction Number

The inter-flock reproduction ratio (R_f) is the average number of flock infections caused by one infectious flock. General reasoning allows one to infer from this definition that when $R > 1$, an outbreak can be sustained and when it is < 1, an outbreak dies out. R_f can be calculated if one knows that average number of new flock infections caused by each infected flock during one time step (transmission coefficient, β) and the average infectious period duration (T) (e.g. Mannelli et al., 2007). Another way to define β is as the rate of effective contacts (contacts that result in transmission) per capita (Vynnycky and White, 2010), which allows us to understand that β is actually composed of two parts, the probability of transmission upon contact and the proportion of susceptible and infectious individuals.

R_f was calculated for the baseline model using two methods, referred to here as the direct SIR method and the GLM method. The direct SIR method was described by Stegeman *et al* (Stegeman et al., 1999). This method has been used by others to calculate R_f from H5N1 outbreak data (Ward et al., 2009). Our estimation was conducted within the SEIR model framework, with β calculated from the number of susceptible (S), newly infected (latent) (E), infectious (I) and total flocks (N) that are present within the same time step. For both methods used to calculate β and R_f, we assume that all flocks are infected by other flocks in the model (and not an outside

source), and that each susceptible flock has equal probability of infection and each infectious flock has an equal probability of causing infection (β is constant and there is random mixing). While we know that our model violates the complete random mixing assumptions, there are likely a sufficient number of random components in the contact structure that the transmission in our model may approximate that of a randomly mixing population (Watts and Strogatz, 1998).

The direct SIR method is based upon the frequency-dependent state transmission principle that the number of newly infected flocks in each time step is dictated by the transmission coefficient and the proportion of infectious and susceptible flocks. Thus, in each step:

$$E_t = \beta_t * \frac{S_t I_t}{N} \qquad (8)$$

Once β was calculated for every outbreak day in the simulation, the average β was calculated, and the following equation used to calculate R_f, where T is the duration of infection.

$$R_f = \beta * T \qquad (9)$$

Because the duration of infection differs for chickens (4 days) and ducks (7 days) (Table 5.3), R_f was calculated for the average shedding time (5.5 days) and for the shedding time of chickens (4 days). The values of R_f calculated for each outbreak simulation were then averaged to obtain the final reproductive number estimate for the model scenario.

The GLM method used here was described by Becker (Becker, 1989) and as has been utilized for veterinary infectious disease transmission studies, including between-flock transmission of foot and mouth disease (Bouma et al., 2003) and avian influenza (Stegeman et al., 2004), and within-flock transmission of avian influenza (e.g. van der Goot et al., 2003; van der Goot et al., 2005; van der Goot et al., 2007) and swine influenza (Romagosa et al., 2011). Based on what we know from equation (8), the probability of a susceptible flock becoming infected in time *t* is represented by:

$$P_{\mathrm{E}}(t) = 1 - e^{-\beta \frac{I(t)}{N}} \qquad (10)$$

The number of new infections in time *t* is represented by a binomial distribution (Stegeman et al., 2004):

$$\mathrm{E}(t) \sim \mathrm{Bin}\left[S(t), 1 - e^{-\beta \frac{I(t)}{N}} \right] \qquad (11)$$

The transmission coefficient (β) was estimated using a GLM with a complementary log-log link (as the dependent parameter was binomially distributed) and an offset parameter of ln[I(*t*)/N], as seen in equation (12).

$$\ln\left[-\ln(1 - e^{-\beta \frac{I(t)}{N}}) \right] = \ln(\beta_0) + \ln\left(\frac{I(t)}{N} \right) \qquad (12)$$

The intercept parameter estimate ($\ln(\beta_0)$) is used to calculate the transmission coefficient, as:

$$\beta = 1 - e^{-e^{\beta_0}} \qquad (11)$$

Once β was calculated for every outbreak day in the simulation, the average β was calculated, and equation (9) was used again to calculate R_f. One assumption of this method that we violate is the independence of each observation (i.e. each time step) and the equal susceptibility of a flock from one period to the next (Velthuis et al., 2007).

Spatial Analysis

In an effort to determine whether the outbreaks resulted in clustering of cases within the subdistrict, a local estimate of spatial clustering, Kuldorff's spatial scan statistic (Kulldorff and Nagarwalla, 1995), was applied to a simulation dataset of case and non-case coordinates. Local spatial statistics look for spatial dependence individually in small sections of the study area. After completion of the 500 baseline model simulations and assessment of the transmission characteristics of the scenario, a list of individual simulations that best-represented the summary model characteristics with regard to outbreak duration, number of flocks infected and transmission types, was developed (data not shown), and four scenarios were selected at random for spatial and temporal analysis. SaTScan version 9.1 (www.satscan.org) was used to look for evidence of local clustering with high rates of infected premises. Circular scan windows with a maximum spatial cluster size of 50% of the population were used as well as a Bernoulli model to compare the number of cases and controls in each scan window and determine if the groups of cases in each window are more than would be expected by chance. Secondary clusters (with no geographic overlap with the primary cluster) were also identified.

Results

Initial FGD Infection (Baseline) Scenario

The results of 500 simulations of the baseline scenario, where one FGD flock was initially infected, can be seen in Table 5.4. Outbreak duration was a median of 49 days (95% PI 8, 108), with a maximum outbreak period of 132 days (Figures 5.2 and 5.7). Figure 5.2 shows a spike in the histogram at 5-10 days. The best-fitting distribution for the simulated outbreak duration can be seen in Figure 5.7 In twenty simulations (4%) there was no transmission of influenza from the initially infected FGD flock to any other flock in the model (data not shown). The median day of peak infection was 15.5 (95% PI 0, 69). The median number of flocks infected in this baseline scenario was 86 (95% PI 1, 239), and 69% of the transmissions were a result of neighbor-to-neighbor visiting. Twenty-three percent of the transmissions were caused by FGD flocks contaminating roadways and adjacent yards, and fewer were caused by egg traders (5%) and live poultry traders (1.6%). Figure 5.3 shows the epidemic curve for the baseline model, comprised of the median number of flocks infected at each time step.

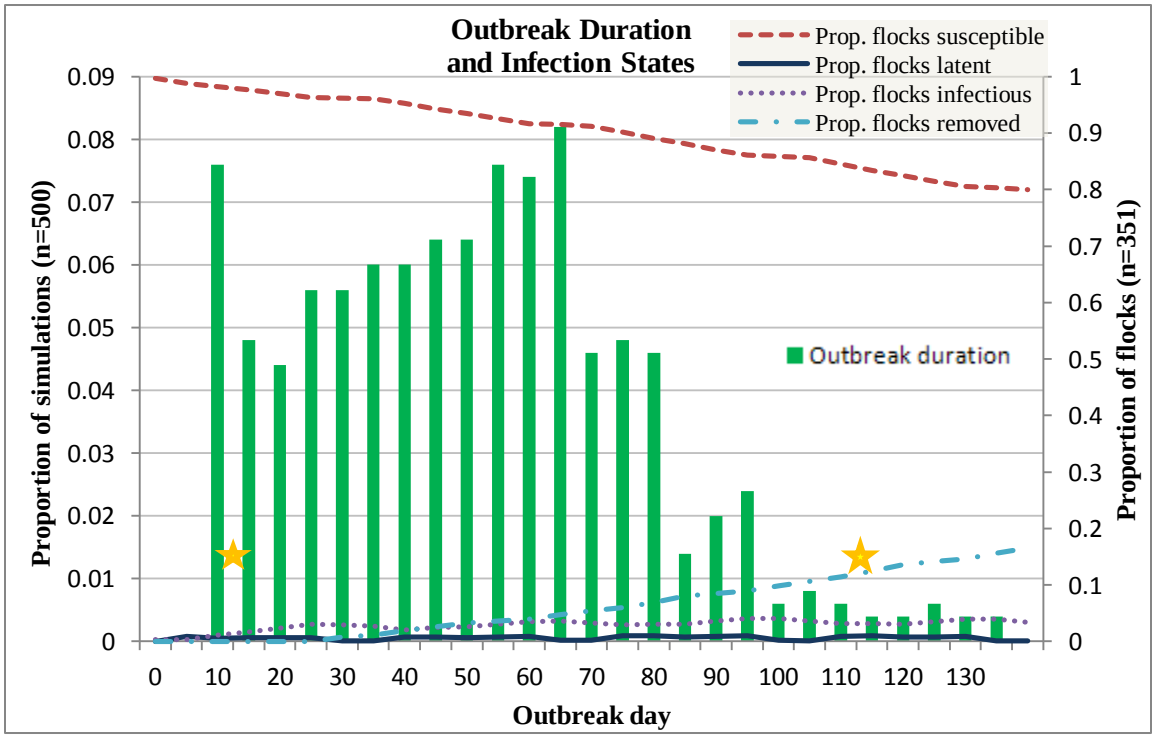


Figure 5. 2 Frequency histogram displaying the outbreak duration over 500 model simulations. Stars indicate 95% PI.

Table 5. 4 Summary of baseline model results, compiled over 500 simulations using the parameters seen in Table 5.3.

Model Component	Median (median % of transmissions)	95% Probability Interval
Outbreak dynamics		
Outbreak duration in days (range)	49 (8 – 132)	8, 108
Day of peak poultry infection	15.5	0, 69
Infected flocks		
No. flocks infected at peak	23	1, 55
Total no. flocks infected (%)	86 (25%)	1, 239
No. FGD flocks infected (% FGD)	3 (33%)	1, 7
No. backyard flocks infected (% backyard)	60 (24%)	0, 171
No. penned ducks infected (% penned ducks)	21 (26%)	0, 56
No. layer farms infected (% layer farms)	3 (33%)	0, 8
Transmission Routes		
No. road-to-flock transmissions (%)*	15 (21%)	0, 76
No. transmissions due to contamination while visiting off-site (%)*	15 (18%)	0, 51
No. transmissions caused by contaminated visitor (%)*	43.5 (53%)	0, 132
No. egg trader-facilitated transmissions (%)*	4 (4.5%)	0, 15
No. live poultry trader-facilitated transmissions (%)*	0	0, 8

* Percent of transmissions (n=85)

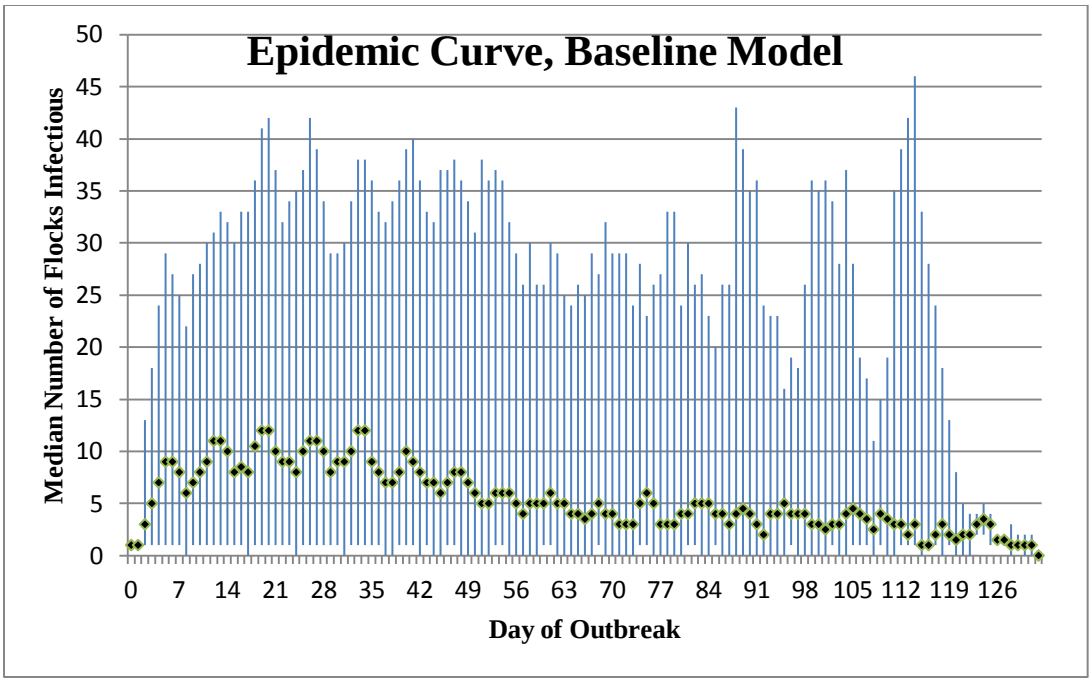


Figure 5. 3 Summary epidemic curve, including number of infected flocks (all flock types) at each time step. Number of infected flocks is the median calculated over 500 model simulations using the parameters in Table 5.3. Median is indicated by the diamond, vertical lines extend between the 2.5 and 97.5 simulation percentiles (95% probability interval).

Sensitivity Analyses

Table 5.5 summarizes the effect of altering the transmission probabilities on outbreak duration, peak, number of flocks infected and distribution of transmission types. Figures 5.4 and 5.5 show the change in the median outbreak duration and size, respectively, when parameter values are changed from the baseline model.

Road-flock transmission probability

To investigate the robustness of the road-flock transmission probability of the PERT probability distribution (Table 5.3), three scenarios were run: low, high and zero risk of transmission to backyard and penned flocks. The low risk scenario resulted in over 50 fewer affected flocks, but shorter median outbreak duration and a sooner median peak infection day. In the high risk scenario, outbreak duration was shortened by 10 days and fewer flocks were infected, but the median time to the outbreak peak was shorter. Despite the higher probability of road-to-flock transmission, the proportion of transmissions of this type did not change. Transmissions were still predominantly caused by people visiting flocks. When road-to-flock

transmission was reduced to zero, median outbreak duration, time to peak and number of flocks infected were even further reduced from baseline, to 30.5 days, 2 days and 19.5 flocks, respectively, and median proportion of transmissions caused by visiting flocks increased. In this model, transmission via road contamination caused by FGD flocks moving between home and field does play a role in maintaining transmission within the poultry sector, but elimination of this transmission probability only mitigates and does not prevent outbreak perpetuation.

Probability of transmission via social visits

Inter-flock transmission was facilitated bidirectionally by visiting neighbors. The probability of transmission by movement of infectious materials from infected premises to non-infected premises (visitor-flock transmission) is slightly higher than contamination of a visitor at an infected premises and subsequent transmission to his or her flock at home (flock-visitor transmission) (Table 5.3). When both visitor-facilitated transmission probabilities were set to the minimum of the PERT probability distribution used in the stochastic baseline model ("low" transmission scenario), outbreak duration was over 30 days shorter, and the number of infected flocks was only 10. The largest proportion of transmissions was still facilitated by visiting. With visitor-facilitated transmission set at the very high level recommended by one subject matter expert (see Appendix F) ("very high" transmission scenario), the number of flocks affected was much higher than in the baseline model (300.5 flocks, >85% of all flocks in the model), an occurrence which may have led to a scarcity of susceptible flocks and, subsequently, a shortened outbreak duration which had a median time of 45 days with a more narrow 95% PI than in the baseline model. When visitor-facilitated transmission was removed completely, the median outbreak duration was four times shorter, and only 5 flocks were involved in the outbreak. These analyses show that the baseline model depends on the contributions of visitor transmission to continue the outbreak beyond a small number of flocks. Based on the results of the "low" and "high" visiting scenarios, the model is robust to the probability of transmission that was used to inform the model (Table 5.3). This is almost certainly due to the high frequency of such contacts that are occurring and the multiplicative effect on the probability of transmission over all time steps (similar to the concept shown in equation (1). However, major model outcomes are sensitive to extreme probabilities of visitor-facilitated transmission ("zero" and "very high" scenarios, Table 5.4).

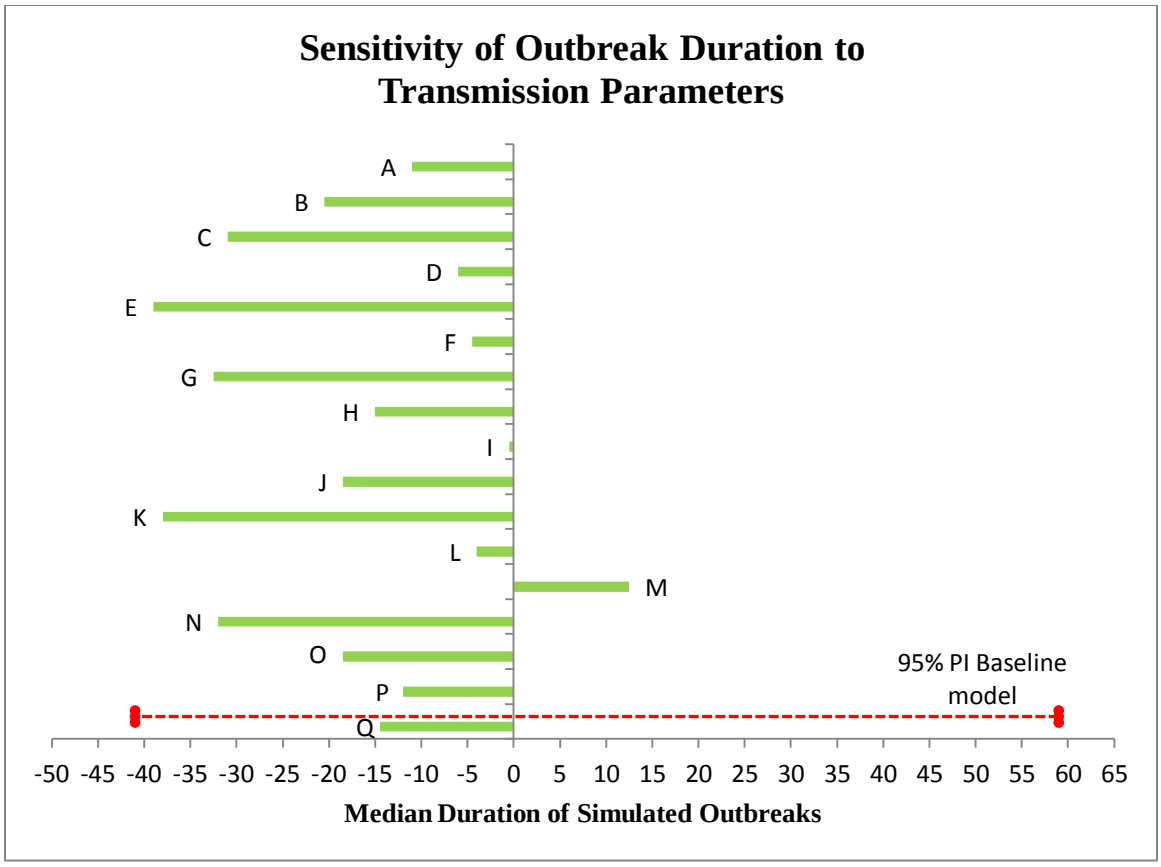


Figure 5. 4 Tornado plot showing the change in median outbreak duration from that of the baseline model when individual parameters are changed at model setup. A) Initial infection is in a layer flock; B) Initial infection is in a penned duck flock; C) Initial infection is in a backyard chicken flock; D) Duck infectious period is 10 days; E) Duck infectious period is 1 day; F) Chicken infectious period is 5 days; G) Chicken infectious period is 1 day; H) Risk of transmission through poultry trading is high; I) Risk of transmission through egg trading is high; J) Risk of transmission through egg trading is low; K) Risk of transmission through visiting is zero; L) Risk of transmission through visiting is very high; M) Risk of transmission through visiting is high; N) Risk of transmission through visiting is low; O) Risk of transmission through road contamination is zero; P) Risk of transmission through road contamination is high; Q) Risk of transmission through road contamination is low.

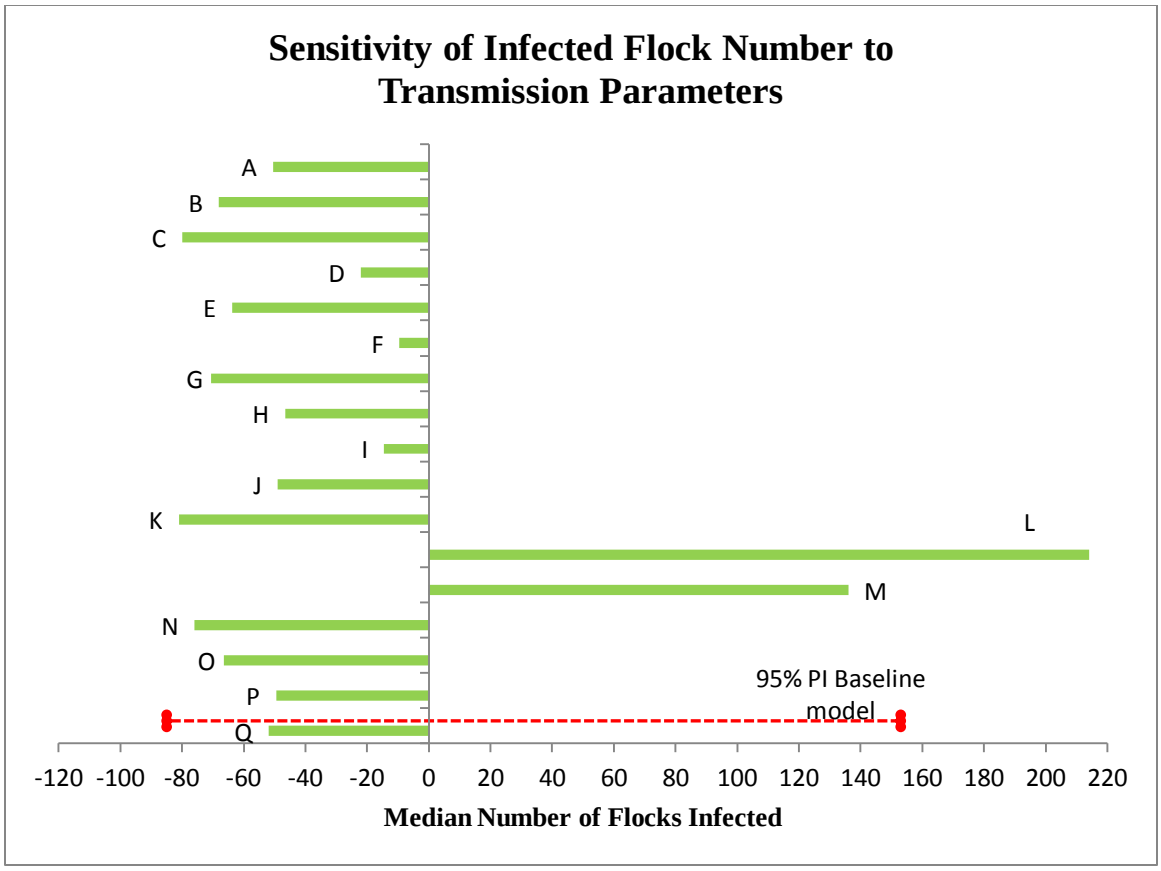


Figure 5. 5 Tornado plot showing the change in median number of flocks infected from that of the baseline model when individual parameters are changed at model setup. A) Initial infection is in a layer flock; B) Initial infection is in a penned duck flock; C) Initial infection is in a backyard chicken flock; D) Duck infectious period is 10 days; E) Duck infectious period is 1 day; F) Chicken infectious period is 5 days; G) Chicken infectious period is 1 day; H) Risk of transmission through poultry trading is high; I) Risk of transmission through egg trading is high; J) Risk of transmission through egg trading is low; K) Risk of transmission through visiting is zero; L) Risk of transmission through visiting is very high; M) Risk of transmission through visiting is high; N) Risk of transmission through visiting is low; O) Risk of transmission through road contamination is zero; P) Risk of transmission through road contamination is high; Q) Risk of transmission through road contamination is low.

Table 5. 5 Effect of changing transmission probabilities on multiple model outcomes over 100 simulations of each scenario. Transmission probabilities for the baseline model can be seen in Table 5.3

Transmission Scenario	Transmission Parameter	Parameter Probability	Median Outbreak Duration (95% PI)	Median Day of Peak Infection	Median No. Flocks Infected (%) (95% PI)	Transmission Routes (Median % of transmissions)	
Probability of transmission from road to nearby flocks							
Low	Road to penned flock	0	34.5 (8, 125)	6 (0, 55)	34 (9.7%) (1, 204)	Road-flock	13.4%
	Road to backyard flock	0.29				Flock-visitor	21.4%
						Visitor-flock	58.6%
						Egg trader-flock	3.7%
						Poultry trader-flock	0%
High	Road to penned flock	0.40	37 (8, 87)	26 (0, 66)	36.5 (10.4%) (1, 223)	Road-flock	14.8%
	Road to backyard flock	0.71				Flock-visitor	18.4%
						Visitor-flock	58.4%
						Egg trader-flock	3.2%
						Poultry trader-flock	0.51%
Zero	Road to penned flock	0	30.5 (8, 100)	2 (0, 54)	19.5 (5.6%) (1, 174)	Road-flock	0%
	Road to backyard flock	0				Flock-visitor	22.5%
						Visitor-flock	67.4%
						Egg trader-flock	4.4%
						Poultry trader-flock	0%

Table 5. 5 (cont.) Effect of changing transmission probabilities on multiple model outcomes over 100 simulations of each scenario. Transmission probabilities for the baseline model can be seen in Table 5.3

Transmission Scenario	Transmission Parameter	Parameter Probability	Median Out-break Duration (95% PI)	Median Day of Peak Infection	Median No. Flocks Infected (%) (95% PI)	Transmission Routes (Median % of transmissions)	
Probability of transmission associated with neighborhood visiting							
Low*	Flock to visitor	0.05	17 (8, 59)	7 (5, 34)	10 (2.8%)	Road-flock	33.3%
	Visitor-to-flock	0.1			(1, 82)	Flock-visitor	7.1%
						Visitor-flock	46.7%
						Egg trader-flock	0%
						Poultry trader-flock	0%
High*	Flock to visitor	0.3	61.5 (8, 93)	26 (0, 66)	222 (63.4%)	Road-flock	9.9%
	Visitor to flock	0.4			(1, 285)	Flock-visitor	23.7%
						Visitor-flock	59.8%
						Egg trader-flock	3.4%
						Poultry trader-flock	1.4%
Very High*	Flock to visitor	0.98	45 (8, 59)	19 (0, 33)	300.5 (85.6%)	Road-flock	5.0%
	Visitor to flock	1			(1, 321)	Flock-visitor	31.67%
						Visitor-flock	60.1%
						Egg trader-flock	2.2%
						Poultry trader-flock	0.68%
Zero*	Flock to visitor	0	11 (8, 31)	6.5 (5, 17)	5 (1.4%)	Road-flock	84.6%
	Visitor to flock	0			(1, 26)	Flock-visitor	0%
						Visitor-flock	0%
						Egg trader-flock	15.4%
						Poultry trader-flock	0%

* Flock to visitor: Infection of home flock after visiting infectious premises; Visitor to flock: Infection of flock by visitor from infectious premises

Table 5. 5 (cont.) Effect of changing transmission probabilities on multiple model outcomes over 100 simulations of each scenario. Transmission probabilities for the baseline model can be seen in Table 5.3

Transmission Scenario	Transmission Parameter	Parameter Probability	Median Outbreak Duration (95% PI)	Median Day of Peak Infection	Median No. Flocks Infected (%) (95% PI)	Transmission Routes (Median % of transmissions)	
Probability of transmission associated with egg trading							
Low	Flock to egg trader contamination	55	30.5 (8, 86)	6 (0, 54)	37 (10.5%) (1, 222)	Road-flock	15.0%
						Flock-visitor	19.1%
	Egg trader to flock infection	53				Visitor-flock	56.3%
						Egg trader-flock	2.1%
						Poultry trader-flock	0%
High	Flock to egg trader contamination	88	48.5 (8, 99)	13 (0, 68)	71.5 (20.4%) (1, 224)	Road-flock	13.6%
						Flock-visitor	18.1%
	Egg trader to flock infection	85				Visitor-flock	51.5%
						Egg trader-flock	7.1%
						Poultry trader-flock	0%
Probability of transmission associated with live poultry trading							
High	Flock to poultry trader contamination	95	34 (8, 39)	6 (0, 54)	39.5 (11.3%) (0, 198)	Road-flock	14.6%
						Flock-visitor	20.8%
	Poultry trader to flock infection	92				Visitor-flock	54.9%
						Egg trader-flock	3.3%
						Poultry trader-flock	0%

Probability of transmission via egg trading

Transmissions facilitated by egg traders were determined by two stochastic probabilities, the probability that an egg trader will be contaminated at an infectious premises and the probability that, once contaminated, the trader will cause infection in a susceptible flock. When both of these probabilities were set to the minimum of their respective PERT probability distributions used in the stochastic baseline model ("low" transmission scenario), the median proportion of transmissions caused by egg traders decreased, the outbreak was shorter and fewer flocks were involved (Table 5.4). Setting the probabilities to their maximum PERT distribution values ("high" transmission scenario) did not appreciably affect the outbreak duration or number of flocks involved, and the proportion of transmissions caused by egg traders did increase. These findings indicate that the model is robust to changes in the egg trader-facilitated transmission probability and that egg traders do not play a dominant role in transmission in this model.

Probability of transmission via live poultry trading

Transmissions facilitated by poultry traders were also determined by two stochastic probabilities, the probability of contamination at infectious premises and the probability that a contaminated trader will cause infection in a susceptible flock. Because the median proportion of transmissions facilitated by live poultry traders in the baseline model was zero, a sensitivity analysis was only conducted using the maximum probabilities in the PERT distributions used for the baseline model (Table 5.4). With a 95% chance of contamination and a 92% probability of transmission once contaminated, the median outbreak duration was considerably shorter, the peak was a week sooner, and over 40 fewer flocks were infected. The median proportion of transmissions facilitated by poultry traders was still zero, indicating that in most scenarios, they were not involved in the outbreak.

Table 5. 6 Effect of changing duration of influenza shedding (infectious period) on model outcomes

Model Parameter	Infectious Period (days)	Median Outbreak Duration (95% PI)	Median Day of Peak Infection	Median No. Flocks Infected (%) (95% PI)	Transmission Routes (Median % transmissions)	
Duration chicken infectious period*	1	16.5 (8, 52)	2 (0, 25)	15.5 (4.4%) (1, 80)	Road-flock	23.3%
					Flock-visitor	13.3%
					Visitor-flock	50%
					Egg trader-flock	2.1%
					Poultry trader-flock	0%
	5	44.5 (8, 108)	19.5 (0, 72)	76.5 (21.8%) (1, 237)	Road-flock	15.3%
					Flock-visitor	20.0%
					Visitor-flock	55.8%
					Egg trader-flock	3.8%
					Poultry trader-flock	0%
Duration duck infectious period*	1	10 (2, 45)	4.5 (0, 26)	22.2 (6.3%) (1, 81)	Road-flock	14.8%
					Flock-visitor	14.3%
					Visitor-flock	52.2%
					Egg trader-flock	0%
					Poultry trader-flock	0%
	10	43 (11, 100)	13 (0, 62)	64 (18.2%) (1, 234)	Road-flock	14.7%
					Flock-visitor	18.4%
					Visitor-flock	56.0%
					Egg trader-flock	4.6%
					Poultry trader-flock	0%

* Baseline infectious periods are 3 days for chickens and 7 days for ducks (Table 5.3)

Table 5. 7 Effect of varying the initial infection from FGD flock to one of the other poultry types

Model Parameter	Median Outbreak Duration (95% PI)	Median Day of Peak Infection	Median No. Flocks Infected (%) (95% PI)	Transmission Routes (Median % transmissions)	
Initial infection in backyard chickens	18 (5, 118)	8 (4, 76)	6 (1.7%) (1, 234)	Road-flock	0%
				Flock-visitor	20%
				Visitor-flock	61.5%
				Egg trader-flock	1.0%
				Poultry trader-flock	0%
Initial infection in penned ducks	28.5 (8, 106)	10.5 (0, 62)	18 (5.1%) (1, 211)	Road-flock	0%
				Flock-visitor	21.5%
				Visitor-flock	62.9%
				Egg trader-flock	3.2%
				Poultry trader-flock	0%
Initial infection in layer flock	38 (5, 97)	13 (0, 69)	35.5 (10%) (1, 225)	Road-flock	7.7%
				Flock-visitor	21.7%
				Visitor-flock	58.7%
				Egg trader-flock	4.7%
				Poultry trader-flock	0.72%

Infectious period

The chicken and duck infectious periods were altered from their baseline values of 4 days and 7 days, respectively, as seen above in Table 5.6. Decreasing the infectious period of chickens or ducks to 1 day (in separate analyses) led to significant decreases in median outbreak duration, days to peak and number of flocks infected. When the duck infectious period was decreased to one day, the mean outbreak duration was only 10 days, and fewer than 7% of flocks were affected. Increasing the infectious periods beyond their baseline values did not significantly change the outbreak dynamics.

Initially Infected Flock

When a backyard chicken flock was set to be the only initially infected flock, outbreak duration and the number of flocks were lower than the baseline scenario (Table 5.7), and visiting was responsible for a higher median proportion of transmissions more likely than in the baseline model. Road-flock transmission was less likely than when a FGD flock is initially infected, with a median proportion of zero such transmissions. The distribution of outbreak duration over the 100 simulations with the initial infection in a backyard flock (Figure 5.6) is visibly distinct from that of the baseline scenario (Figure 5.3). The former is right skewed, with most outbreaks lasting less than 5 days. In >25% of cases (data not shown), the outbreak does not spread past the initially infected flock, thus ceasing after the 4 days of initial flock shedding. The difference in these histograms was hypothesized to be due to the fact that when a FGD flock is infected at the start of a simulation, it has the opportunity to infect multiple other flocks in the first few days, by road-flock transmission. In fact, on average, the initially infected FGD flock infects 2.5 backyard flocks on the first day of the model (data not shown). The baseline outbreak duration distribution could be approximately replicated by running simulations with three initial backyard chicken flock infections (data not shown). Simulations run with initial infections in penned duck flocks and layer farms also resulted in shorter outbreaks and fewer infected flocks.

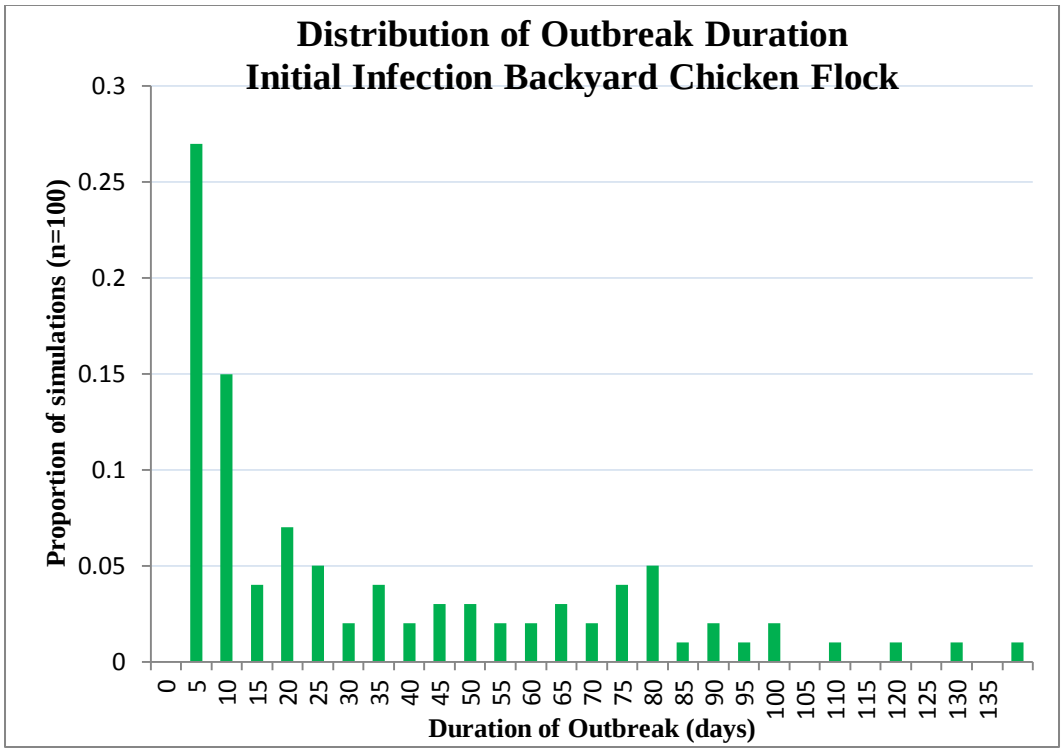


Figure 5. 6 Frequency histogram displaying the outbreak duration over 100 model simulations when the initially infected agent is a backyard chicken flock.

Hypothesis Testing

Table 5.8 displays the results of the scenarios run to test the three hypotheses. The first hypothesis stated that if all FGD flocks are kept on the rice field where they are grazing both day and night (eliminating daily road travel), the duration and number of flocks involved in the outbreak will be lower than in the baseline model. As the baseline scenario featured 6 of the 9 FGD flocks moving back and forth daily, we also ran a scenario that included all flocks moving daily to provide a more complete contrast for the flock restriction scenario. There was no appreciable difference from baseline when all flocks moved from home to field daily. Our hypothesis was correct in that, when flocks stayed on the field at night rather than returning home each day, the median outbreak duration was decreased by 35 days and 80 fewer flocks were affected. Road-flock transmissions were eliminated by keeping the flocks on the rice fields at all times and moving only to new fields when the rice supply was exhausted.

The second hypothesis stated that if all eggs from FGD flocks are delivered by the owners to a centralized egg cooperative, the duration and extent of the outbreak will be mitigated.

This change resulted in slightly shorter median outbreak duration, but, as we cannot compare our results statistically, it is difficult to tell if the difference is meaningful (Table 5.8). The effect on the size of the outbreak was greater, with the median number of infected flocks decreased by 50%.

The third hypothesis stated that the number of egg pickup locations serviced by egg traders in one day is directly related to the extent of the outbreak, and limiting traders to egg pickup from only one location per day will alleviate the outbreak duration and spread. Restriction of the number of pickup sites to one on each trading day did decrease the median outbreak duration by over 2 weeks and cut the median number of infected flocks by greater than half (Table 5.8).

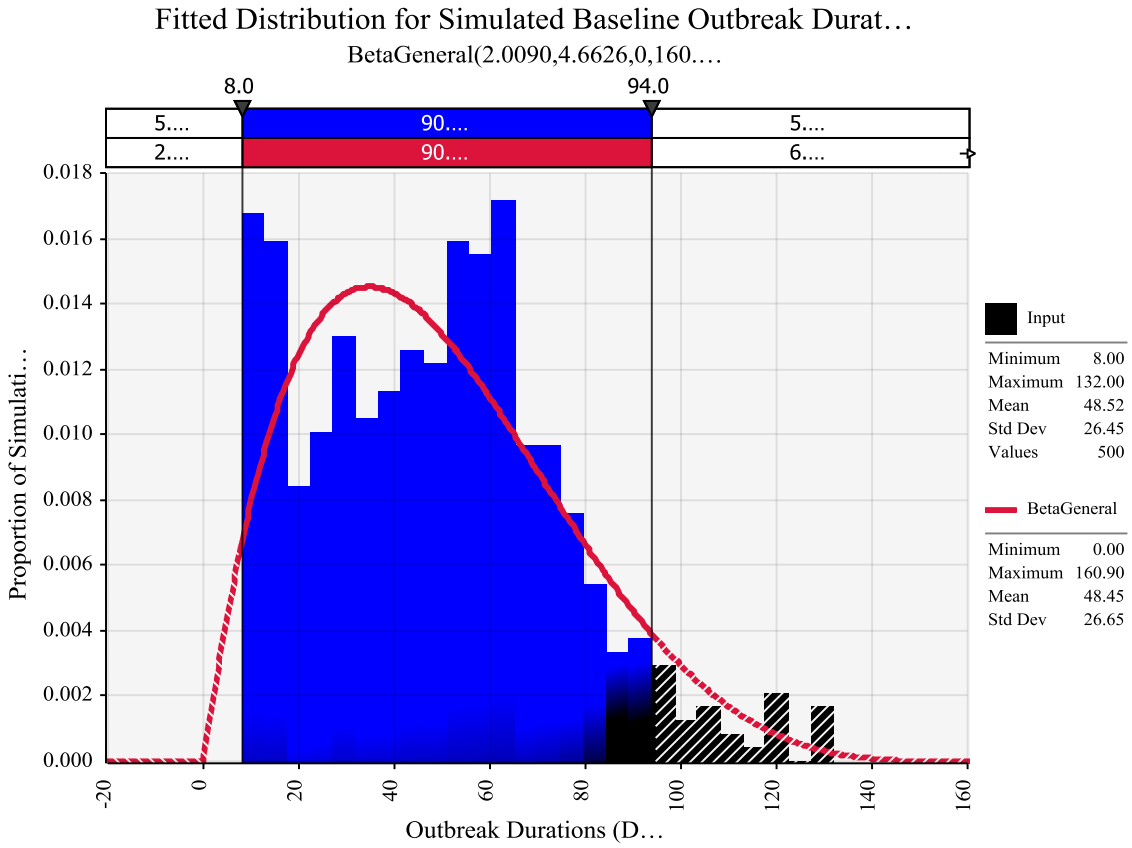
Estimation of the Reproduction Ratio

The transmission coefficient and R_f were calculated as described above for each simulation of the baseline model scenario (Table 5.3). Figure 5.8 shows the distribution of R_f estimates. Using the SIR direct method, the mean R_f over the 500 simulations was 1.4 when the infectious period was considered to be 4 days and 1.9 when the infectious period was considered to be 5.5 days (Figure 5.9). Using the GLM method, the estimates were 0.68 (T= 4) and 0.94 (T= 5.5). We may overestimate the value by calculating R_f using a mean duration of infectiousness (T = 5.5 days) for duck infectious period (4 days) and chicken infectious period (7 days), as most of the infected flocks were chickens (>70%). Table 5.9 shows the calculated R_f for the hypothesis testing scenarios. R_f can be brought to < 1 when the probability of transmission by visiting is decreased or eliminated (reflecting improved biosecurity), and when FGD flock movement between the owner's home and the rice field is minimized.

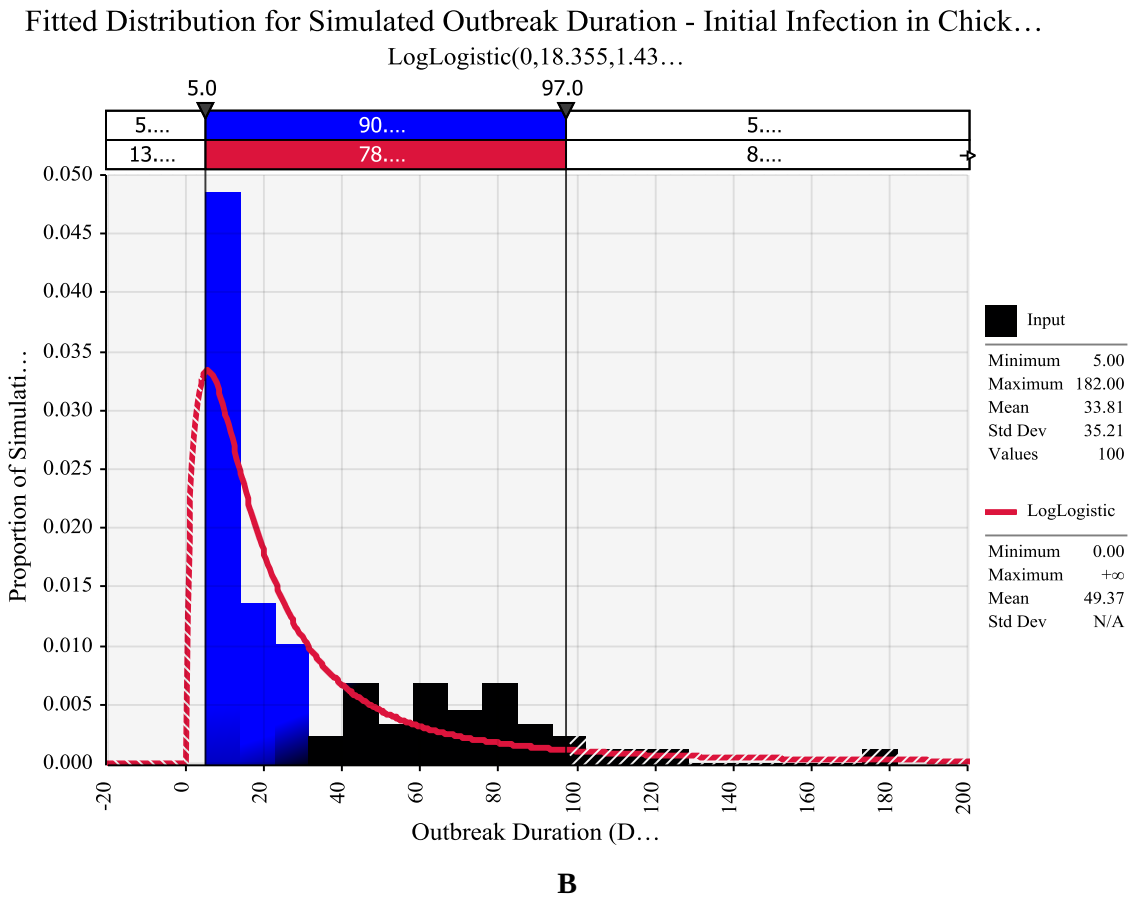
Local Cluster Analysis

The simulations that were used for spatial analysis resulted outbreaks with primary clusters and, in some cases, secondary non-overlapping spatial clusters with a high frequency of infected flocks. One simulation resulted in a primary cluster with 19 infected flocks and four non-infected flocks and a secondary cluster of 8 infected and 7 non-infected flocks (of 46 total infected flocks). The second simulation resulted in a primary cluster of 4 infected and no non-infected and a secondary cluster of 11 infected and 8 non-infected flocks (of 115 total infected flocks). The third simulation resulted in a only a primary cluster with 63 infected and 67 non-

infected flocks (of 65 total infected flocks). The fourth simulation resulted in a single cluster of 59 infected and 26 non-infected flocks (of 116 total infected flocks).



A



B

Figure 5. 7 Fitted distributions for outbreak duration (days). (A) The best-fitting distribution for baseline model duration output is a beta general distribution with min = 0, max = 161, mean = 48.5, standard deviation = 26.7. (B) The best-fitting distribution when backyard chickens are initially infected is a log logistic distribution with min = 0, max = + ∞, mean = 49.4.

Table 5. 8 Effect of scenarios on model outcome.

Model Parameter	Parameter Value	Median Outbreak Duration (95% PI)	Median Day of Peak Infection (95% PI)	Median No. Flocks Infected (%) (95% PI)	Transmission Routes (Median % transmissions)	
Proportion of FGD flocks moving between barn and field*	0	14.5 (8, 90)	11.1 (0, 47)	6.5 (1.9%) (1, 188)	Road-flock	0%
					Flock-visitor	23.0%
					Visitor-flock	67.9%
					Egg trader-flock	2.8%
					Poultry trader-flock	0%
	1.0	45 (8, 114)	19.6 (0, 60)	75.5 (21.5%) (1, 234)	Road-flock	24.1%
					Flock-visitor	17.7%
					Visitor-flock	50%
					Egg trader-flock	4.1%
					Poultry trader-flock	0.76%
Use of egg cooperative by all FGD owners**	-	42 (8, 93)	10 (0, 63)	43 (12.3%) (1, 201)	Road-flock	13.6%
					Flock-visitor	20.0%
					Visitor-flock	58.0%
					Egg trader-flock	1.8%
					Poultry trader-flock	0%
Number of flocks egg collectors visit in one day	1.0	31 (8, 101)	5 (0, 76)	30.5 (8.7%) (1, 99)	Road-flock	11.5%
					Flock-visitor	20.4%
					Visitor-flock	60.8%
					Egg trader-flock	0%
					Poultry trader-flock	0%

* In the baseline model, 6 flocks move between barn and field daily and 3 flocks stay on the field at all times.

** All FGD owners deliver all eggs to one centralized egg cooperative (no egg pickup at barn or field

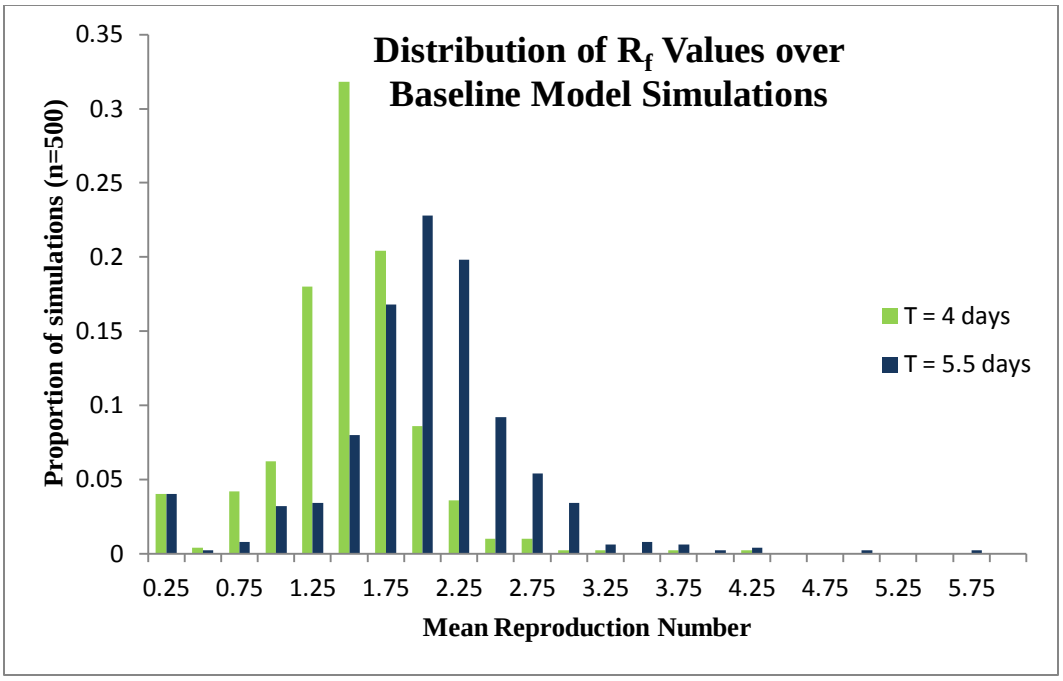
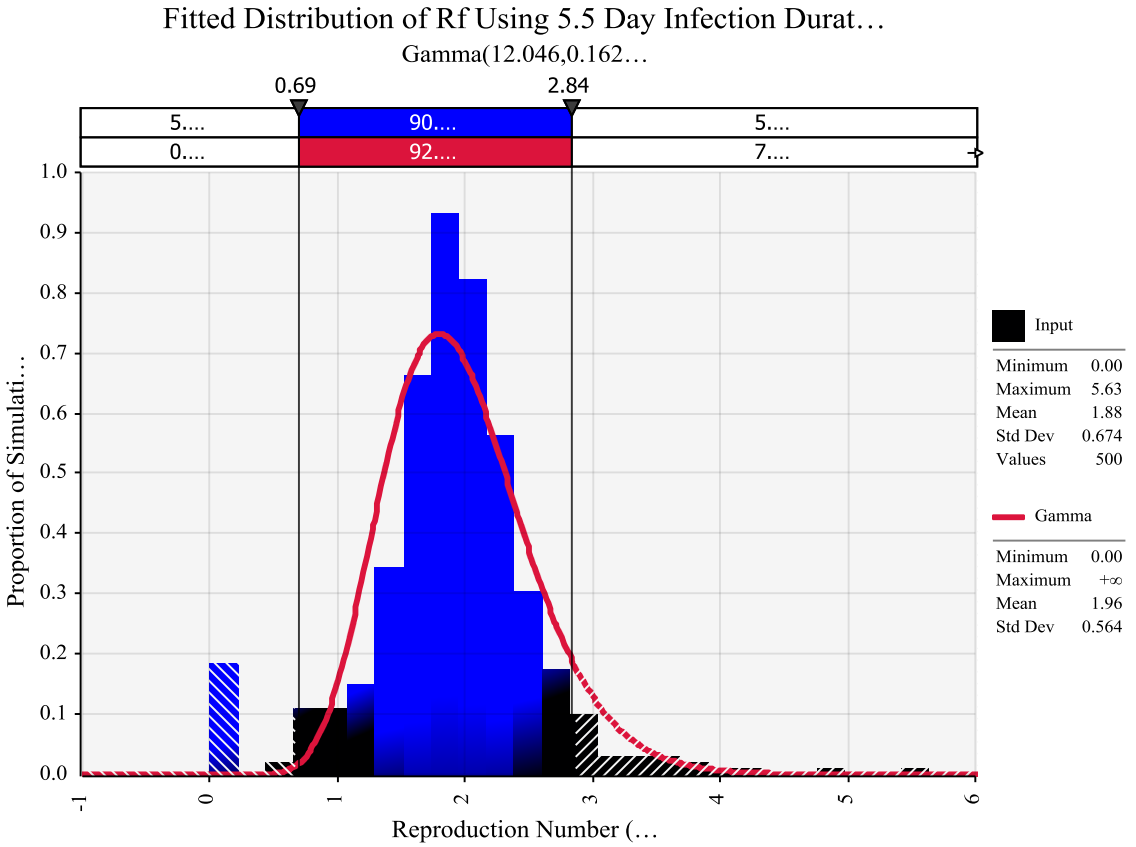


Figure 5. 8 Histogram showing the range of R_f values calculated from the baseline model simulations for T=4 days and T=5.5 days using the direct SIR method to calculate R_f

Table 5. 9 Estimates of the mean reproduction number (averaged over all model simulations for each scenario) from the baseline ABM and selected model scenarios

Scenario	SIR Direct Method		GLM Method	
	T=4 days	T = 5.5 days	T=4 days	T = 5.5 days
Baseline model*	1.4	1.9	0.68	0.94
Low visitor risk of transmission	1.0	1.4	0.73	0.73
Zero visitor risk of transmission	0.94	1.3	0.38	0.52
FGD stay on field only	0.73	1.0	0.43	0.59
FGD egg cooperative	1.1	1.8	0.60	0.82
One egg trader pickup location	1.0	1.4	0.55	0.76
Initial infection in BYC	0.78	1.1	0.48	0.66

*N=500 simulations; the rest of the estimates are averages generated over 100 simulations


Fitted Distribution of Rf Using 5.5 Day Infection Durat…
Gamma(12.046,0.162…
0.69
2.84
5....
90....
5....
0....
92....
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Proportion of Simulati…
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Input
Minimum 0.00
Maximum 5.63
Mean 1.88
Std Dev 0.674
Values 500
Gamma
Minimum 0.00
Maximum +∞
Mean 1.96
Std Dev 0.564

A

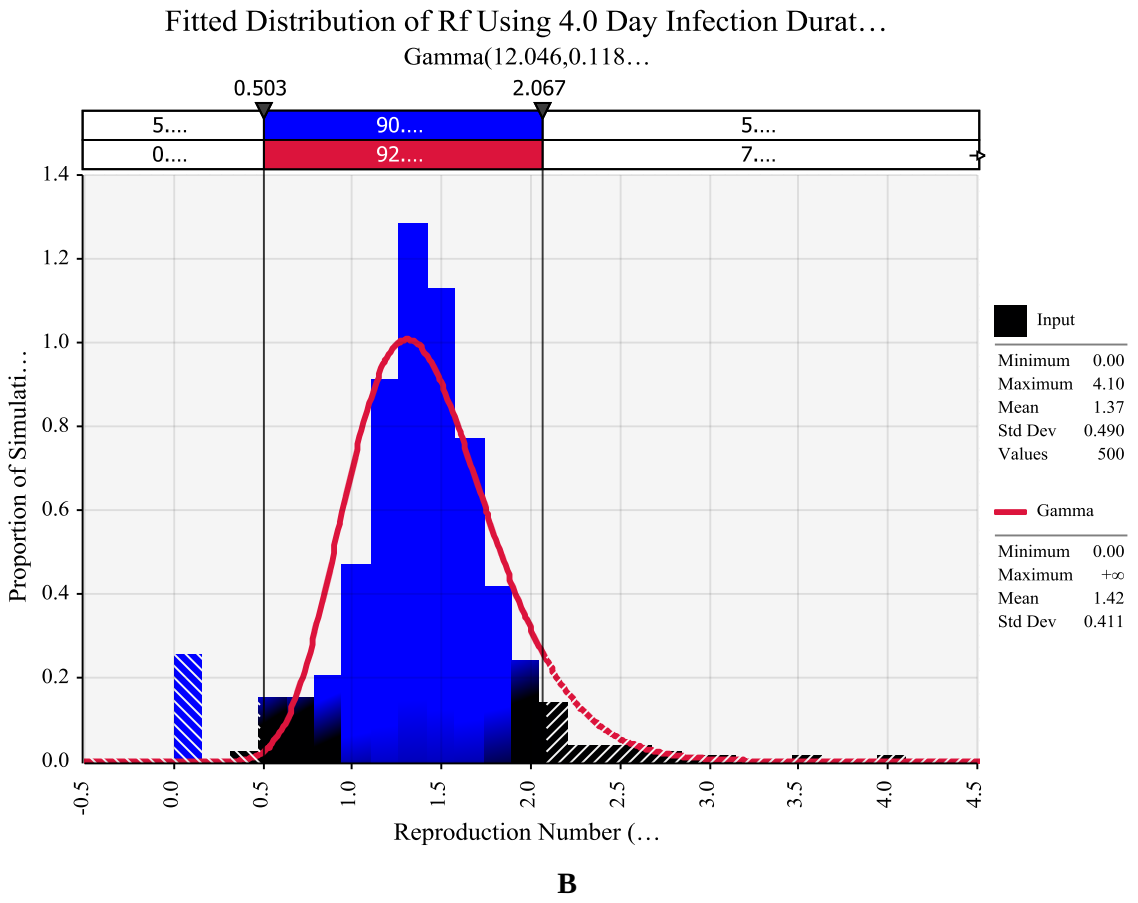

Figure 5. 9 Fitted distributions for the reproduction number as calculated with the direct SIR method. (A) The best-fitting distribution for baseline model R_f is a gamma distribution with min = 0, max = + ∞, mean = 2.0, standard deviation = 0.56. (B) The best-fitting distribution for R_f when the duration of infection = 4 days is a gamma distribution with min = 0, max = + ∞, mean = 1.4, standard deviation = 0.41.

Discussion

Model Performance

Agent-based modeling is well-suited to investigation of flock-to-flock transmission of avian influenza because of the following characteristics: 1) An ABM allows the simplification of the complex real-life system of poultry production and includes the aspects of the system with the greatest implications for disease spread; 2) Agent-based modeling allows the investigation of heterogeneous populations with contacts that vary in time and space; 3) Once the ABM framework was put into place, we were easily able to manually vary the values of certain parameters and inform others stochastically; 4) By varying individual model components, we are able to appreciate the contribution of each interaction type on system-wide disease transmission.

Overall, this model appears to sufficiently capture the frequency and stochasticity of interactions within the local Thai poultry sector. Smallholder flocks are connected by both poultry-specific and social contacts, manipulations of which, on the whole, result in predictable deviations from the baseline model. The model also reflects the movements of FGD flocks, a component that has been shown to be influential on model outbreak dynamics. We are satisfied that the key components of the system are sufficiently represented, although some may be overly simplified (see discussion of limitations below). Lastly, the transmission parameter outcomes appear to be reasonable with regard to previous investigations into inter-flock influenza transmission (Garske et al., 2007; Mannelli et al., 2007; Ward et al., 2009)

Transmission Characteristics

In this chapter, R_f was estimated from simulation output using two previously published methods (Becker, 1989; Stegeman et al., 1999; Stegeman et al., 2004). The mean R_f value calculated using the 4 day infectious period of chickens is closer to other estimates and is more appropriate for the primary flock types affected in our simulated outbreaks. Using the SIR direct method, R_f was estimated to be 1.4. In one analysis that also used this method, the reproduction number of a Romanian HPAI H5N1 outbreak was estimated to be 2.68 (Ward et al., 2009). Other published values of the reproduction number have been calculated using generalized linear models. One study using this method estimated the reproduction number to be between 1.5 and 1.8 in a 1999-2000 outbreak of HPAI H7N1 in northern Italy (Mannelli et al., 2007). A

reproduction number range of 3.1 to 6.5 was estimated for an HPAI H7N7 outbreak in the Netherlands, prior to interventions (Stegeman et al., 2004), followed by a reduction to 1.3 after control measures began. The estimates for R_f in this study are likely lower than published estimates because we conducted the value using all simulations, including both those that resulted in transmission past the initial infected flock and those that did not. Estimates of the reproduction number made from real-world outbreak data are by definition generated from an outbreak that has spread beyond the initially infected flock(s).

In this study, the estimates of R_f were much lower when calculated using the GLM method (Table 5.9). This may be because we violate the GLM assumption of constant variance across observations (time steps). Residual deviance plots and residual goodness of fit tests were generated for each simulation, and in multiple cases, certain time steps are poorly accounted for by the model and cause instability of the parameter estimate. Depending on the direction of the residual deviance, such findings can have an unpredictable effect on the estimate. Future work involving the calculation of R_f by simulation modeling may be enhanced by thorough assessment residual deviance and removal of simulations that may have unstable parameter estimates.

In light of the critical threshold theory (Kermack and McKendrick, 1991) and equation (13) (Keeling and Rohani, 2008), we can estimate that with $R_f = 1.4$, the ability to decrease between-flock transmission by $p_c = 29\%$ would assist in limiting between-flock influenza transmission during an outbreak by reducing R_f to <1. In equation (13), which is typically used to calculate the critical proportion of the population that must be vaccinated, p_c could be inferred to represent the critical threshold proportion of the population that needs to be protected by any intervention. Since vaccination is illegal in Thailand, this decreased transmission would need to be based upon improved biosecurity. In our model, R_f was brought < 1 by decreasing the probability of transmission during social visits and by minimizing the road transit of FGD flocks (Table 5.9).

$$p_c = 1 - \frac{1}{R_0} \qquad (13)$$

The cluster analysis allows us to take advantage of the spatial capabilities of agent-based modeling. In addition, as mentioned in the Introduction, one of the most important qualities of an ABM is the ability to include a heterogeneous population with heterogeneous contacts and

observe how micro-level interactions lead to a macro-level pattern. During infectious disease outbreaks, the detection of disease clusters allows us to better understand what may be an unpredictable pattern of disease spread. In the case of agent-based modeling, the model programmer can understand and relate both the interactions that contribute to transmission (by writing the model code) and the system-wide pattern that emerges (by assessing the outcome). While the configuration of cases can be observed using the graphic user interface of the modeling software, having a way to statistically measure the spatial relation of cases and non-cases is of benefit. The results of this model indicate that while not all cases are clustered together, there is a tendency for cases to be located near other cases in some locations, with cluster radii ranging from 2-25 patches (equivalent to 1-12.5 km). Knowing that social visiting contributed the largest proportion of effective contacts and is carried out within a defined radius of the home (within 10 patches, or 5 km, in this model), it is possible for us to hypothesize that this visitor-facilitated transmission plays a role in disease cluster formation. Future work with this model might investigate whether the presence of infected flock clusters differs with the type of initially infected flock (e.g. FGD or backyard flock).

Human-Facilitated Transmission

Visiting was shown in the model simulations to have a proportionally large impact on transmission. We know that the transmission of influenza from farm to farm by neighbors, friends and relatives has historical context in the 1983-84 HPAI H5N2 outbreaks in Pennsylvania (Fichtner, 1987). Outbreak intensity was decreased when the probability of transmission due to visiting was decreased (Table 5.5), indicating that there would be opportunity to make strides with regard to limiting farm-to-farm transmission if smallholders are educated about the importance of poultry biosecurity, including penning or restricting the range of backyard birds, preventing the movement of potentially infectious materials among farms by shoes and motorbikes.

The role of visitors in house-to-house transmission is an especially important point of discussion for Thailand, as identification of poultry and human cases of HPAI H5N1 has depended largely upon the work of the village health volunteer system during X-ray campaigns. X-ray campaigns are country-wide, simultaneous periods of targeted surveillance that include door-to-door surveys to identify sick poultry and humans and testing of every flock (Tiensin et

al., 2005). Five X-ray campaigns were carried out over the course of widespread outbreaks in 2004-2006 (Buranathai et al., 2007), and since that time, they have been conducted twice-yearly in areas considered to be at highest risk (Mana Keawyai, personal communication 2012). Village health volunteers are local community members who have been trained in disease detection. While the X-ray campaigns and local disease surveillance efforts are likely helpful in detecting disease, the findings from this model indicate that perhaps once an area is known to be affected by HPAI H5N1, such door-to-door surveillance could also assist in virus transmission among households. In such cases (when an outbreak is ongoing), education about the importance of self-reporting by smallholders with ill or dying poultry may be preferred.

Limiting egg traders' daily pickup to one location resulted in a > 50% decrease in the median number of infected flocks and a shortened median outbreak period in the model simulations. While it is unreasonable to think that the number of suppliers that small egg traders deal with daily can be regulated, there may be some basis for the promotion of centralized egg sale locations. Currently, there is one egg cooperative near DKY where owners of layer duck flocks can deliver their eggs (Appendix D). Other such locations for drop-off of eggs by owners and pick-up by traders may be beneficial during times of high avian influenza risk. Such an effort could be as informal as recommending that all flock owners in a neighborhood bring their eggs to one house or another nearby location for pickup by traders.

Live poultry traders were responsible for a small proportion of effective contacts in our model. Likely, this is due to the small number of trading days and flocks visited per trading day. During Chinese New Year and other holidays, the sales of live poultry to traders increases dramatically (Appendix D), and, were a model scenario run with increased contacts reflective of this, it is likely that the role of live poultry traders in influenza transmission would be greater.

While this model focuses on poultry infection only, it is important to realize that we have also laid out a detailed picture of human poultry contacts. This model does not quantify or characterize the poultry contacts made within a household, but we have learned that Thai poultry owners do have close contacts with their poultry and are often unprotected from potential exposures (Chapter 4). The baseline model identified a median number of 28.5 incidents of egg trader contamination, 173 incidents of contamination while visiting a neighbor, two incidents of live poultry trader contamination during an outbreak (data not shown). This is in addition to

environmental contamination of roads and rice fields. Continued education of poultry owners as to the risk factors of human infection with HPAI H5N1 and the protective measures that can be taken is very important.

FGD Flocks

One of our primary interests was to observe the potential role of FGD in transmission of influenza to nearby poultry. We saw that road-to-flock transmission does influence outbreak dynamics in this model, and if a FGD is primarily infected, several flocks along the roadway may be infected early in the outbreak, leading to a faster and more wide-spread outbreak. The model shows that keeping flocks on the field night and day, without daily movement from home to field (movement only to new fields when feed is exhausted), is effective in decreasing outbreak duration, shortening the time to peak infection and decreasing the total number of flock infected.

This scenario of keeping flocks on fields night and day was investigated specifically to determine if there is merit to suggesting this as an alternate policy recommendation. During the widespread outbreaks in Thailand during 2004 and 2005, FGD flock owners were told to keep their flocks at their homes instead of grazing them on rice fields. This was economically prohibitive to many flock owners, as many are attracted to this form of agriculture by the low overhead that comes with free-grazing feeding systems. During the in-depth interviews to inform this model, FGD owners were asked about their willingness to keep flocks on the rice fields day and night if asked to by the livestock officers (Appendix D). When asked what they would do if the livestock officer told them to stop grazing (n=15 responses), eight (53%) said that they would do nothing different, two (13%) said that they would stop moving to fields, three (20%) would keep the ducks at home, and two (13%) would keep the ducks on the field at all times. When asked if they would be willing to raise the ducks at their home at all times (n=18 responses), eleven (61%) said that they would if provided with feed by the government, five (28%) said they could not because they don't have a barn, and one (5.6%) said he or she was not willing despite having a place at home to keep the ducks. Only one (5.6%) respondent said he or she would be willing and did not mention the provision of feed. When asked if they would be willing to keep the ducks on the field day and night (n=14 responses), thirteen owners (93%) said that they would be, and only one owner (7%) was not willing. This general unwillingness or inability of flock owners to keep ducks at home is consistent with owner responses to regulations in 2004 and 2005

(Safman, 2010). The readiness of owners to keep flocks on the fields day and night if asked and the evidence from the ABM that this may be an effective way to decrease influenza transmission indicate that this may be a more acceptable regulation implemented at times of known local HPAI H5N1 circulation. The combination of this type of movement restriction with intensified surveillance could perhaps be a useful intervention in such times.

FGD owners (n=17) were also asked in interviews about their willingness to use a dedicated FGD egg cooperative (Appendix D), and 35% of owners (n=6) said that they would be interested in using one, while 65% (n=11) said that they would not be. Most disinterest was based upon egg pricing, not convenience. FGD eggs have specialty uses, such as in the making of traditional Thai desserts. Egg cooperatives that manage eggs from farmed ducks do not take eggs from FGD flocks. For this reason, FGD owners would need to establish their own cooperative to get appropriate prices and to target suitable buyers. In our model, use of an egg cooperative (one central location for egg drop-off) by FGD owners did, indeed, result in shorter outbreaks and fewer infected flocks, although the effect was not great, and the model does not assume any risk of owner or egg pallet contamination (and subsequent flock infection) at the egg cooperative. As the Thai poultry sector continues to become more compartmentalized and structured (Rushton, 2005; NaRanong, 2007), the use of more egg cooperatives could be considered for smallholder flocks to both control pricing and mitigate infectious disease transmission through the egg trading network.

The model may have underrepresented the time spent by FGD owners in each field grazing location, a measure determined by the "size" of each grazing area in the model. During the simulations, flocks stayed in one location for a mean and median 16.2 and 15.7 days, respectively (range 4.5 to 49 days). Interviews with a small number of flock owners (Appendix D) revealed that, for those owners that answered this question (n=14), the mean minimum time that they spend in one contiguous rice field area was 65 days (range 4-365, median 38) and the mean maximum number of days was 91 (range 4-365, median 53). However, it was unclear in these interviews whether these contiguous areas have multiple access points, which would necessitate the flocks to utilize different routes to the grazing location every few days or weeks. This is not uncommon if the flocks are grazing over a very large area. The implication of underestimating the time spent of the rice fields is the generation of an increased number of road-

based movements for "field-only" flocks (those that stay on the field day and night until the feed supply is exhausted) and an increased number of novel routes traveled by flocks that move between home and field. This may be important, because, as discussed above, these road movements have the potential to influence transmission.

As compared to the simulations where a FGD flock was initially infected, there was a shorter duration and smaller size of the outbreaks caused by an initial infection in backyard chickens, penned backyard ducks and layer farms. Findings in Chapter 2 indicate that FGD flocks are widely exposed to influenza viruses, including H5 subtype viruses. Our model shows that on average, on the first day of infectiousness, a FGD infects 2.5 backyard chicken flocks. Because of the mobility of the FGD flocks and the role of ducks as a natural reservoir for influenza, this type of transmission scenario may occur with a low pathogenicity avian influenza (LPAI) virus or even a high pathogenicity virus such as H5N1. While HPAI H5N1 viruses are of current primary concern to livestock officials in Thailand, some LPAI viruses that circulate in domestic poultry have the potential to become highly pathogenic. In the light of the findings from these two chapters, the potential for avian disease and human disease in the case of HPAI H5N1, are an indicator that ongoing rigorous surveillance of FGD flocks is imperative. This surveillance should be conducted not only for HPAI H5N1 viruses but for LPAI viruses as well, in an effort to increase the knowledge of circulating viruses and help to identify risks for poultry infection.

Limitations

This work has several limitations. We were unable to incorporate every aspect of poultry sector contacts, including feed pickup and delivery and manure removal. The exclusion of these contacts is unlikely to have a large impact on transmission among smallholder flocks. Interviews indicate that feed is often picked up by owners at a market, rather than at a location with poultry (Appendix D). We also did not include any contacts that occur because of manure management. During our interviews, none of the modeled flock types had structured manure management. The Sector 3 flocks that we interviewed (those with the largest flock size in our model) were positioned over bodies of water, thereby negating the need for manure trading. Another limitation of this model is the lack Sector 1 and 2 flock agents (duck, chicken and quail farms), which, while largely compartmentalized, may have some indirect contacts with the rest of the local poultry sector. Although these types of facilities are rare in DKY, their exclusion from the model

may attenuate the ability to generalize findings to areas with more such facilities. If this model is to be used to investigate interactions and influenza transmission in areas other than those that are similar to DKY, components like Sector 1 and 2 farms and live bird markets will need to be added to the model. Based on the impact of live bird markets in HPAI H5N1 outbreaks, it is likely that the relative importance of transmission types would be significantly altered. Additionally, we do not consider holdings with multiple poultry types, a situation which would change the transmission dynamics from the standpoint of infectious period, poultry contacts and poultry mobility. We did not include canals as a method for FGD transport. When canals run alongside the road that ducks are traversing, flock owners often prefer to have the ducks swim, as it is faster and is easier for the ducks. Swimming and bathing in natural bodies of water has been identified as risk factors for human infection with HPAI H5N1 (Vong et al., 2006; Vong et al., 2009), and water can be a source of influenza transmission for poultry.

Because there are no wild resident or migratory birds included in the current version of this model, we do not take into consideration the potential for transmission among FGD flocks as a result of contaminated rice field water and infection of free-living birds. Likewise, we do not specify where the FGD flock obtains its initial infection, although it is speculated that this might be facilitated by movement of virus by wild ducks or other waterfowl.

As with all models, the results and conclusions from this ABM are only as reliable and complete as the conceptual framework and data that we used to inform it (Reeves et al., 2011). The contact frequencies were informed by in-depth interviews, but the sample size was fairly small. Also, the study designer was not a native Thai speaker, and interview responses were passed through an interpreter. The use of stochastic parameter inputs allows us to compensate for the small number of interviews and account for the normal variation in poultry owners' tendencies. In regard to transmission among and within flocks, represented in our model by a simple SEIR transmission pattern, we have set single transmission parameters for chickens and ducks, consistent with infection in an individual animal. By doing this, we may (and perhaps it is even likely we do) underestimate the duration of shedding for infected flocks. This is decidedly of more concern with regard to the large FGD flocks (up to 11,000 ducks/ flock), which may be able to sustain transmission within the flock beyond 7 days due to staggered infections, stressful transport situations and environmental persistence (Ziegler et al., 1999).

Future Work

Now that the model has been developed, verified and its component parameters assessed for their role in model outcome, the model should be put through conceptual validation by other researchers with intimate knowledge of the Thai local poultry sector and by other researchers with an understanding of influenza and risk factors from inter-flock transmission. All models can benefit from ongoing evaluation and critique, and, in the specific case of the agent-based model presented here, feedback from other well-informed parties will assist in determining whether appropriate assumptions and parameter estimates have been used.

There are aspects that were not included in the scenarios described here that may be of use in the future. For example, there was no risk of infection to fighting cocks at practice and match arenas. In future versions of this model, it may be useful to include a second SEIR model of transmission that drives intra-flock transmission. This would be easily facilitated by NetLogo. In future work, it would be interesting to see if there is spatial autocorrelation of infectious flocks and roadways, a finding which has been reported (Gilbert et al., 2006; Ward et al., 2008; Paul et al., 2009; Rivas et al., 2009; Tiensin et al., 2009; Loth et al., 2010; Yupiana et al., 2010). As mentioned previously, this model is designed to represent a particular subdistrict's population (poultry and human), size, road structure and contact patterns. While DKY is likely representative of nearby areas, in order to make this model work for other locations in Thailand or other countries in South East Asia, additional data must be collected and the model altered accordingly.

The use of ABM is appealing because they can be easily understood as a simplified simulation of actors in the real world. However, because this type of modeling is carefully informed by the most accurate deterministic or probabilistic data available, it can also be as rigorous a method as mathematical and statistical modeling (Gilbert, 2008). While the setup of an agent-based model and the makeup of the real world system it represents may be easily described, the ability to run the model depends on specifically and fully informing all aspects of agent interactions and immediate outcomes from those interactions. The practical (and exciting) aspect that sets agent-based modeling apart from mathematical modeling is the ability to model heterogeneous populations and movement of agents within those populations and observe the emergence of potentially unexpected results. These attributes should be appealing to researchers in livestock diseases. There is a place for ABM in describing and understanding agricultural

systems as well as in generating hypotheses for disease transmission and control, identifying economic impacts of potential interventions and, with sufficient detail of the data and processes in the system, potentially serve as a tool for prediction of disease spread.

Chapter 6: General Discussion and Conclusions

The document presented here has provided a review of avian influenza, with specific regard to highly pathogenic avian influenza (HPAI) H5N1, a description of the Thai poultry system and the country's experiences with influenza outbreaks, and discussions of both poultry and human exposure to and infection with HPAI H5N1. In addition, four research manuscripts provided insight into the relationship between influenza A, including HPAI H5N1, and poultry and human health in rural Thailand. A major goal of this work was to learn more about free-grazing duck (FGD) flocks and their role in avian influenza virus maintenance and transmission. FGD flocks are critical to the livelihood of thousands of rural Thais, and they can be produced without the need for a sophisticated barn or large amounts of purchased feed, making them ideal for persons with limited financial resources. In Thailand's most recent outbreaks, avian influenza has become an infection of small poultry holdings and backyard and FGD flocks (Auewarakul, 2008), presenting a risk to poultry production and human health.

FGD flocks have been identified as a potential reservoir for HPAI H5N1 virus in Thailand (Chaichoune et al., 2009; Amonsin et al., 2010). In addition to the more traditional understanding of a single reservoir host species, the term reservoir can be defined as one or more epidemiologically connected populations or environments in which the pathogen can be maintained and from which infection is transmitted to a target population (Haydon et al., 2002). When considering reservoirs in this way, one might imagine that a reservoir could include multiple species as well as the environment in which they live (Figure 6.1). It is this type of approach that must be taken to analyze the role of FGD flocks in the maintenance and transmission of influenza viruses, including HPAI H5N1. In Central Thailand, FGD flock density increases with rice field density (Gilbert et al., 2007), a finding explained simply by the fact that where there are rice fields, there is opportunity for residents to graze ducks, and both FGD density and rice cropping density are associated with outbreaks of HPAI H5N1 in Thailand (Gilbert et al., 2006; Gilbert et al., 2007). Rice fields provide a feeding ground for other avian species as well, including migratory and resident waterfowl, leading to the potential for shared infection.

In Chapter 2, we determined that ducks in Central Thailand are widely exposed to influenza A viruses, including viruses of the H5 subtype. We were not surprised to find a high

level of influenza A seropositivity, including some H5 seropositivity. Because ducks are a natural host for influenza viruses, this serologic information does not tell us much on its own. The way to determine if FGD and their rice field system are a potential reservoir for HPAI H5N1 viruses is to accumulate epidemiological evidence for or against the case (Haydon et al., 2002). The risk factor analysis that was presented in Chapter 2 gives an indication of what management factors are important in flock seropositivity. Because ducks rarely show clinical signs of influenza infection, routine active surveillance of this population is critical to understanding when and how they become infected with these viruses. Thai FGD flocks should be tested twice yearly during flock registration and sampled prior to inter-province movement, as discussed in Chapter 2, flocks often are not registered and do not undergo pre-movement testing. Sampling a limited number of flocks through a prospective approach may be rewarding, allowing diagnostic testing at specified intervals as well when otherwise indicated (e.g. illness in ducks, reports of illness in other nearby poultry).

If FGD flocks are capable of maintaining HPAI H5N1 viruses, then a basic, but critical, question is: Where does the virus come from? One study during early Thai outbreaks found that young domestic ducks were negative for H5N1 until they began grazing (Songserm et al., 2006). In addition to routine surveillance of mobile FGD flocks, there is a place for sentinel surveillance, including both serologic and antigen-based tests, in areas of high HPAI H5N1 risk. Sentinel systems are often used to define the prevalence of an agent or disease or to confirm hypotheses about the ecology of infectious diseases (McCluskey, 2008). Because sentinels are in place for extended periods of time, surveillance from such systems can provide insight into the temporal patterns of pathogen presence. Prospective surveillance of FGD flocks could be logistically and financially difficult due to frequent and potentially unpredictable movement. Sentinel flocks, on the other hand are stationary, and they could be owned by the Thai Department of Livestock Development or by a private owner. Samples collected could contribute to laboratory viral isolate panels for use in future diagnostic testing.

The ability of a maintenance population to sustain circulation of a pathogen depends on contact patterns (Craft et al., 2009). Contacts within FGD flocks are readily apparent and include both direct and indirect (e.g. shared water, fecal material) interactions. Such contacts are exacerbated by penning and transport, as discussed in Chapter 2. The direct and indirect contacts

between ducks and wild birds are likely not quantifiable, and the impact of these contacts will depend on multiple conditions, including the infectious state of the birds and ducks, the number present, physical conditions of the field water, and the size of the space in which the birds interact. With all these factors in consideration, are the contacts achieved in one FGD flock-rice field ecological unit alone enough to maintain virus circulation above the epidemic threshold (reproduction number > 1)? As the number of ducks in a flock is limited and new (naïve) ducks are purchased only once every two years, we are pressed to try to identify the contacts that link each FGD flock and its environment with the other FGD flocks in the area at risk.

In Chapter 5 we enumerated and investigated the contacts among FGD flocks and other members of the local Thai poultry sector. FGDs were shown through agent-based modeling to be connected to other FGD flocks via routine travel along roads and interaction of flock owners. Thus, it is possible that if FGD flocks do maintain influenza viruses, the true reservoir is comprised of a web of links between multiple FGD flocks and rice fields over a large geographic area, all connected by human contacts and poultry movements. Interacting with this web at the periphery are wild waterfowl, which may move directly among FGD flocks, and non-grazing duck flocks, infected by indirect contacts, as discussed in Chapter 5.

The interest in understanding the role of FGD flocks is related to the need to prevent infection of other domestic poultry as well as infection of their human caretakers. The agent-based model of the local poultry sector (Chapter 5) revealed that once a flock of any kind is infected with HPAI H5N1, in most situations ($\geq$ 70%), transmission occurs beyond the initially infected flock, facilitated by the contacts between flock owners and other members of the poultry sector and by poultry movement. In Thailand and other South East Asian countries, poultry infection not only means that humans may be exposed to influenza virus during poultry-related tasks, but also, because many people own backyard poultry, they may be exposed near their homes at virtually all times. Our surveys with local poultry owners (Chapter 4) indicate that poultry owners conduct high-contact activities such as slaughtering and cooking poultry and cleaning the mucous discharge of fighting cocks, as well as other activities that entail exposure to potentially contaminated materials, such as gathering eggs and moving fecal material. Despite these contacts, many reported limited use of personal protective equipment and, at times, do not practice good hand hygiene. Because rural Thai poultry owners find protective measures

uncomfortable and unfamiliar (Somrongthong et al., 2010), and because many believe that HPAI H5N1 is not a new disease and is a normal cyclical event (Liao et al., 2009), they may not take appropriate protective measures. It is important for poultry owners to be educated about the etiology of avian influenza infection in poultry, because when the general understanding of transmission is inconsistent with reality, compliance with recommended practices will be poor (Liao et al., 2009).

As follows, the findings from our agent-based model indicate that poultry owners should be advised to be mindful when moving among households in their immediate area and be aware of the health status of nearby flocks. While the village health volunteer system was effective in identifying infected premises during the large scale outbreaks in 2004-2005 (Auewarakul, 2008), persons should be cautioned that movement from house-to-house may put their own flocks and those of others at risk when HPAI H5N1 viruses are in the area. Having visitors come to the flock was identified as a risk factor for FGD seropositivity in Chapter 2, as well. In Chapter 3, we reported poultry owner seropositivity to HPAI H5N1 in five of 20 villages. All study villages were in areas that had experienced poultry infection, however not all villages had seropositive participants. In addition to individual host immune factors, subclinical seropositivity in humans is likely related to the number of infected birds in close proximity and the amount of virus exposure. The agent-based model revealed that infected poultry flocks tend to be clustered within the subdistrict, mainly because of the role of local movement of poultry owners. The human serologic findings could be reflective of this local transmission phenomenon.

There is much that we do not yet understand about human infection with avian influenza viruses, both because surveillance has not been routinely practiced and because detection of human exposure to avian viruses has been difficult in the past (Hinshaw et al., 1981). The level of human subclinical infection with HPAI H5N1 has been the topic of much discussion as of late, with concerns centering on the overall risk of these viruses to humans and our ability to efficiently and accurately detect subclinical exposure (Cohen, 2012; Osterholm and Kelley, 2012; Palese and Wang, 2012). There are now sufficient laboratory assays to detect these antibody responses (Rowe et al., 1999; Kayali et al., 2008), and surveillance of persons with and without poultry exposure must be carried out and be both consistent among laboratories and complete in obtainment of exposure histories.

The work presented in this dissertation adds only small amount of understanding to the complex world of avian influenza, but it is hoped that the observations and recommendations made herein may be useful in the improvement of detection and control of avian influenza in rural Thailand.

Figure 6. 1 Interrelatedness of FGD flocks, wild birds, other poultry flocks and humans. Diagram created by Kira Beaudoin and used with permission.

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Appendix A: Summary of human seroprevalence studies investigating poultry-human transmission of HPAI H5N1

Table A. 1 Summary of human seroprevalence studies investigating poultry-human transmission of HPAI H5N1

Author	Study Year, Location	Population (sample size)	Time Since Exposure	Serologic Tests (cutoff value)	Seroprevalence	Clade of Diagnostic Antigen	Risk factors	Associated Animal Testing
Katz et al. (1999)	1997 Hong Kong China	Confirmed H5N1 cases, household and social contacts (124)	11-21 days after exposure to case patient	MNT (≥1:80) or ELISA, WB to confirm	12% (6/51) household contacts 4% (1/26) social contacts 0% (0/47) coworkers	Clade 0	N/A	None
Bridges et al. (2002)	1997-1998 Hong Kong China	Poultry and government workers (1,525)	Up to 2.5 months since first poultry cases for poultry workers; Start of culling, then 2 weeks later for gov. workers	MNT (≥1:80) , WB to confirm	10% poultry workers (est.) 3.1% gov. workers	H5N3 for MNT Clade 0 expressed protein for WB	Work in retail poultry operations; mortality >10% in poultry operation; butchering; feeding; preparing poultry for restaurants	Prior to culling, 20% of chickens found infected

Wang et al. (2006)	2006 Guangdong China	Poultry sellers (110)	9 days after onset of human infection, one day after death	HI w/ turkey RBCs (cutoff not provided) MNT	.01% (1/110) sample was >320 by HI, > 640 by MNT	Clade 0 Clade 1	N/A Seropositive person slaughtered 100 birds/ day for 5 years	Environmental sample (1 cage) RT-PCR positive in market
Vong et al. (2006)	2005 Cambodia	Households within 1 km of confirmed case (351)	2 months after high poultry deaths; Animal testing days after deaths	MNT (≥1:80), WB to confirm	0% (0/351)	Clade 1	N/A	42 with > 60% flock mortality; chickens next to case property infected
Ortiz et al. (2007)	2006 Nigeria	Poultry workers at confirmed/ suspected farms; lab workers w/ 4 hours virus contact (320)	Minimum 3 weeks after last possible exposure	MNT (≥1:80), HI w/ horse RBCs to confirm (no cutoff provided)	0% (0/295) poultry workers (0%) (0/25) lab workers	Clade 2.2	N/A No association between titer >1:20 and PPE/ hand washing	None
Lu et al. (2008)	2004 Guangdong China	Persons living w/in 3km of poultry outbreaks (1,214)	Unknown	MNT (≥1:20), HI w/ chicken RBCs (≥1:20)	3% (7/231) by occupational exp. 2.3% (12/983) general population. (14 HI titers ≥1:80)	Unknown	None provided	None
Hinjoy et al. (2008)	2004 Thailand	Poultry farmers (322)	Poultry infections in province w/in 6 months	MNT (≥1:80), HI or WB to confirm	0% (0/322) 4 had detectable, negative titers	Clade 1	N/A	None

Wang et al. (2009)	2008 China	Poultry workers (2,191)	Unknown	MNT, HI (no cutoff provided)	0.20% (4/2,191)	Clade 0 Clade 1	N/A	None
Dejpichai et al. (2009)	2005 Thailand	Rural Thais in 4 villages w/ confirmed 2004 human case (901)	12-14 months after confirmed human case in village	MNT (≥1:40), Immunofluores-cence to confirm	0% (0/901)	Clade 1	N/A	None
Vong et al. (2009)	2006 Cambodia	Persons living w/in 1km of household with human cases (674)	7 weeks after confirmation of first poultry outbreaks	MNT (≥1:80), WB confirmation	1% (7/674) Serum collected 10 mo later had 4-32 fold reduction in MNT; only the highest initial titers (1:640-1280) ≥1:80	Clade 1	Bathing/ swimming in community pond; gathering poultry into cages	70% of ducks sampled seropositive
Schultsz et al. (2009)	2005 Vietnam	Poultry workers involved in culling job; cullers in long-term culling operations (317)	Median time for workers 164 days; median time undetermined for cullers	MNT (≥1:80) Analyzed for cross-reactivity to H1N1, H3N2	0% (0/500) poultry workers (0.9%) 3/317 cullers	Clade1 Clade2.3.4	N/A	None

Cai et al. (2009)	2006 Germany	Gov. workers, firemen, vets collecting dead wild birds (78)	1 year	Plaque neutralization, MNT (≥1:20)	0% (0/78)	Clade 2.2	N/A PPE compliance also assessed	Known infected wild birds, mostly swans
Santhia et al. (2009)	2005 Indonesia	Poultry owners, market vendors (928)	18 months after first outbreaks, but outbreaks ongoing during study	MNT (≥1:80)	0% (0/928)		N/A	Market birds had higher isolation rate than home. Pigs seroneg.
Robert et al. (2010)	2007 Indonesia	Poultry farmers (495)	6 months	MNT (≥1:80) HI (≥1:160)	0% (0/495)	Clade 2.1	N/A	One w/ outbreak during study
Cavailler et al. (2010)	2007 Cambodia	Villagers near confirmed human case (700)	9 weeks after H5N1 human fatality in village	MNT (≥1:80) HI (≥1:160)	2.6% (18/700) MNT becomes negative after 1 year (unpublished)	Clade 1	Bathing or swimming in community pond; gathering poultry into cages	None
Khuntirat et al. (2011)	2008 Thailand	Rural Thais, 65% of which had ever been near poultry (800)	3 years	MNT (≥1:10) using 2 test isolates	3.5 (45/800) - 5.6% (28/800) Did not provide titers for positive participants	Clade 1	Age > 60 yrs; seropositivity to seasonal H1N1 flu; no indoor water; poultry presence not a risk factor	None

MNT- microneutralization; ELISA- enzyme-linked immunosorbent assay; HI- hemagglutination inhibition; WB- western blot

Appendix B: ROC analysis of FlockCheck AI MultiS-Screen kit for ducks

Background

As discussed in Chapter 2, Brown *et al* (2009) evaluated the IDEXX FlockCheck AI MultiS-Screen diagnostic kit (ELISA), finding that the sensitivity of the ELISA was higher than AGID for both LPAI and HPAI infected birds, especially for ducks. Using the manufacturer's cutoff value of 0.5, sensitivity of the ELISA for LPAI-experimentally-infected birds was 75% and for HPAI-infected birds was 96%. Total sensitivity was 82%. In addition, the test was 100% specific. ROC analysis of the results indicated that the percentage of correctly classified birds was maximized at a cutoff higher than the recommended manufacturer cutoff, and that sensitivity and specificity were maximized at a cutoff of 0.6. The use of a higher cutoff would result in a small drop in specificity while gaining in sensitivity, as samples with a value below the cutoff are considered positive. The goal of the current analysis is to determine what the most ideal S/N value cutoff is for ducks and to estimate the test sensitivity and specificity using that cutoff.

Methods

Presented here is a receiver operating characteristic (ROC) curve analysis of the duck subset of birds tested in Brown *et al* (2009). Data for 198 ducks of known infection status was obtained from the corresponding author and is included in this analysis. Data provided include inoculum type (LPAI virus, HPAI virus or sham inoculum), duck species and S/N value from ELISA analysis. Using the known infection status and the test-positive and test-negative status based upon ELISA over a range of cutoffs, an ROC curve (sensitivity as a function of 1-specificity) was created. The curve was used to evaluate the optimum cutoff for S/N value for duck samples, indicated by the point where the sum of sensitivity and specificity is maximized. Samples with an S/N value below the cutoff value are considered positive. The analysis was conducted using SAS 9.2 (SAS Institute, Inc.; Cary, NC).

Results

The mean S/N value for infected ducks was 0.30 (range 0.046-1.05), and the mean value for uninfected ducks was 0.90 (range 0.577-1.24). The mean value for uninfected ducks was significantly higher than that for infected ducks (t-value 22.5, $p < 0.0001$). The mean S/N value for HPAI-infected ducks (0.21) was significantly lower than the mean value for LPAI-infected ducks (0.33) (t-value -3.96, $p = 0.0002$). These findings were consistent with the results from the Brown *et al.* (2009b) analysis with multiple avian species. Distributions of the S/N values for LPAI, HPAI and negative duck samples can be seen in Figure B.1.

The ROC curve for all 198 ducks can be seen in Figure B.2, and the characteristics of the curve can be seen in Table B.1. The cutoff that maximizes sensitivity and specificity is 0.60, with S/N values higher than the cutoff identified as negative for influenza antibodies and values lower than the cutoff identified as positive. Because the distributions of the HPAI and LPAI S/N values

overlap and the mean values are significantly different, an ROC curve was created for each of these categories of infected ducks. The cutoff that maximizes sensitivity and specificity for the HPAI-infected ducks is 0.55 (Table B.2), and for the LPAI-infected ducks is 0.6, though 0.55 is only minutely more effective than 0.6 for ducks with HPAI (Table B.3).

Discussion

The cutoff value that maximizes sensitivity and specificity in duck serum samples is 0.6. Though the S/N values for HPAI virus infection are in general lower than this cutoff, the test needs to be maximized for all potential virus infections, as it is frequently unknown which virus isolate, if any, ducks have been exposed to in the field. The choice of a cutoff value of 0.6 will mean that all samples with an S/N value lower than 0.6 will be called positive. This would include 92% of all positive ducks, 97% of HPAI-infected ducks, and 90% of LPAI-infected ducks in this study. The overall specificity at this cutoff is 97%.

While it is necessary to have one cutoff value to determine definitively positive samples, it is often useful to also establish a suspect positive zone. The results from this analysis indicate that suspect S/N values should include those >0.6 and <0.7. The use of this buffer zone would improve the overall sensitivity for positives or suspect-positive samples to 96% of all positive ducks, 100% of HPAI-infected ducks, and 95% of LPAI-infected ducks in this study. Using this buffer zone decreases the overall specificity to 88%, though this is acceptable if the goal is not to miss positive samples, as it is with surveillance-based studies.

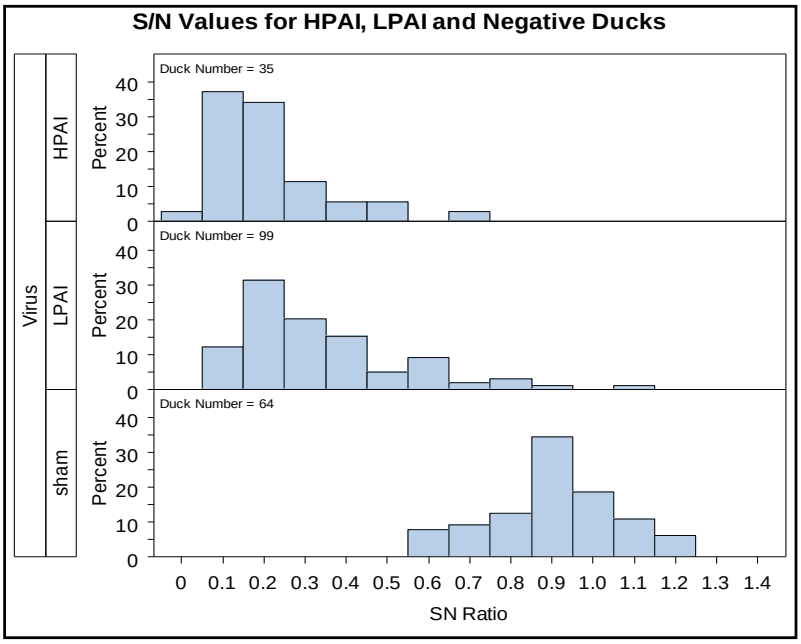


Figure B. 1 Comparative histogram of S/N values for ducks infected with HPAI, LPAI and sham inoculum.

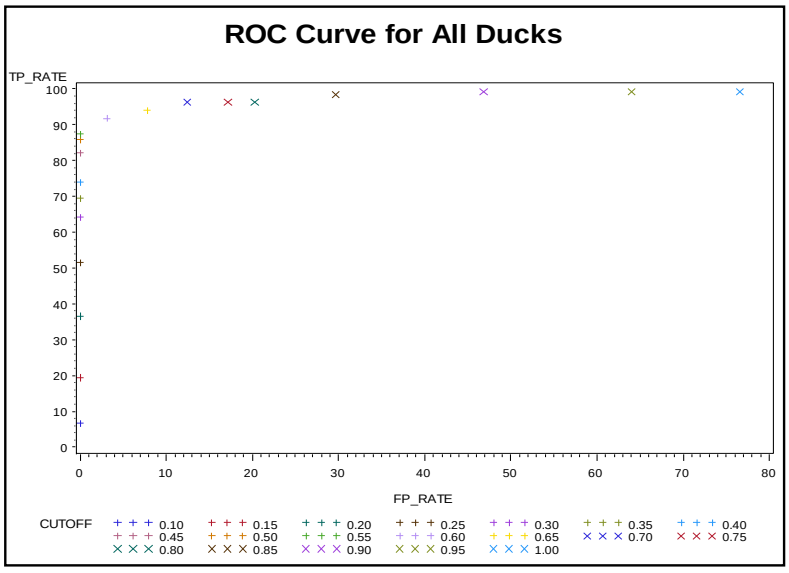


Figure B. 2 ROC curve for IDEXX ELISA evaluated with 198 ducks of known status for previous AI infection. Points on the curve indicate ELISA S/N cutoff values.

Table B. 1 Characteristics of the ELISA kit for the identification of influenza A antibodies over a range of cutoffs in ducks with antibodies to HPAI or LPAI viruses.

Cutoff (S/N ratio)	Sensitivity (%)	Specificity (%)	Sensitivity + Specificity
0.10	6.7	100.0	106.7
0.15	19.4	100.0	119.4
0.20	36.6	100.0	136.6
0.25	51.5	100.0	151.5
0.30	64.2	100.0	164.2
0.35	69.4	100.0	169.4
0.40	73.9	100.0	173.9
0.45	82.1	100.0	182.1
0.50	85.8	100.0	185.8
0.55	87.3	100.0	187.3
0.60	91.8	96.9	188.7
0.65	94.0	92.2	186.2
0.70	96.3	87.5	183.8
0.75	96.3	82.8	179.1
0.80	96.3	79.7	176.0
0.85	98.5	70.3	168.8
0.90	99.3	53.1	152.4
0.95	99.3	35.9	135.2
1.00	99.3	23.4	122.7

Table B. 2 Characteristics of the ELISA kit for the identification of influenza A antibodies over a range of cutoffs in ducks infected with HPAI

Cutoff (S/N ratio)	Sensitivity (%)	Specificity (%)	Sensitivity + Specificity
0.10	14.3	99.9	114.2
0.15	40.0	99.9	139.9
0.20	60.0	99.8	159.8
0.25	74.3	99.8	174.0
0.30	85.7	99.7	185.4
0.35	85.7	99.7	185.4
0.40	85.7	99.6	185.3
0.45	91.4	99.6	191.0
0.50	94.3	99.5	193.8
0.55	97.1	99.5	196.6
0.60	97.1	99.4	196.5
0.65	97.1	99.4	196.5
0.70	100.0	99.3	199.3
0.75	100.0	99.3	199.3
0.80	100.0	99.2	199.2
0.85	100.0	99.2	199.2
0.90	100.0	99.1	199.1
0.95	100.0	99.1	199.1
1.00	100.0	23.4	123.4

Table B. 3 Characteristics of the ELISA kit for the identification of influenza A antibodies over a range of cutoffs in ducks infected with LPAI

Cutoff (S/N ratio)	Sensitivity (%)	Specificity (%)	Sensitivity + Specificity
0.10	4.0	100.0	104.0
0.15	12.1	100.0	112.1
0.20	28.3	100.0	128.3
0.25	43.4	100.0	143.4
0.30	56.6	100.0	156.6
0.35	63.6	100.0	163.6
0.40	69.7	100.0	169.7
0.45	78.8	100.0	178.8
0.50	82.8	100.0	182.8
0.55	83.8	100.0	183.8
0.60	89.9	96.9	186.8
0.65	92.9	92.2	185.1
0.70	94.9	87.5	182.4
0.75	94.9	82.8	177.8
0.80	94.9	79.7	174.6
0.85	98.0	70.3	168.3
0.90	99.0	53.1	152.1
0.95	99.0	35.9	134.9
1.00	99.0	23.4	122.4

Appendix C: Don Kam Yan Subdistrict Poultry Census Data

Table C.1 Poultry census data for Don Kam Yan Subdistrict in Muang Suphanburi District, Suphanburi Province, Thailand, 2011

Village (Moo)	Total households	Total households w/ livestock	Quail	Total chicken owners	Backyard Chickens	Meat Chickens	Egg Chickens	Total non-FGD duck owners	All meat ducks*	Egg Ducks	FGD, Egg
1	1060	14	0	14	13	0	0	1	1	0	-
2	341	41	0	38	35	0	1	11	3	8	-
3	217	40	3	28	27	0	1	18	2	17	-
4	185	9	0	7	7	0	0	0	0	0	-
5	240	59	0	59	59	0	0	9	4	5	-
6	314	47	0	47	43	2	3	9	5	5	-
7	344	44	0	35	35	0	1	14	10	5	-
8	136	16	0	15	13	0	0	6	6	6	-
9	210	35	0	31	28	2	1	13	11	5	-
	3047	305 (10% of households)	3	274	260 (8.5% of households)	4	7	81	42	51	6

*Most of the meat duck flocks are owned by people without egg ducks (approx. 70%).

Appendix D: Local Thai Poultry Sector In-Depth Interview Summaries

Free-Grazing Duck Flocks

We interviewed 25 free grazing duck (FGD) owners specifically to inform the agent-based model. The duck flocks were registered in seven subdistricts within Muang District in Suphanburi Province, but the majority of the surveys were conducted in three subdistricts: Don Kam Yan, Sala Khao and Suan Taeng. According to the subdistrict livestock officers, at the time of the surveys, Don Kam Yan Subdistrict had 6 registered flocks, Sala Khao Subdistrict had five to six registered flocks, and Suan Taeng Subdistrict had ten registered flocks, and we interviewed nine, two and eight flocks in each subdistrict, respectively. The number of interviews conducted in Don Kam Yan was higher than the number of registered flocks, indicating imprecision of the livestock officer's FGD flock number estimates. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- Ducks are purchased from a duck farm or another FGD owner. In most cases (57%), FGD owners pick up their new ducks from the seller, as opposed to having them delivered (43%).
- In 56% of cases, the owner will use different sellers each time they purchase new ducks.
- Most FGD owners (88%) use their ducks for egg production and sell the eggs.
- Of those that sell eggs, 55% have the eggs picked up at the flock location, and 41% deliver the eggs to the buyer or another trader. Some flock owners (4%) use both methods.
- For those who have the eggs picked up, they work with between 1 and 4 traders that pick up eggs, with a mean of 1.5 traders and a median of 1 trader.
- Daily onsite egg trader contacts occur with a mean of 1.3 egg traders come each day, but this number can range from 1 to 3, with a median of 1.
- Weekly onsite egg trader contacts occur with a mean of 3 trader contacts per week, ranging from 1 to 7, with a median of 2.5.
- Most FGD owners (60%) pick up their own feed at a market or other location. A smaller proportion (15%) has the feed delivered, while 25% of owners do not give the ducks any supplemental feed.
- For those who have feed delivered, the mean number of onsite feed delivery contacts is 0.92, with a range from 0.25 to 1.5, and a median of 1.25. In other words, most owners have feed delivered about once weekly.
- For those who pick up their feed, the mean number of offsite feed contacts is 2, with a range from 0.25 to 3, and a median of 2.5. In other words, most owners pick up feed 2-3 times weekly. In 27% of these flocks, feed is purchased at the same place where the eggs are sold, in 20% of the flocks it is not, and in 53% of the cases, the data was missing.
- Ducks are sold in equal proportions to traders for slaughter (38%) and to new flock owners (38%) for continued egg production. There were 5 flock owners (24%) that reported selling to both types of buyers.

- When sold, 81% flock owners have the ducks picked up, 14% deliver them, and 4.8% use a combination of pickup and delivery to get the flock to the buyer(s).
- The number of buyers for each flock varies. The mean response for the minimum number of buyers per flock was 1.5 (range 1-5, median 1), and the mean response for maximum number of buyers per flock was 2.9 (range 1-20, median 1). In other words, most people sell each flock to 1-3 people.
- Ducks are sold by their owners at intervals between 3 and 24 months, with a mean and median interval of 14.3 and 16, respectively.
- FGD owners have friends, relatives, neighbors and other acquaintances that own poultry. The mean response for the minimum number of such relationships was 6.6 (range 2-10, median 5.5), and the mean maximum number was 8.3 (range 3-20, median 7). The number of meetings per day had a mean minimum of 2.5 (range 0-10, median 1.5) and a mean maximum of 4.4 (range 1-10, median 1.5).
- Visits with poultry-owning contacts occur on a mean minimum of 3 days per week (range 1-7, median 3) and on a mean maximum of 3.6 days per week (range 1-7, median 3). Respondents reported visits at the home (52%), field (56%), market (20%), at the homes of others (8%), and at unnamed places outside the household (16%).
- Ducks are usually brought back to the home (57%) when grazing in the home subdistrict, as opposed to being penned by the field (43%). Ducks get back to the barn on foot (33%), by truck (8%) or a combination of the two methods (60%). If brought back to the barn, a truck was used for a mean distance of 1.9km or more (range 1-5, median 1). In general, ducks walked if the distance from the barn was 1km or less. The mean distance usually traveled back and forth from the home was reported to be 0.55km (range 0.05-1, median 0.5).
- Flock owners like to find a large contiguous area where they can graze for days at a time. For those owners answered this question (n=14), the mean minimum time that they spend in one contiguous rice field area was 65 days (range 4-365, median 38) and the mean maximum number of days was 91 (range 4-365, median 53).
- Some flock owners reported moving out of Suphanburi Province at some point during the year (38%). When grazing in their home district, 48% of owners reported moving out of their subdistrict, and while grazing within Suphanburi Province, 26% reported grazing outside of Muang District. Everyone interviewed said that they use the same grazing locations every year.
- There was quite a range for the estimates given for the amount of rice the ducks eat each day. The amount eaten will depend on the individual field and the amount of rice, snails and other food sources that are there. It is likely that this also depends on the age and the egg production status of the ducks. The median from this set of interviews was 0.009 rai/duck/day, and the mean was twice that at 0.018 rai/duck/day. Information from the larger survey conducted in 2010 (see Chapter 2) is likely more reliable due to the larger sample size. That study indicated that ducks eat an average of 0.006 rai/duck/day (range 0.0006-0.06, median 0.005).
- When asked what they would do if their own duck flock was sick or dying suddenly, most (84%) said that they would do nothing different, some said that they would keep the ducks at home (5.3%), keep the ducks on the field only (5.3%), or vaccinate (5.3%).

- The mean time to inform the livestock officer about sick or dying birds in their flock would be 2.1 days (range 1-4). Three respondents said they would not tell the livestock officer.
- When asked what they would do if another duck flock nearby was sick or dying suddenly, most (78%) said that they would do nothing different, some said that they would keep the ducks at home (5.6%), or move the flock far away (17%).
- When asked what they would do if nearby chickens were sick or dying suddenly, most (78%) said that they would do nothing different, some said that they would keep the ducks at home (5.6%), keep the ducks on the field only (5.6%), or move far away (11%).
- When asked what they would do if the livestock officer told them to stop grazing, most (53%) said that they would do nothing different, some said that they would stop moving to fields (13%), keep the ducks at home (20%) or keep the ducks on the field only (13%).
- When asked if they would be willing to raise the ducks at home only, most (61%) said that they would if provided with feed by the government, others said they could not because they don't have a barn (28%) or said they were not willing despite having a place at home to keep the ducks (5.6%). Only one said he would be willing and did not mention the provision of feed (5.6%).
- When asked if they would be willing to keep the ducks on the field day and night, 93% of owners said that they would be, and only one owner was not willing (7%).
- When asked about interest in an egg cooperative for FGD eggs, 35% said that they would be interested in using one, and 65% said that they would not be. Most disinterest was based upon egg pricing, not convenience.

Backyard Duck Flocks

We interviewed nine people with penned duck flocks specifically to inform the agent-based model. The duck flocks were all located in Don Kam Yan Subdistrict of Muang District. According to the data provided by the subdistrict livestock officer, at the time of the surveys (Table C.1), 81 households in Don Kam Yan Subdistrict owned duck flocks. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- All penned duck flocks except one were kept for the purpose of egg production (n=8, 89%). One flock was exclusively meat ducks (11%). Three flocks had both meat and egg ducks. One flock owner had geese penned with the ducks.
- Ducks are purchased from duck farms, neighbors with duck flocks or family. One owner maintains the flock by letting the ducks breed themselves. Owners reported picking up their ducks from the seller in most cases. Two owners bought ducks from the FGD flocks of near neighbors, and it is likely that the ducks walked to their new home.
- The owners of all eight egg-producing flocks sell the eggs. Half of them have traders or neighbors pick up the eggs, and half deliver the eggs themselves for sale.
- Two flock owners sell the eggs to traders that pick up the eggs. One flock owner uses one trader, and the other uses two traders. In both cases, however, only one trader comes each day. The owner that uses one trader has the eggs collected once each week, and the owner

with two traders has the eggs picked up seven days each week. Two flock owners do not sell to traders but, rather, have neighbors pick up the eggs.

- Four flock owners (50%) deliver the eggs offsite for sale. Three of these owners bring the eggs to the egg co-operative, which is in Bang Pla Ma District, and the other person brings the eggs to a trader in Bang Pla Ma. It is possible that this is the cooperative as well, although this was not made clear in the interview. The mean number of contacts with offsite traders per week is 3.8 (range 2-7, median 2.5).
- Six owners (86%) pick up their duck feed, and one owner does not use purchased feed (14%). The feed is picked up with a mean frequency of 1.2 times per week (range 1-2.5, median 1). Four of the six owners (75%) always pick up feed at the same place, and the other two flock owners go to different places depending on price and availability.
- All of the flock owners sell their ducks to traders, and traders come to the home to pick up the ducks when it is time to sell. The owners use different traders each time they sell.
- Ducks are sold on average every 16 months (range 6-24, median 20).
- The duck owners reported knowing an average minimum of 4 people with poultry (range 1-8, median 4) and an average maximum of 5.4 people (range 3-10, median 5) that own poultry.
- The average minimum number of visits with other poultry owners per day is 3 (range 1-10, median 2), and the average maximum is 7 (range 3-30, median 4).
- Owners visit with other poultry owners on a mean minimum of 4 days each week (range 1-7, median 3), and on a mean maximum of 5.6 days each week (range 1-7, median 7).
- Backyard duck owners meet with other poultry owners at home (89%), at others' homes (33%), at the egg cooperative (33%) and at the market (22%).

Backyard Chicken Flocks

We interviewed 13 people with backyard chicken flocks specifically to inform the agent-based model. The backyard flocks were all located in Don Kam Yan Subdistrict of Muang District. According to the data provided by the subdistrict livestock officer, at the time of the surveys, Don Kam Yan Subdistrict had 260 backyard duck flocks. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- The mean flock size was 43 chickens (range 20-100, median 40). All of the flocks roam around the property.
- All flocks breed themselves, so the owners do not purchase any chickens.
- None of these owners sell the chicken eggs.
- Six owners do not buy feed for the chickens. Of the four owners that do buy feed, three (60%) of them pick it up and one (30%) has it delivered. The owner that has feed delivered gets it once each month, and those that pick up the feed do so, on average, twice each month (range 1-4, median 1). At least two of the three pick up at the same place every time.
- Most of the owners (83%) sell the chickens to traders, and only two (17%) sell to new owners. In both these cases, the owner sells fighting cocks. In most cases (92%), the chickens are picked up by the trader. Only one owner delivers the chickens for sale.

- The mean number of sales per year is 2 (range 0.5-4, median 2). Most people try to sell chickens at festival times, especially Chinese New Year in February.
- The chicken owners reported knowing an average minimum of 7.8 people with poultry (range 0-30, median 5) and an average maximum of 9.1 people (range 0-30, median 6).
- The average minimum number of visits with other poultry owners per day is 1.4 (range 0-5, median 1), and the average maximum is 2.7 (range 0-10, median 2).
- Owners have visitors on a mean minimum of 2.4 days each week (range 0-7, median 2), and on a mean maximum of 3.1 days each week (range 0-7, median 3).
- Backyard chicken owners meet with other poultry owners at home (54%) and at others' homes (46%).

Large Chicken and Duck Farms

We conducted interviews with seven large poultry farm owners. We interviewed four people with chicken layer farms (2,000-12,000 chickens with a mean flock size of 5,400), one with a duck layer farm (2,000 ducks), one with a meat chicken farm (15,000 broilers), and one with a duck breeding farm (20,000 ducks). All chicken and duck layer farms were in sector 3, with the birds under a roofed barn with open, netted (or partially netted) sides. Layer chickens were in individual cages in the barns. Layer ducks were free to roam around on the floor of the barn and had access to an exit to swim in the pond beneath the barn. The broiler farm was in sector 2, with biosecurity practices in place and a fully-enclosed barn. This farm is a contract farm, where the young chicks, feed, veterinary care and flock removal are all provided by a large poultry corporation. The farms were all located in Don Kam Yan Subdistrict of Muang District. According to the data provided by the subdistrict livestock officer, at the time of the surveys, Don Kam Yan Subdistrict had seven chicken layer flocks, but only 5 had more than 100 birds, indicating that we interviewed most, if not all, large layer chicken owners in the subdistrict. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- All owners have poultry delivered to the farm after purchase, rather than going to pick up the poultry from the seller. In general, the farm owners always use the same poultry source.
- The farms have a mean number of 4.4 employees (range 1-12, median 3).
- All but one (meat chicken farm) of the interviewed farms sells eggs. Farm owners have the eggs picked up (50%), deliver them to the buyer (16.7%) or use a combination of both sales methods (33.3%).
- For those farms that use a trader to pick up the eggs, there is a mean number of 4 traders per farm (range 1-10, median 1) that go to the farms with a mean frequency of 3.4 times each week (range 2-7, median 2.5). On each day of egg collection, the farms have a mean minimum number of 2.7 egg collectors come to get eggs (range 1-6, median 1) and a mean maximum number of 3 traders (range 1-7, median 1).
- Farm owners that deliver their eggs offsite for sale do so on average 4 time each week (range 2-7, median 3).
- Most farm owners have the feed delivered to the farm (n=6, 86%) as opposed to going to pick it up (n=1).

- The mean number of feed deliveries made to the farms each week is 1 (range 0.5-2, median 1).
- The owner that picks up feed offsite gets it at the same place that the eggs are delivered for sale. The frequency of these trips is three times each week.
- Feed is not always purchased from the same place. While most owners (n=5, 71%) do use the same supplier each time, some (29%) do not.
- We did not ask about manure removal specifically, but as five (71%) of the farms had barns positioned over bodies of water where fish are may be farmed, it is expected that these owners do not have manure removed.
- Most farm owners (n=6, 86%) sell poultry to large-scale traders that take them to slaughter. One owner, the duck breeder sells ducks both to traders (when the male breeding ducks are one year old) and to new owners (one day old ducklings and one year old female breeding ducks).
- When poultry is sold, all layer chicken owners sell to a single trader. Sales are made at a mean frequency of every 16 months (range 12-18 months, median 18). Most owners (75%) always sell to the same trader.
- When ducks are sold, the layer duck owner sells to a single trader and sells the ducks every 20 months.
- The broiler chickens are picked up at 45 days of age by a trader employed by the overhead company for the contact farmer.
- The duck breeder sells one-day old ducklings to 146-730 people each year. Between 2 and 10 customers buy ducklings every 5 days. Also, every 2-3 months, she sells the older female breeding ducks to 2 or 3 new owners and sells the older males to slaughter.

Fighting Cocks

We interviewed six people with fighting cocks specifically to inform the agent-based model. These owners all live in Don Kam Yan Subdistrict of Muang District. The number of fighting cock owners in Don Kam Yan is unknown, but it was estimated by the livestock officer to be approximately 300, and most people that won fighting cocks also own a flock of backyard chickens. The percentages below are calculated from the non-missing responses for each category.

- The mean number of practice fight locations attended by the respondents is 1.5 (range 1-4, median 1).
- Cock owners go to the practice arenas on average 1.7 times each week (range 1-4, median 1).
- All respondents go to just one match arena, and they go to watch fights on average 2.5 times each month (range 1-4, median 2.5).

We discussed fight cock practice and match arenas, the number of attendees and the frequency of fights in detail with one of the respondents that hosts practice matches at his home. A summary of these findings follows.

- Each Tuesday and Wednesday, approximately 30 people who own fighting cocks come to his home for practice cock fights. Usually people will bring only one fighting cock, but sometimes they bring two.
- People that come here to practice on Tuesdays and Wednesdays come from a variety of places.
- Everyone who has a FC goes someplace to practice, and everyone nearby comes to his house on Tuesdays and Wednesdays.
- There is only one other practice facility like his in Muang District. The other one is in U Ya subdistrict, where practice occurs on Sundays and Mondays and there are more than 100 people per day. People that come to his house for practice may also go to U Ya.
- On practice day, there are ten fights. It is up to the participants if they want to bring a fighting cock on that day. Usually there are about 20 cocks there, but there have been as many as 40. Sometimes people will bring two or three fighting cocks, but usually just one. After fighting, the cock must rest for two weeks. The fights have six 20 minute periods, separated by a 20 minute rest period, for a 120-minute fight.
- There is at least one match arena in each district, and they have matches on different days of the week. The Muang District match arena is open every Saturday and Sunday. This owner goes at least once/ month, (when he doesn't have to work in the field).
- There are approximately 300 people inside the match arena, with others outside watching on televisions.
- There are only 20 fighting cocks that fight each day (10 fights), and the rest of the people are just spectators. Last year this owner brought a fighting cock to the match arena on two different occasions.
- People who make a living from fighting cocks (10-20% of attendees) will go to the arena every week to gamble, but most people (80-90%) are like him and only go about once/ month.

Egg Traders

We interviewed seven egg traders to inform the agent-based model. Five of the traders live in Don Kam Yan Subdistrict of Muang District, one lives in Sala Khao Subdistrict and one in Suan Taeng Subdistrict. Subdistrict livestock officers do not know how many egg traders there are in their areas. The traders do not need to be registered, and there is no regulation of the industry. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- Most of the traders move chicken eggs (71%), while some transport duck eggs (57%) and quail eggs (14%) as well.
- While most traders just pick up the eggs after they have been collected and crated, two traders go into the barn to collect eggs (40%) if they are not ready.
- The mean number of trading days per week is 3.3 (range 1-7, median 2).
- On average, traders go to 2.8 sites (range 1-10, median 2) to pick up eggs on each trading day. Most traders go to the same sites to pick up eggs every day (67%), while others have different pick-up locations each day.

- The mean number of delivery sites to which traders deliver eggs is 5.3 (range 1-25, median 1). The mean minimum number of these sites visited in one trading day is 2.3 (range 1-10, median 1), and the mean maximum number of sites visited per day is 4.4 (range 1-25, median 1).
- Most (86%) egg traders deliver to the same locations every day.

One egg trader described how she thinks most egg traders and sellers work: There are 4-5 other egg traders like her at the large markets, and at the small markets, maybe only one. Some traders do as she does and go to only one large farm, while others go to multiple small farms. Some traders have 1-2 stops to get the eggs. Chicken owners may have 1-3 traders per day come. Usually there are 1-2, but if there are eggs left, they will have a third come. Sometimes if there are extra eggs, the farmer will call a neighbor and arrange to have the eggs picked up with theirs.

Live Poultry Traders

We interviewed six live poultry traders to inform the agent-based model. The interviewed traders live in Don Kam Yan (n=4) and Rua Yai (n=1) Subdistricts of Muang District, and Song Phi Nong (n=1) Subdistrict of Bang Pla Ma District. Subdistrict livestock officers do not know how many live traders there are in their areas. The traders do not need to be registered, and there is no regulation of the industry. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- The traders transport chickens (n=3, 50%), ducks (n=17, %) or both (33%).
- Most traders catch the poultry at the yard or barn (n=4, 67%).
- The mean number of trading days per week is 4 (range 1-7, median 3.5).
- The number of collection sites is difficult to estimate for some traders. Often, traders are picking up birds from household flocks, and the number of stops will depend on the number of birds that each household wants to sell. One trader reported having 20 sites from which he may pick up poultry.
- Only one trader (17%) goes to the same sites (two) to pick up chickens every day. The mean minimum number of pick-up sites per day is 1.2 (range 1-2, median 1), and the mean maximum number of sites is 4.5 (range 1-10, median 2.5).
- For live poultry traders that service backyard flocks (n=3), the average minimum number of birds transported each day is 18 (responses: 5, 5, 40 birds/day), and the average maximum number is 28 (responses: 10, 10, 50 birds/day).
- For traders that service large chicken and duck flocks, on average, the minimum number of birds transported each day is 933 (responses: 300, 1000, 1500birds/day), and the maximum number is 1033 (responses: 400, 1200, 1500 birds/day).
- The mean number of delivery sites to which traders deliver poultry is 1.8 per day (range 1-5, median 1). Most (83%) egg traders deliver to the same locations every day.
- Two of the 3 traders that transport backyard birds bring them to their homes for slaughter. One brings them to a market in another province. The traders that collect birds from large poultry facilities deliver them to one or multiple slaughterhouses.
- Two of the backyard poultry traders informed us that they are the only ones that do the poultry collection and slaughter in their area on a regular basis, but before Chinese New

Year, many other people collect poultry from their villages and bring it to slaughter for sale the day before the festival.

Slaughterhouses

We interviewed five people that slaughter poultry to inform the agent-based model. The interviewed persons all live in Muang District, in Don Kam Yan (n=2), Suan Taeng (n=1), Ban Pho (n=1) and Rua Yai (n=1) Subdistricts. Subdistrict livestock officers do not know how many persons slaughter in their areas. There are no large chicken or duck slaughterhouses in Don Kam Yan, Suan Taeng or Sala Khao Subdistricts, although there are a small number of small household slaughterhouses in these areas. These are some of the interviews summarized below. The small slaughterhouses do not need to be registered, and there is no regulation of the industry at the household level. We interviewed one large quail slaughterhouse owner. This location contributes to the large average number of birds per delivery. Most large chicken and duck slaughterhouses are located outside of Suphanburi Province. A summary of the results follows. The percentages are calculated from the non-missing responses for each category.

- The slaughterhouses are slaughter chickens (n=3, 60%), chickens and ducks (n=1, 20%) or quail (n=1, 20%).
- The mean number of slaughter days per week is 6.1 (range 2.5-7, median 7).
- On average, the minimum number of origination sites for the poultry slaughtered at each facility each day is 2.5 (range 1-8, median 2), and the maximum number is 4 (range 1-10, median 2.5).
- The mean minimum number of live deliveries per week is 4.6 (range 0-7, median 7), and the maximum number is 6.4 (range 1-14, median 7).
- The quail slaughterhouse has 3,000 birds brought for slaughter per delivery. The other slaughterhouses have an average minimum of 66 birds per delivery (range 5-100, median 80) and an average maximum of 90 (range 10-150, median 100).

Pictures of Thai Poultry Holdings and Poultry-Related Activities

FGD Flocks

Figure D. 1 Pictures of free-grazing ducks

Backyard Chicken Flocks

Figure D. 2 Pictures of backyard chickens

Fighting Cocks

Figure D. 3 Pictures of fighting cocks and a practice fight arena

Small Duck Flocks


Figure D. 4 Pictures of small duck flocks

Duck and Chicken Layer Farms, Sector 3


Figure D. 5 Pictures of Sector 3 layer farms

Egg Traders


Figure D. 6 Pictures of local egg traders

Live Poultry Traders


Figure D. 7 Pictures of live poultry traders

Appendix E: Agent-Based Model Agent Rules and NetLogo Code

Rules for Agents in the Local Thai Poultry Sector Agent-Based Model

Table E. 1 Agent rules for FGD flocks and owners

Rule	FGD Flocks	FGD Owners
1	Determine if will move daily from home to field or stay on field.*	Set pickup, delivery or both for the flock's eggs.*
2	Set target rice field for the day. Acceptable fields have sufficient feed supply (rai), and closest acceptable field to present location is field for day. Same field is visited daily until feed supply depleted, then new location selected.	If eggs to be picked up, set number of traders to deal with.*
3	If moving from home to field, move to the field to graze for the day, over the road system, choosing the shortest path.	If eggs to be delivered, set number of days/ week to bring to market.*
4	If infectious, contaminate the roadway with feces and other infectious debris.	Set number of days/ week to make visits to neighbors.*
5	If infectious, contaminate the rice field where grazing.	If flock is infectious, cause infection of neighbors based on sampling from distribution of Pr(inf) for visitors.
6	When infected, move through SEIR transition using duck shedding period.	

* Rules that are established at model setup and hold their values/ links for the entire simulation

Table E. 2 Agent rules for small duck flocks and owners

Rule	Small (Penned) Duck Flocks	Small Duck Flock Owners
1	Set duck type as egg or meat.*	If egg ducks, set pickup, delivery or both for eggs.*
2	If home patch is adjacent to road contaminated with infectious debris from traveling FGD flock, become infected based on Pr(inf) by contaminated road.	If eggs to be picked up, set number of days/ week to sell.*
3	When infected, move through SEIR transition using duck shedding period.	If eggs to be picked up, set trader number to 1.*
4		If eggs to be delivered, set number of days/ week to bring to market.*
5		Set number of days/ week to make visits to neighbors.*
6		If flock is infectious, cause infection of neighbors based on sampling from distribution of Pr(inf) for visitors.

* Rules that are established at model setup and hold their values/ links for the entire simulation

Table E. 3 Agent rules for layer flocks and owners

Rule	Chicken and Duck Layer Flocks	Layer Farm Owners
1	Set poultry type as chicken or duck.*	Set pickup, delivery or both for eggs.*
2	If home patch is adjacent to road contaminated with infectious debris from traveling FGD flock, become infected based on Pr(inf) by contaminated road.	If eggs to be picked up, set number of days/ week to sell.*
3	When infected, move through SEIR transition using duck or chicken shedding period, accordingly.	If eggs to be picked up, set number of traders to deal with.*
4		If eggs to be delivered, set number of days/ week to bring to market.*
5		Set number of days/ week to make visits to neighbors.*
6		If flock is infectious, cause infection of neighbors based on sampling from

			distribution of Pr(inf) for visitors.

* Rules that are established at model setup and hold their values/ links for the entire simulation

Table E. 4 Agent rules for backyard chicken flocks, backyard chicken owners and fighting cock owners (subset of backyard chicken owners)

Rule	Backyard Chicken Flocks	Backyard Flock Owners	Fighting Cock Owners
1	If home patch is adjacent to road contaminated with infectious debris from traveling FGD flock, become infected based on Pr(inf) by contaminated road.	Determine if own fighting cocks; if so also follow rules for fighting cock owners.*	Set chosen practice arena as the closest practice arena.*
2	If owner returns with infected fighting cock, become infected based on Pr(inf) by returning cock.	Set number of days/ week to make visits to neighbors.*	Go to practice arena with fighting cock on Wednesdays once every 5 weeks. *
3	When infected, move through SEIR transition using chicken shedding period.	If flock is infectious, cause infection of neighbors based on sampling from distribution of Pr(inf) for visitors.	Set match frequency at twice yearly *
4			On Sundays, go to match arena only if it is agent's week to compete.
5			If an infectious fighting cock is at arena, become infected based on Pr(inf) for arena.

* Rules that are established at model setup and hold their values/ links for the entire simulation

Table E. 5 Agent rules for egg traders

Rule	Egg Traders	Live Poultry Traders
1	Set target market randomly.*	Set number of days per week to pick up live birds.*
2	Establish number of suppliers/day (*s*).*	Each collecting day, make new trading links with 4 poultry suppliers at minimum distance to trader's home patch.
3	Make links with *s* suppliers in the model that haven't reached trader quota.*	Go to all suppliers for that day to pick up birds.
4	Each day go to all suppliers that need pickup.	If flock at pickup site is infectious, become contaminated with influenza virus based on sampling from distribution of Pr(contam) for live traders.
5	If flock at pickup site is infectious, become contaminated with influenza virus based on sampling from distribution of Pr(contam) for egg traders.	If contaminated, when at supplier with susceptible birds, cause infection based on sampling from distribution of Pr(inf) for live traders.
6	If contaminated, when at supplier with susceptible birds, cause infection based on sampling from distribution of Pr(inf) for egg traders.	After collection from all suppliers, return home to slaughter birds, remove supplier links.
7	After collection from all suppliers with pickups that day, go to assigned market.	If contaminated with influenza virus by infectious flock, set status to uncontaminated at end of day.
8	If contaminated with influenza virus by infectious flock, set status to uncontaminated at end of day.	

* Rules that are established at model setup and hold their values/ links for the entire simulation

Appendix F: Summary of Expert Opinion Elicitation for Thai Local Poultry Sector Agent-Based Model

Table F. 1 Transmission probability estimates from subject matter experts used to inform the agent-based model (Chapter 5)

Probability that…	Components	Probability Estimates	Expert 1	Expert 2	Expert 3	Aggregate (mean)*
Fecal material and feathers deposited by infectious FGD on road (walk/ truck) will infect backyard poultry along route	Direct contact between FGD and backyard chickens	Maximum	80%	80%	70%	71%
		Minimum	20%	50%	30%	29%
		Most Likely	50%	75%	50%	55%
	Indirect contact between feces, feathers and backyard chickens-	Maximum	40%	80%	70%	
		Minimum	0%	50%	30%	
		Most Likely	25%	75%	50%	
Egg trader will become contaminated when on infected premises	Direct contact between trader and eggs, chickens/ducks when collecting eggs from barn	Maximum	100%	100%	90%	88%
		Minimum	50%	70%	60%	53%
		Most Likely	75%	85%	75%	70%
	Indirect contact with feces in yard/ barn	Maximum	100%	60%	80%	
		Minimum	50%	40%	50%	
		Most Likely	75%	45%	65%	
Live poultry trader will become contaminated when on infected premises	Direct contact between trader and chickens/ ducks when collecting birds	Maximum	100%	100%	90%	95%
		Minimum	70%	80%	60%	68%

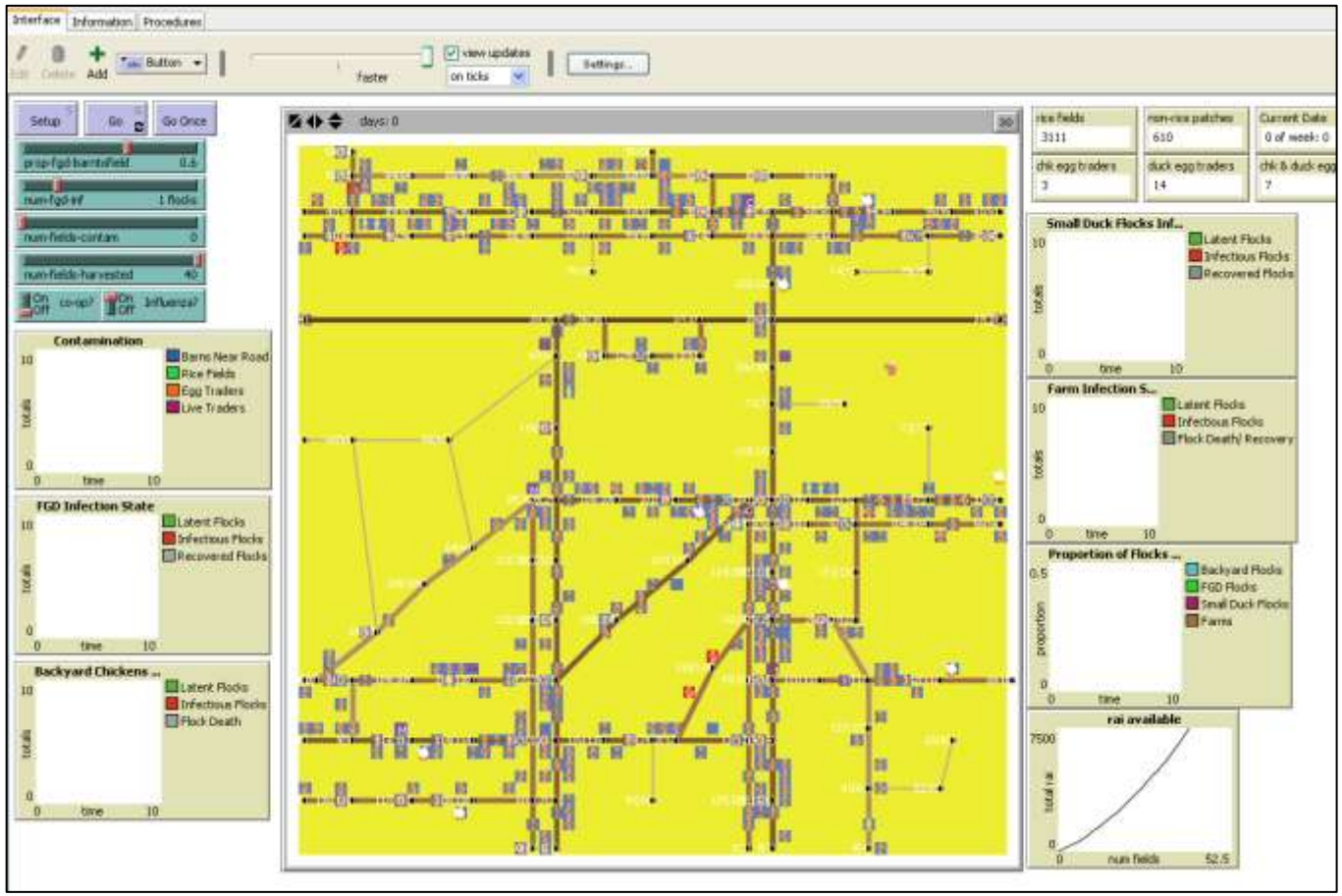
		Most Likely	90%	90%	75%	83%
	Indirect contact with feces in yard/ barn, birds on truck	Maximum	100%	100%	80%	
		Minimum	70%	80%	50%	
		Most Likely	90%	90%	65%	
Egg trader will bring contaminated material to a new yard/ barn	Direct contact between trader and chickens/ ducks when collecting eggs	Maximum	80%	100%	100%	85%
		Minimum	40%	80%	70%	55%
		Most Likely	60%	90%	85%	70%
	Indirect contact by bringing contaminated material on shoes, clothes, vehicles	Maximum	80%	60%	90%	
		Minimum	40%	40%	60%	
		Most Likely	60%	50%	75%	
Live poultry trader will bring contaminated material to a new yard/ barn	Direct contact between trader and chickens/ ducks when collecting birds	Maximum	100%	100%	100%	92%
		Minimum	70%	80%	80%	68%
		Most Likely	90%	90%	90%	82%
	Indirect contact by bringing contaminated material on shoes, clothes, vehicles	Maximum	100%	60%	90%	
		Minimum	70%	40%	65%	
		Most Likely	90%	50%	80%	
Poultry owners will become contaminated during visits to infected premises and will expose their flock	Indirect contact with feces in yard/ barn	Maximum	20%	40%	100%	53%
		Minimum	0%	10%	95%	35%
		Most Likely	10%	20%	98%	43%

Contaminated poultry owners from infected premises will cause infection in another flock during a visit	Indirect contact with feces in yard/ barn	Maximum	40%	40%	100%	60%
		Minimum	10%	10%	80%	33%
		Most Likely	20%	20%	100%	46%
Infectious fighting cock will expose its opponent during 120 minutes of fighting	Direct contact with infectious bird and body fluids and indirect contact by being in stressful environment near other birds	Maximum	40%	20%	100%	57%
		Minimum	20%	0%	90%	38%
		Most Likely	30%	10%	100%	48%
Newly exposed and infected fighting cock will infect rest of flock at home	Direct and indirect contacts within flock	Maximum	100%	100%	100%	100%
		Minimum	50%	60%	90%	67%
		Most Likely	75%	80%	95%	83%

* The aggregate estimates are the mean of the direct and indirect contact estimates for each expert, averaged over the three experts.

Appendix G: NetLogo Code and GUI for Baseline Agent-Based Model of the Local Thai Poultry Sector

Figure G. 1 Graphic user interface of NetLogo for the Thai local poultry sector agent-based model described in Chapter 5



NetLogo Programming Code for the Agent-Based Model Simulating Influenza Transmission in the Thai Local Poultry Sector

```
extensions [table]

;;human-types
breed [eggtraders eggtrader]          ;; egg traders
breed [livetraders livetrader]        ;; live poultry traders
breed [byowners byowner]              ;; backyard poultry owners (the owners move w/out poultry to other households,   market, arena, etc)
 breed [farmowners farmowner]                ;; owners of large farms
breed [byduckowners byduckowner]          ;; owners of small duck flocks/ backyard ducks
breed [fgd-owners fgd-owner]              ;; FGD owners (can move w/out ducks to households, egg cooperative, market, etc)
;;poultry-types
breed [fgds fgd]                      ;; free-grazing duck flocks (FGD)
breed [bychickens bychicken]          ;; backyard chicken flocks (BYC)
breed [byducks byduck]                ;; penned ("backyard") duck flocks (BYD)
breed [farms farm]                    ;; layer farms, duck or chicken
;;inanimate-types
directed-link-breed [egg-links egg-link]              ;; links between egg traders and pickup flocks
directed-link-breed [livebird-links livebird-link]    ;; links between poultry traders and pickup flocks
undirected-link-breed [fgd-links fgd-link]            ;; links between FGD flocks and their owners
undirected-link-breed [newroads newroad]              ;; links that serve as roads for movement of FGDs
breed [intersections intersection]                    ;; intersections positioned to link roads

 globals
[
  ;; Constants                  ;; number of each agent type
  N-FGD
  N-LIVETRADERS
  N-FGDOWNERS
  N-BYOWNERS
  N-FCOWNERS
  N-FARMOWNERS
```

```
N-BYDUCKOWNERS
N-BARNS
N-PRACTICE-ARENAS
N-MATCH-ARENAS
N-MARKETS
N-SLAUGHTERHOUSES
N-COOPERATIVES
RAI-PER-DUCK                ;; per duck daily consumption rate

location-datafile           ;; string, filename for location data
;; prop-fgd-barntofield     ;; (slider) controls pct of fgds in barns and on field only
num-bychick-inf
num-byduck-inf
num-farm-inf

COLOR-FALLOW
COLOR-HARVESTED
COLOR-GROWING

debug

runsim?                     ;; boolean for simulation control
stop-sim-reason             :: string for diagnostics

;; patch agentsets
rice-fields                 ;; agentset containing the patches that can belong to rice fields, which can be in 3 states
access-fields               ;; agent set of num-fields-harvested rice-field patches; these provide field access
roads                       ;; patch agentset containing the patches that are roads
barns                       ;; patch agentset containing the patches that are FGD barns
edge-patches                ;; patch agentset for the edge of the world
agents
match-arenas
```

```
  practice-arenas
  markets
  slaughterhouses
  cooperatives
  out-of-subdistrict-patches
  arenas
;; non-patch agentsets
  human-types
  humans
  fcowners
  livebird-suppliers
  poultry-types
  poultry-owners
  trader-types

  ;; influenza toggle switch will control whether influenza transmission is included or not (on or off,           respectively)
  ; field-infection-prob       ;; probability that an exposed flock will be infected by a rice field
  latent-period               ;; length of the latent period in days
  shedding-period-duck        ;; length of the shedding period in days for ducks
  shedding-period-chicken     ;; length of the shedding period in days for chickens
  road-contam-period          ;; time that the road will stay contaminated with viable virus
  trader-contam-period        ;; time that traders will stay contaminated after contact with infectious birds
  field-contam-period         ;; time that fields will stay contaminated after infected FGD flock on there

  road-infect-byc-prob        ;; probability that BYC flock infected via infected FGD flocks passing on road
  road-infect-pen-prob        ;; probability that BYD or farmed layer flock infected via flocks passing on road
  flock-eggtrader-contam-prob       ;; probability that an egg trader will be contaminated when picking up eggs from an infected premises
  live-trader-contam-prob           ;; probabiltiy that a live trader will be contaminated when picking up birds from an infected premises
  eggtrader-flock-infect-prob       ;; probability that a contaminated egg trader will cause infection of a susceptible flock he visits
  livetrader-flock-infect-prob      ;; probability that a contaminated live trader will cause infection of a susceptible flock he visits
  from-visit-infect-prob            ;; probability that a visitor that goes to an infected flock will infect its susceptible flock
  to-visit-infect-prob        ;; prob that someone from an infectious flock will infect a neighbor's flock
```

```
  arena-fc-infect-prob       ;; probability that a FC becomes infected while at the arena
  fc-bkyd-infect-prob        ;; probability that a FC infected at the arena will infect his home flock

  ;; Tables
  days-on-field               ;; table mapping FGD's who numbers to lists
  transmissions              ;; table mapping transmission types to daily numbers
  contaminations             ;; table mapping contamination types to daily numbers

  ;; calendar variables
  DAY-NAMES
  LIVETRADER-PICKUP-SCHEDULE  ;; list, specifies for each week day who will do live-bird pickup
  DAY-SCHEDULE               ;; list, specifies for each day of the week which flocks want egg pickup
  day-idx                    ;; int in range 0 to 6
  day-name                   ;; string, one item of DAY-NAMES (i.e., a weekday name)
  week
  livetrader-pickup-days     ;; sub-list of LIVETRADER-PICKUP-SCHEDULE, updated by update-calendar
  eggtrader-pickup-days      ;; sub-list of DAY-SCHEDULE, updated by update-calendar
  visit-days                 ;; sub-list of DAY-SCHEDULE, updated by update-calendar
]

newroads-own [
  roadtype
  road-patches ]

intersections-own [
  name
  int_type ]

patches-own [
  rai
  field-contaminant          ;; extent of contamination in [0,1] in rice field
                             ;; state 1: harvested: These are the fields that duck flocks move to
```

```
                        ;; state 2: growing. Ducks do not graze in these fields
                        ;; state 3: fallow. After ducks graze, fields are left until the next planting
  field-patches         ;; agentset of patches; ``nobody`` for all but access fields
  field-num             ;; unique field identifier
  field-state           ;; "", "harvested", "growing", or "fallow"

  occupant              ;; field is the target of occupant (who may not be physically present)
  road-contam-time      ;; time (days) road has been contaminated (+ 1 per tick)
  field-contam-time     ;; time (days) field has been contaminated (+ 1 per tick)
  patch-type            ;; rice field, barn, market, practice-arena, match-arena, etc
  road-access
  pcontaminated?        ;; patches will be contaminated if next to a road with infectious ducks passing by; rice field contaminated by grazing infectious FGD
                        flock
  infected-outsider-FC-here?        ;; is there an infected fighting cock (FC) at the arena?
  my-practice-group
  practice-arena-number
]

turtles-own             ;; attributes common to all agent types
[
infectious?              ;; is the agent infectious?
can-contaminate?        ;; can this agent contaminate patches if infectious?
home-patch              ;; location of barn for each FGD flock; household and yard for backyard poultry; household and yard for FC; household/home
                        slaughterhouse for local traders
egg-pickup?              ;; does the turtle have eggs picked up?
  egg-delivery?          ;; does the turtle deliver eggs to the market?
  onsite-trader-num     ;; number of traders that come to flock to pick up eggs
  eggs-gone?            ;; eggs have been picked up for the day
  egg-type              ;; can be duck, chicken or both egg types
  livebirds-gone?       ;; has this turtle had live poultry collected?
  livebird-selldate     ;; date of last live poultry sale
]
```

```
fgds-own                ;; attributes held by FGD flock agents
[
  owner                  ;; turtle, the owner of the FGD flock
  n-ducks                ;; number of ducks in flock
  exposed?               ;; exposed but not necessarily infected
  latent?                ;; infected but not yet infectious
  recovered?             ;; no longer infectious
  transport-type         ;; ducks transported by walking or by truck
  target-field           ;; target is a harvested rice field (i.e., a dayfield)
  infection-time         ;; "days" since infection; Increases by one day with each tick.
  shedding-time          ;; shedding time (infectious period)
  target-road-access
  field-only?
]

fgd-owners-own          ;; attributes held by FGD owner agents
[

  pickup-days           ;; days that eggs are picked up by trader
  delivery-days         ;; days that eggs are delivered by owner to market/ egg cooperative
  links-in              ;; number of trader that come to collect eggs
  target-cooperative    ;; location to which owner delivers eggs
  target-market         ;; location to which owner delivers eggs
  contaminated?         ;; is owner contaminated with influenza-containing debris?
  visit-list            ;; patch agentset of people owner will visit on each day
  n-visit-days-per-week ;; number of days each week owner visits neighbors
  field-only?           ;; does this owner keep the flock on the field day and night?
  own-infected-flock?   ;; does this owner own an infected flock?
  exposed?
  latent?
  recovered?
```

```
  infection-time ]

bychickens-own
[
  exposed?
  latent?
  recovered?
  death?                    ;; when chicken flocks become “recovered”, are dead
  infection-time
  shedding-time ]

byowners-own
[
  contaminated?
  target-visit
  visit-list
  n-visit-days-per-week
  own-fightingcocks?
  target-practice-arena
  target-match-arena
  links-in
  pickup-days
  delivery-days
  practice-fight-week           ;; 0 or 1 for even or odd weeks
  practice-fight-week?
  match-fight-week?
  own-infected-flock?
  latent?
  recovered?
  infection-time
  shedding-time
  own-infected-fc?
```

```
]

farms-own
[
  exposed?
  latent?
  recovered?
  death?
  infection-time
  shedding-time  ]

farmowners-own
[
  contaminated?
  target-visit
  visit-list
  n-visit-days-per-week
  links-in
  pickup-days
  delivery-days
  target-cooperative
  target-market
  own-infected-flock?
]

byducks-own
[
  exposed?
  latent?
  recovered?
  infection-time
  shedding-time
```

```
  byduck-type  ]              ;; duck type is egg or meat

byduckowners-own
[
  contaminated?
  target-visit
  visit-list
  n-visit-days-per-week
  links-in
  pickup-days
  delivery-days
  target-market
  own-infected-flock?
  byduck-type ]

eggtraders-own
[
  contaminated?               ;; has egg trader been contaminated at an infected premises?
  target-eggfarm              ;; next pickup location for egg trader
  collecting?                 ;; egg trader actively collecting eggs
  n-eggtrader-links           ;; number of flocks from which the egg trader gets eggs
  trader-contam-time          ;; time (days) trader has been contaminated (+1 with each tick)
  target-market ]             ;; market to which egg trader will deliver eggs


livetraders-own
[
  target-livefarm             ;; next location for poultry pick up
  contaminated?               ;; has poultry trader been contaminated at an infected premises?
  livetrader-links            ;; number of flocks from which the trader gets poultry
  n-bird-pickup-days          ;; number of days/week that the trader collects poultry
  trader-contam-time
```

```
]


;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;    UTILITIES   ;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;

;; return a single draw from a binomial distribution:

to-report binomial [n p] ;; written by Seth Tisue
  report length filter [? < p] n-values n [random-float 1]
end

;; return a result (value) based on the defined probabilities (#weights) of each value written by Nick Bennet 2009 http://groups.yahoo.com/group/netlogo-users/message/9091:

to-report random-weighted [#values #weights]
  let selector (random-float sum #weights)
  let running-sum 0
    (foreach #values #weights [
      set running-sum (running-sum + ?2)
      if (running-sum > selector) [
        report ?1
      ]
    ])
end

;;``split`` was written by Jim Lyons (2007):

to-report split [ #string #sep ] ; #sep must be non-empty string
  let result [] ; return value
  let w length #sep
```

```
  loop ; exit when done
  [ let next-pos position #sep #string
  if not is-number? next-pos
  [ report reverse (fput #string result) ]
  set result fput (substring #string 0 next-pos) result
  set #string substring #string (next-pos + w) (length #string) ]
end

;; use law of sines to report determine minimum distance from patch to link (no wrapping):

to-report patch-near-link? [#patch #link]
  let max-distance 1
  let eps 0.0001
  set max-distance 1 + eps
  let dist 0
  let angles (list )
  let result true
  ask #link
    [
      ask both-ends
      [
        if (patch-here != #patch)
        [
          let angle abs (subtract-headings towards #patch towards other-end)
          ifelse (angle > 90)
          [set result false]
          [
            set dist (sin angle) * distance #patch
            if (dist > max-distance) [set result false]
          ] ] ] ]
  report result
end
```

```
;use pert params to draw from a beta distribution:

to-report random-pert [#minval #likeval #maxval]
    let pert-var 1. / 36
  let pert-mean (#maxval + 4 * #likeval - 5 * #minval) / (6 * (#maxval - #minval))
  let temp pert-mean * (1 - pert-mean) / pert-var
  let alpha1 pert-mean * (temp - 1)
  let alpha2 (1 - pert-mean) * (temp - 1)
  let x1 random-gamma alpha1 1
  let x2 random-gamma alpha2 1
  report (x1 / (x1 + x2)) * (#maxval - #minval) + #minval
end

to-report random-visit-int
  report (random-pert 0 1 10)
end

to export-transmissions-and-contaminations
  file-open "transmissions-and-contaminations.txt"
  let %ct behaviorspace-run-number
  ;file-print "bsnum,t-or-c,type,sum"
  foreach table:keys transmissions [
    file-print (word %ct ",transmission," ? "," (sum table:get transmissions ?))
  ]
  foreach table:keys contaminations [
    file-print (word %ct ",contamination," ? "," (sum table:get contaminations ?))
  ]
  file-close
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
```

```
;;  SETUP PROCEDURES  ;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

;;;;;;;;;;;;;;;
;;Globals;;;
;;;;;;;;;;;;;;;

to startup                      ;;  this procedure will run automatically when the model loads
  set-default-shape byowners "person"
  set-default-shape eggtraders "car"
  set-default-shape fgds "duck"
  set-default-shape fgd-owners "person"
  set-default-shape livetraders "person"
  set-default-shape farmowners "person"
  set-default-shape byduckowners "person"
  set-default-shape livetraders "truck"
  set-default-shape farms "farm"
  set-default-shape byducks "bird side"
  set-default-shape bychickens "backyard"
end


to setup                        ;; setup the display with globals, patches, agents
  clear-turtles
  clear-patches
  clear-drawing
  clear-all-plots
  clear-output

  ;;preliminaries
  set debug 0                   ;; set to 0 during Bahavior Space to speed runs
  if (debug > 5) [print "enter proc: setup"]
```

```
  set runsim? true              ;; set runsim? false for programmatic stop of simulation
  set stop-sim-reason ""

  ;; file for event logging (please do not delete)
  carefully [file-delete "birdflu-xxx.log"] []
  file-open "birdflu-xxx.log"
  file-print date-and-time
  file-print ""
  file-close


  ;;define agent types
  set human-types (list eggtraders livetraders byowners farmowners byduckowners fgd-owners fcowners)
  set humans turtles with [member? breed human-types]
  set poultry-types (list fgds bychickens byducks farms)
  set trader-types (list eggtraders livetraders)

  setup-globals
  setup-newroads  ;;must come before setup-patches!
  setup-patches   ;;calls place-along-roads!
  setup-agents
  ask fgds [table:put days-on-field ([who] of self) (list )]  ;;initialization of table
  reset-ticks  ;;needed at the end of setup, as of version 5
  do-plots   ;chk: plot initial state
  if (debug > 5) [print "exit proc: setup"]
  initialize-locations-file
  test-setup
end

to initialize-locations-file              ;; creates the file for transmission locations and records initial locations
   let %fnum (word "000" behaviorspace-run-number)
  let %len length %fnum
```

```
  let %fname (word "locationdata" substring %fnum (%len - 3) %len ".txt")
  set location-datafile %fname
  carefully [file-delete %fname][]
  file-open %fname
  file-print "positions after setup:"
  ask turtle-set poultry-types [
    if (latent? or infectious?) [file-type "infected "]
    file-show patch-here]
  file-close
end

;; Setup the enumerations
to setup-globals                    ;; set values of constants
  set N-FGD 9
  set N-FGDOWNERS 9
  set RAI-PER-DUCK 0.005            ;; median value from large flock survey
  set N-LIVETRADERS 4
  set N-BYOWNERS 250
  set N-FCOWNERS 201
  set N-FARMOWNERS 9
  set N-BYDUCKOWNERS 80
  set N-BARNS 360
  set N-MATCH-ARENAS 1
  set N-PRACTICE-ARENAS 3
  set N-MARKETS 3
  set N-COOPERATIVES 1
  set N-SLAUGHTERHOUSES 3

  set num-bychick-inf 0             ;; set number of initially-infected poultry
  set num-byduck-inf 0
  set num-farm-inf 0
  set latent-period 1
```

```
  set field-infection-prob 100
  set road-contam-period 1
  set field-contam-period 30
  set trader-contam-period 1

  set arena-fc-infect-prob random-pert 38 48 57
  set fc-bkyd-infect-prob random-pert 67 83 100

  set access-fields nobody                ;; field initialization

  set days-on-field table:make          ;; make tables
  set transmissions table:make
  foreach ["road-flock" "after-visit" "visitor-flock" "eggtrader-flock" "livetrader-flock" "arena-fc" "fc-    flock"] [  table:put transmissions ? (list ) ]
  set contaminations table:make
  foreach ["flock-livetrader" "flock-eggtrader" "flock-visitor" "flock-road" "flock-field"] [
    table:put contaminations ? (list ) ]

  ;; calendar variables
  set DAY-NAMES ["Monday" "Tuesday" "Wednesday" "Thursday" "Friday" "Saturday" "Sunday"]
  set LIVETRADER-PICKUP-SCHEDULE [[3] [2] [3] [2] [1 3] [] []]
  set DAY-SCHEDULE [[3 5 7] [1 2 5 7] [3 5 7] [2 5 7] [3 5 7] [7] [7]]

  setup-bs-globals              ;; globals for behavior space utility
end

to setup-bs-globals
  ;; ROAD-INFECT-PEN-PROB
  if (road-infect-pen-prob = 0) [ set road-infect-pen-prob random-pert 0 25 40 ]
  if (road-infect-pen-prob = 999) [ set road-infect-pen-prob  0 ]              ;;test with zero value
   ;; ROAD-INFECT-BYC-PROB
  if (road-infect-byc-prob = 0) [ set road-infect-byc-prob random-pert 29 55 71 ]
  if (road-infect-byc-prob = 999) [ set road-infect-byc-prob  0 ]
```

```
  ;;FROM-VISIT-INFECT-PROB
  if (from-visit-infect-prob = 0) [ set from-visit-infect-prob random-pert 5 15 30 ]
  if (from-visit-infect-prob = 999) [ set from-visit-infect-prob  0 ]
  ;;TO-VISIT-INFECT-PROB
  if (to-visit-infect-prob = 0) [ set to-visit-infect-prob random-pert 10 20 40]
  if (to-visit-infect-prob = 999) [ set to-visit-infect-prob  0]
  ;; FLOCK-EGGTRADER-CONTAM-PROB
  if (flock-eggtrader-contam-prob = 0) [ set flock-eggtrader-contam-prob random-pert 53 70 88]
  ;; EGGTRADER-FLOCK-INFECT-PROB
  if (eggtrader-flock-infect-prob = 0) [ set eggtrader-flock-infect-prob random-pert 55 70 85]
  ;; FLOCK-LIVETRADER-CONTAM-PROB
  if (live-trader-contam-prob = 0) [ set live-trader-contam-prob random-pert 68 83 95]
  ;; LIVETRADER-FLOCK-INFECT-PROB
  if (livetrader-flock-infect-prob = 0) [ set livetrader-flock-infect-prob random-pert 68 82 92]
  ;; CHICKEN SHEDDING PERIOD
  if (shedding-period-chicken = 0) [ set shedding-period-chicken 4]
  ;; DUCK SHEDDING PERIOD
  if (shedding-period-duck = 0) [ set shedding-period-duck 7]

end

;;;;;;;;;;;;;;;
;;Patches;;
;;;;;;;;;;;;;;;

to setup-newroads                              ;; make road system
  let nodes table:make  ;;
  file-open "intersections.txt"
  while [not file-at-end?]
  [
    let data file-read-line
    if not (first data = "#")
```

```
  [
    set data split data " "
    ;; cast the co-ordinates from strings to numbers
    let x read-from-string item 0 data
    let y read-from-string item 1 data
    ;; get the intersection name and intersection type
    let iname item 2 data
    let itype last data
    let ilocation list (item 0 data) (item 1 data)
    ask patch x y [
      sprout-intersections 1 [
        setxy x y
        table:put nodes iname self  ;;map name to intersection (node)
        set color black
        set shape "target"
        set size 0.5
        set label (word who "(" iname ")")
      ] ]
    ask intersections [set home-patch patch-here ]
  ] ]
file-close
print nodes
file-open "roads.txt"
while [not file-at-end?]
[
  let data file-read-line
  if not (first data = "#")   ;; prevent reading of the first line
  [
    set data split data " "   ;; split location indicated by a space
    let node1 table:get nodes item 0 data
    let node2 table:get nodes item 1 data
    ask node1 [create-newroad-with node2
```

```
      [
        set roadtype last data
      ]]
    ask newroads
      [
        if roadtype = "highway" [set color 33 set thickness 0.5]
        if roadtype = "major_road" [set color 34 set thickness 0.5]
        if roadtype = "loose_road" [set color 35 set thickness 0.5]
        if roadtype = "small_road" [set color 36 set thickness 0.5]
        if roadtype = "field_road" [set color 37 set thickness 0.25]
      ]
  ]
 ]
 file-close
 print "roads: "
 print newroads
 print [roadtype] of newroads
 ;;ai:20120118
 ask newroads [set road-patches patches with [patch-near-link? self myself]] ;;ai: every road keeps a set of neaby patches
 ask newroads with [roadtype = "major_road"] [ask road-patches [set pcolor red]]
 ask newroads with [roadtype = "loose_road"] [ask road-patches [set pcolor violet]]
 ask newroads with [roadtype = "small_road"] [ask road-patches [set pcolor pink]]
end

to setup-patches
 ask patches                          ;; initialization: all patches are growing rice fields
 [
   set patch-type "ricefield"         ;; this inital value will change when we change the patch type
   set field-state "growing"
   set field-patches nobody
   set occupant nobody
   set road-access min-one-of ( intersections )[ distance myself ]
```

```
  set pcontaminated? FALSE
]

setup-edge-patches

;;;; PLACE OBJECTS ALONG ROADS
let my-major-roads newroads with [roadtype = "major_road"]
let my-loose-roads newroads with [roadtype = "loose_road"]
let my-small-roads newroads with [roadtype = "small_road"]

;; place match arenas
place-along-roads N-MATCH-ARENAS "match-arena" my-loose-roads
set match-arenas patches with [ patch-type = "match-arena" ]
ask match-arenas [set infected-outsider-FC-here? FALSE ]

;; place practice arenas
place-along-roads-north (N-PRACTICE-ARENAS / 3) "practice-arena" my-loose-roads
place-along-roads-south (N-PRACTICE-ARENAS / 3) "practice-arena" my-loose-roads
place-along-roads (N-PRACTICE-ARENAS / 3) "practice-arena" my-loose-roads
set practice-arenas patches with [ patch-type = "practice-arena" ]
ask practice-arenas [set infected-outsider-FC-here? FALSE set practice-arena-number ""]

ask one-of practice-arenas [set practice-arena-number 1]
ask one-of practice-arenas with [practice-arena-number = ""] [set practice-arena-number 2]
ask one-of practice-arenas with [practice-arena-number = ""] [set practice-arena-number 3]

;; place markets
place-along-roads N-MARKETS "market" my-major-roads
set markets patches with [ patch-type = "market" ]

;; place cooperatives
place-along-roads-north N-COOPERATIVES "cooperative" my-major-roads
```

```
  set cooperatives patches with [ patch-type = "cooperative" ]

 ;; place barns (60%, 30% and 10% around major, loose and small roads)
  place-along-roads round (0.6 * N-BARNS) "barn" my-major-roads
  place-along-roads round (0.3 * N-BARNS) "barn" my-loose-roads
  place-along-roads round (0.1 * N-BARNS) "barn" my-small-roads
  set barns patches with [ patch-type = "barn" ]

  ;; place out-of-subdistrict-patches
  set out-of-subdistrict-patches (patch-set patch -29 15 patch 29 15)
  ask out-of-subdistrict-patches [set patch-type "out" set field-state ""]

  setup-ricefields
  ask patches [ set-patch-visuals ]
end

to setup-edge-patches
  ;; make all edge patches growing fields so that turtles don't hit wall
  set edge-patches patches with [count neighbors != 8]
  ask edge-patches [
    set patch-type "edge"
    set field-state ""
  ]
end

to place-along-roads [#num #type #roads]
  let %patches ((patch-set [road-patches] of #roads) with [(not any? turtles-here) and (patch-type = "ricefield")])
  ask n-of #num %patches [
    set patch-type #type
    set field-state ""
  ]
end
```

```
to place-along-roads-north [#num #type #roads]
  let %patches ((patch-set [road-patches] of #roads) with [(not any? turtles-here) and (patch-type = "ricefield") and ( pycor > 0)])
  ask n-of #num %patches [
    set patch-type #type
    set field-state ""
  ]
end

to place-along-roads-south [#num #type #roads]
  let %patches ((patch-set [road-patches] of #roads) with [(not any? turtles-here) and (patch-type = "ricefield") and ( pycor < 0)])
  ask n-of #num %patches
  [
    set patch-type #type
    set field-state "" ]
end


to setup-ricefields ;;observer proc
  set rice-fields (patches with [ field-state = "growing" ])  ;;intialize global variable

  ;create-fields num-fields-harvested   ;; augments the ``access-fields`` global
  new-create-fields
  ask access-fields [
    harvest-field                        ;;changes field-state (and color), makes rai available
  ]

  show (word "total rai available at sim start is " sum [rai] of access-fields)
  show (word "this shd match " sum [rai] of patches)

  ;; make number of harvested fields determined by slider to be contaminated
  if influenza? [
```

```
    ask n-of num-fields-contam access-fields [
      contaminate-field ] ]
end

to new-create-fields  ;; observer proc
  if (debug > 5) [show "enter proc: create-fields"]
  let %avradius 9
  ;; create the field-access patches
  let %y min-pycor + (%avradius / 2)
  while [%y < max-pycor] [
    let %x min-pxcor + (%avradius / 2)
    while [%x < max-pxcor] [
      let %new-access-field patch %x %y
      if ([field-state] of %new-access-field = "growing") [
        set access-fields (patch-set access-fields %new-access-field)
      ]
      set %x (%x + %avradius)
    ]
    set %y (%y + %avradius)
  ]
  show (word (count access-fields) " access fields created")
  let %n-access-fields 0
  ;; number the access fields and set their size
  ask access-fields [
    set %n-access-fields (%n-access-fields + 1)
    set field-num %n-access-fields
    set rai random-pert 80 150 610
  ]
  ;; make minimal fields around the access patch
  ask access-fields [
    let %field patch-set [neighbors] of neighbors
    ask %field with [field-state = "growing" and field-num = 0] [ ;; does *not* include the access-field patch
```

```
    set field-num [field-num] of myself
   ]
  ]
  ;; fill in the fields
  ask access-fields [
   let %n-patches (rai / 2)
   let %radius ceiling sqrt (%n-patches / 3)
   let %field patches in-radius %radius with [field-state = "growing" and field-num = 0]  ;; does *not* include the access-field patch
   ask %field [
    set field-num [field-num] of myself
   ]
  ]
  ;; attach remaining rice-field patches to fields
  ask patches with [field-state = "growing" and field-num = 0] [
   let %field-patches patches with [field-state = "growing" and field-num != 0]
   set field-num [field-num] of min-one-of %field-patches [distance myself]
  ]
  ask access-fields [
   set field-patches (patches with [field-num = [field-num] of myself])    ;; *include* the access patch
  ]
  if (debug > 5) [show "exit proc: create-fields"]
end


; to create-fields [#num]  ;; observer proc
;   if (debug > 5) [show "enter proc: create-fields"]
;   ;; randomly create growing fields
;   let %new-access-fields n-of #num rice-fields with [field-state = "growing" and field-num = 0]
;   set access-fields (patch-set access-fields %new-access-fields)
;   ;; number the field-access patches
;   ask %new-access-fields [
;     set n-access-fields (n-access-fields + 1)
```

```
;    set field-num n-access-fields               ;; unique field identifier
;   ]
;   ;; create the fields
;   ask %new-access-fields [
;    let %field patch-set [neighbors] of neighbors
;    set %field %field with [field-state = "growing" and field-num = 0]  ;; does *not* include the access-field patch
;    ask %field [
;      set field-num [field-num] of myself
;    ]
;    set field-patches (patch-set self %field)    ;; *include* the access patch
;   ]
;   if (debug > 5) [show "exit proc: create-fields"]
; end

to harvest-field                              ;;patch (access field) proc
  if (debug > 3) [show "enter proc: harvest-field"]
  if (field-state != "growing") [ __error (word "field-state '" field-state "' shold be 'growing'")]
  if (field-patches = nobody) [ __error "apply harvest-field proc only to access fields"]
  set rai random-pert  80 150 610
  show (word "provides access to " rai " rai")
  ask field-patches [ set field-state "harvested" set pcolor COLOR-HARVESTED]
  if (debug > 3) [show "exit proc: harvest-field"]
end

to fallow-field                               ;;patch (access field) proc
  if (debug > 3) [show "enter proc: fallow-field"]
  if (field-state != "harvested") [
    let %msg (word "patch " self " has field-num " field-num " and field-patches " field-patches)
    show %msg
    __error (word %msg "field-state '" field-state "' shold be 'harvested'")
  ]
  if (field-patches = nobody) [ __error "apply fallow-field proc only to access fields"]
```

```
  set rai 0               ;; "wasted rai" chk!
  ask field-patches [ set field-state "fallow" set pcolor COLOR-FALLOW]
  if (debug > 3) [show "exit proc: fallow-field"]
end

to set-patch-visuals          ;; initialize color globals (for patches that may change color)
   set COLOR-FALLOW 48
  set COLOR-HARVESTED yellow
  set COLOR-GROWING 57 ;; light green
  ifelse (field-state != "")
  [
    if (field-state = "fallow") [ set pcolor COLOR-FALLOW ]
    if (field-state = "harvested") [ set pcolor  COLOR-HARVESTED]
    if (field-state = "growing") [ set pcolor COLOR-GROWING ]
  ]
  [
    if (member? self match-arenas) [ set pcolor red set plabel "A"]
    if (member? self practice-arenas) [ set pcolor red set plabel "P" ]
    if (member? self barns) [ set pcolor (blue + 1) ]
    if (member? self markets) [ set pcolor violet set plabel "M"]
    if (member? self cooperatives) [ set pcolor violet set plabel "C"]
    if (member? self edge-patches) [ set pcolor white ]
    if (member? self out-of-subdistrict-patches) [set pcolor brown set plabel "OUT"]
  ]
end

;;;;;;;;;;;;;;;
;; Agents ;;
;;;;;;;;;;;;;;;

to setup-agents
  if (debug > 3) [show "enter proc: setup-agents"]
```

```
setup-livetraders
setup-fgds

;;BYOWNERS: sprout backyard poultry owners from barn patches, assign a certain number to have fighting cocks
ask n-of N-BYOWNERS barns with [ not any? turtles-here ] [
  sprout-byowners 1 [ set home-patch patch-here init-byowner ]
  sprout-bychickens 1 [ init-bychickens ]
]
ask n-of N-PRACTICE-ARENAS practice-arenas with [ not any? turtles-here ] [
  sprout-byowners 1 [ set home-patch patch-here init-byowner ]
  sprout-bychickens 1 [ init-bychickens ]
]
ask n-of num-bychick-inf bychickens [infect-initial]

set fcowners (turtle-set (byowners with [patch-type = "practice-arena"])
(n-of (N-FCOWNERS - N-PRACTICE-ARENAS) (byowners with [patch-type = "barn"])))
ask fcowners [ set own-fightingcocks? true ]            ;; fcowners membership won't change

;;BYDUCKOWNERS: sprout backyard duck owners from barn patches
ask n-of N-BYDUCKOWNERS barns with [ not any? turtles-here ] [
  sprout-byduckowners 1 [ set home-patch patch-here init-byduckowner ]
  sprout-byducks 1 [ init-byducks ]
]
ask n-of num-byduck-inf byducks [infect-initial]        ;; set some of the small duck flocks as infectious

;;FARMOWNERS: sprout farm owners from barn patches
ask n-of N-FARMOWNERS barns with [ not any? turtles-here ] [
  sprout-farmowners 1 [ set home-patch patch-here init-farmowner ]
  sprout-farms 1 [ init-farms ]
]
ask n-of num-farm-inf farms [infect-initial]        ;; set some of the egg farms as infectious
```

```
  ;;;; Establish visit-lists for all poultry owners, no visit-list for eggtraders and livetraders)
; let humans turtles with [member? breed human-types]
  set poultry-owners (turtle-set byowners fgd-owners byduckowners farmowners)
  ask poultry-owners [
    let my-home-patch home-patch
    ;; visit-list is a patch-set
    ;; (note: all neighbors within a radius of 10 are equally likely to be in the visit-list)
    set visit-list min-n-of random-visit-int
      (patch-set [home-patch] of other turtles with [ member? breed human-types])
      [int (distance my-home-patch / 10)]
    if (debug >= 5) [ show (word "turtle " who " with type " breed " has visit list: " [self] of visit-list) ]
    if (debug >= 5) [ ask visit-list [show (word "\tdistance: " (distance myself))] ]
  ]

  set-egg-types
  set-egg-transfer-days
  make-egg-pickup-network
  set-eggtrader-type
  setup-fighting-cocks

  if (debug > 3) [show "exit proc: setup-agents"]
end

to setup-livetraders
  ;; distribute livetraders roughly evenly spaced on a circule around the center of the subdistrict
  ;; (livetraders trade live birds)
  let %cx ((min-pxcor + max-pxcor) / 2)
  let %cy ((min-pycor + max-pycor) / 2)
  let %center patch %cx %cy
  let %radius int min (list (max-pxcor - min-pxcor) (max-pycor - min-pycor)) / 3
```

```
  let %locations (list )
  let %rotation (360 / N-LIVETRADERS)
  let %offset (%rotation / 2)
  foreach n-values N-LIVETRADERS [?] [
    ask %center [set %locations lput patch-at-heading-and-distance (%offset + ? * %rotation) %radius %locations]
  ]
  foreach %locations [
    let %location ?
    let %okbarns barns with [not any? turtles-here]
    ask min-one-of %okbarns [distance %location] [
      sprout-livetraders 1 [ set home-patch patch-here init-livetrader ]
    ]
  ]
end

to setup-fgds                    ;;FGD: sprout num-fgd FGD flocks from barn (home patch) patches
  let %owner nobody
  let %fgd nobody
  let %n-barn-fgds (ceiling ((prop-fgd-barntofield) * N-FGD ))
  let %n-field-fgds (N-FGD - %n-barn-fgds)
  ;; some fgds will have barns to go to
  ask n-of %n-barn-fgds barns with [ not any? turtles-here ] [
    sprout-fgd-owners 1 [
      init-fgdowner
      set field-only? FALSE
      set %owner self
    ]
    sprout-fgds 1 [
      init-fgd
      set field-only? FALSE
      set %fgd self set owner %owner
    ]]                                          ;; some fgds will stay on their fields (field-only)
```

```
    ask n-of %n-field-fgds access-fields with [not any? turtles-here] [
    sprout-fgd-owners 1 [
      init-fgdowner
      set field-only? TRUE
      set %owner self
    ]
    sprout-fgds 1 [
      init-fgd
      set field-only? TRUE
      set %fgd self set owner %owner
    ]
    ask %owner [create-fgd-link-with %fgd [tie hide-link show (word self " tied")]]
  ]
  ;; set some of the fgd flocks as infectious
  ;; ai chk: I'd like to move this to a "setup-infection" proc
  ask n-of num-fgd-inf fgds [infect-initial]

  ;;old version of tie creation
  ;ask fgds with [field-only?] [
  ;   create-fgd-link-with one-of turtles-here with [breed = fgd-owners] [tie]
  ;   print (word who " is tied")
  ; ]
  ;ask fgd-links [ hide-link ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;; Agent Initializations ;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to init-fgdowner                        ;; initializations for FGD owners
  set home-patch patch-here
  set can-contaminate? FALSE
```

```
  set egg-type "duck"
  set egg-pickup? FALSE
  set egg-delivery? FALSE
  set eggs-gone? FALSE
  set contaminated? FALSE
  set infectious? FALSE
  set onsite-trader-num random-weighted [ 1 2 3 4 ] [ 0.513 0.38 0.099 0.0078 ]   ")
  set livebirds-gone? FALSE
  set n-visit-days-per-week binomial 7 0.42857
  ;; attributes for visualization
  set size 1
  set color white
end

to init-fgd                                ;; initializations for FGD flocks
  set home-patch patch-here
  set n-ducks 2000
  set infectious? FALSE
  set exposed? FALSE
  set latent? FALSE
  set recovered? FALSE
  set heading 270
  set color white
  set size 2
  set target-field nobody  ;;ai20110815
  set can-contaminate? TRUE
  set egg-type ""
  set eggs-gone? FALSE
  set egg-pickup? FALSE
  set egg-delivery? FALSE
end
```

```
to init-byowner                         ;; initializations for BYC owners
  set color (orange + 2)
  set size 1
  set can-contaminate? FALSE
  set egg-type ""
  set egg-pickup? FALSE
  set egg-delivery? FALSE
  set eggs-gone? FALSE
  set own-fightingcocks? FALSE
  set livebirds-gone? FALSE
  set practice-fight-week? FALSE
  set match-fight-week? FALSE
  set onsite-trader-num 0
  set own-infected-flock? FALSE
  set contaminated? FALSE
  set latent? False
  set infectious? FALSE
  set recovered? FALSE
  set n-visit-days-per-week binomial 7 0.42857   ;; number of visiting day (add explanation chk )
end

to init-byduckowner                     ;;initializations for penned duck (BYD) owners
  set color (brown + 2)
  set can-contaminate? FALSE
  set egg-type ""                       ;; 28 will be meat only, 41 will be egg only, 11 will be egg and meat
  set egg-pickup? FALSE
  set egg-delivery? FALSE               ;; of those that will have eggs (52 flocks), half will pick up and half will deliver
  set eggs-gone? FALSE
  set onsite-trader-num 1               ;; the number of traders per penned duck flock will be 1
  set livebirds-gone? FALSE
  set own-infected-flock? FALSE
  set contaminated? FALSE
```

```
  set byduck-type ""
  set n-visit-days-per-week binomial 7 0.42857
end

to init-farmowner                    ;;initializations for layer farm owners
  set color (yellow + 2)
  set can-contaminate? FALSE
  set egg-type ""
  set egg-pickup? FALSE
  set egg-delivery? FALSE
  set eggs-gone? FALSE
  set onsite-trader-num random-weighted [ 1 2 3 4 5 6 7 8 ] [ 0.2216 0.3602 0.2156 0.1171 0.0548 0.0223 0.0071 0.0013 ]
  set livebirds-gone? FALSE
  set own-infected-flock? FALSE
  set contaminated? FALSE
    set n-visit-days-per-week binomial 7 0.42857
end

to init-bychickens                   ;; initializations for BYC flocks
  set color orange
  set infectious? FALSE
  set exposed? FALSE
  set latent? FALSE
  set recovered? FALSE
  set death? FALSE
end

to init-byducks                      ;;initializations for penned duck (BYD) flocks
  set color brown
  set infectious? false
  set exposed? FALSE
  set latent? False
```

```
  set recovered? FAlse
  set byduck-type ""
end

to init-farms                          ;;initializations for layer flocks
  set color yellow
  set infectious? FALSE
  set exposed? FALSE
  set latent? FALSE
  set recovered? FALSE
  set death? FALSE
end

to init-eggtrader                      ;;initializations for egg traders
  set color (pink + 1)
  set size 1
  set can-contaminate? FALSE        ;; traders cannot contaminate
  set contaminated? FALSE           ;; traders may contaminate the target-farm
  set n-eggtrader-links RANDOM-PERT 1 1 10
  set egg-type ""
end

to init-livetrader
  set color (magenta + 1)
  set size 1
  set label-color (blue - 2)
  set can-contaminate? FALSE
  set egg-type ""
  set contaminated? FALSE
  set egg-pickup? FALSE
  set egg-delivery? FALSE
  set n-bird-pickup-days random 3     ;; Each live trader will have 1-3 trading days
```

```
end

;;;;;;;;;;;;;;;;;;;;;;;;
;; Egg Network ;;
;;;;;;;;;;;;;;;;;;;;;;;;

to set-egg-types
  ifelse co-op?  [
    ask fgd-owners [set egg-delivery? true]
  ] [
    ask fgd-owners [set egg-pickup? true set egg-delivery? false]
    ask n-of (floor N-FGDOWNERS / 2) fgd-owners [set egg-delivery? true set egg-pickup? false ]
  ]
                          ;; of those byduck flocks that will have eggs (52 flocks), half will pick up and half will     deliver
  ask n-of 26 byduckowners [set egg-pickup? true set byduck-type "egg" set egg-type "duck"]
  ask n-of 26 byduckowners with [egg-pickup? = false] [set egg-delivery? true set byduck-type "egg"
    set egg-type "duck"]
  ask byduckowners with [byduck-type = "" ] [set byduck-type "meat"]
  ask byducks with [any? byduckowners-here with [byduck-type = "egg"]] [set byduck-type "egg"]
  ask byducks with [byduck-type = ""] [set byduck-type "meat"]

  ask farmowners [set egg-type "chicken"]
  ask n-of 3 farmowners [set egg-type "duck"]
                                  ;; of the 6 large chicken egg farms, 2 have pickup, 2 delivery and 2 both
                                   ;; of the 3 large duck egg farms, 1 has pickup, 1 delivery and 1 both
  let my-ct 0
  foreach ["chicken" "duck"] [
    let my-egg-type ?
    ask farmowners with [egg-type = my-egg-type] [
      let my-type (my-ct mod 3)
      if (my-type = 0) [set egg-pickup? true set egg-delivery? false]
      if (my-type = 1) [set egg-pickup? false set egg-delivery? true]
```

```
    if (my-type = 2) [set egg-pickup? true set egg-delivery? true]
    set my-ct (my-ct + 1)
   ]
   set my-ct 0
  ]

  ask farms with [any? farmowners-here with [egg-type = "chicken"]] [ set egg-type "chicken"]
  ask farms with [any? farmowners-here with [egg-type = "duck"]] [ set egg-type "duck"]
end

to set-egg-transfer-days
                                        ;; The number of pickup days will vary for each egg supplier;
  ask farmowners with [egg-pickup?] [
   set pickup-days random-weighted [ 2 3 7 ] [ 0.33 0.33 0.33 ]
  ]
  ask fgd-owners with [egg-pickup?] [
   set pickup-days random-weighted [ 1 2 3 7 ] [ 0.073 0.635 0.197 0.095 ]
  ]
  ask byduckowners with [egg-pickup?] [
   set pickup-days random-weighted [ 1 2 3 7 ] [ 0.073 0.635 0.197 0.095 ]
  ]

                                        ;; The number of delivery days will vary for each supplier
  ask farmowners with [egg-delivery?] [
   set delivery-days random-weighted [ 2 3 7 ] [ 0.33 0.33 0.33 ]
  ]
  ask fgd-owners with [egg-delivery? ] [
   set delivery-days random-weighted [ 1 2 3 7 ] [ 0.087 0.522 0.174 0.217 ] ;; based on large survey data
  ]
  ask byduckowners with [egg-delivery? ] [
   set delivery-days random-weighted [ 1 2 3 7 ] [ 0.087 0.522 0.174 0.217 ] ;; same distribution as FGD
  ]
```

```
end

;;Goals for ``make-egg-pickup-network``:
;;1. set the number of egg-traders "needed" by each turtle (i.e., onsite-trader-num)
;;2. set the number of sites each eggtrader can visit to 2 now, later randomly (i.e., n-eggtrader-links)
;;3. create just as many eggtraders as are needed
;;4. create eggtraders fairly close to their suppliers
;;5. associate each eggtrader with a barn, where a barn is associated with 0 or 1 eggtraders

to make-egg-pickup-network
  let %suppliers turtles with [ egg-pickup? = True ]
  ;start with every barn having a potential egg-trader
  ask barns
  ;with [not any? turtles-here]              ;; 20120327 ab: We did not set up enough barns to support eggtraders alone; they can share patches.
  [
    sprout-eggtraders 1 [ set home-patch patch-here init-eggtrader ]
  ]

                                ;; each egg trader makes links to suppliers (until suppliers all taken)
                                ;; eggtraders with no suppliers are removed from the simulation

  ask eggtraders [
    carefully [
      create-egg-links-from min-n-of n-eggtrader-links %suppliers with [ count my-out-links < onsite-trader-num ]
      [distance myself]
    ] [
      die
    ]
  ]
  ask egg-links [ hide-link ]
  ask eggtraders [ set target-market one-of markets ]
end
```

```
to set-eggtrader-type
  ask eggtraders
  [
    ifelse all? in-link-neighbors [egg-type = "chicken"]
    [ set egg-type "chicken"]
    [
      ifelse all? in-link-neighbors [egg-type = "duck" ]
      [set egg-type "duck"]
      [
        if any? (in-link-neighbors with [egg-type = "duck"]) and any? (in-link-neighbors with [egg-type = "chicken"])
        [set egg-type "duck and chicken"]
      ]
    ]
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;
;;Fighting Cocks;;
;;;;;;;;;;;;;;;;;;;;;;;;


to setup-fighting-cocks
ask fcowners
  [
    set target-match-arena min-one-of match-arenas [ distance myself ]
    set target-practice-arena one-of practice-arenas
  ]
  end
```

```
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;; Go procedures  ;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to go
  file-open location-datafile
  file-print (word "day " (ticks + 1))              ;; advance day of the week
  file-close
  ifelse runsim? [
    update-calendar
    ask eggtraders [ pickup-eggs ]                  ;; egg traders pick up eggs from suppliers, deliver to markets
    ask fgds [go-to-field]                          ;; FGD flocks move from home to field unless already there
    ask fgd-owners [ fgd-deliver-eggs ]             ;; owners of egg-producing poultry deliver eggs to market
    ask farmowners [deliver-eggs ]
    ask byduckowners [deliver-eggs ]
    ask (turtle-set byduckowners byowners) with [livebirds-gone?] [ set-livebird-availability ]
    ask livetraders [livetrader-pickup]             ;; live poultry traders pick up from their nearby suppliers
    ask links with [ color = blue ] [ die ]         ;; clear the live poultry links after the trading day
    fight-cocks                                     ;; on appropriate days, FC owners go to practice or match
    ask fgds with [not field-only?] [               ;; FGD flocks return home
      travel-roads-to home-patch
    ]
    ask fgd-links [untie print (word end1 "untied for visiting")]  ;; all owners visit without their flock
    ask (turtle-set byowners fgd-owners byduckowners farmowners) [visit]
    ask fgd-links [tie print (word end1 "tied after visiting")]

    if influenza? [                                 ;; transmission steps occur for those exposed
      ask bychickens [ road-infect-backyard ]

      ask byducks [road-infect-penned]
      ask farms [road-infect-penned]
```

```
    ask barns [set-road-contam-time]
    ask practice-arenas [set-road-contam-time]
    ask turtles with [member? breed poultry-types] [add-influenza-day change-state ]
    ask turtles with [member? breed trader-types] [ set-trader-contam-time]
    ask byowners [add-influenza-day change-state]
   ]
   do-plots                                  ;; plots generated on the graphic user interface
   ask turtles with [member? breed trader-types] [decontaminate-traders]
   ask patches [decontaminate-roads decontaminate-fields]       ;; decontamination of traders and patches
   tick
   test-for-endsim
  ][
   print (word "Simulation stopped: " stop-sim-reason)
   stop
  ]
  test-go
end

to update-calendar                                   ;; determine day of the week
  type (word "ticks is " ticks ". ")
  set day-idx (ticks mod 7)
  set day-name item day-idx DAY-NAMES
  set week int (ticks / 7)
  set livetrader-pickup-days item day-idx LIVETRADER-PICKUP-SCHEDULE
  set eggtrader-pickup-days item day-idx DAY-SCHEDULE
  set visit-days item day-idx DAY-SCHEDULE
  print (word day-name " of week " week " is starting!!")
  foreach table:keys transmissions [
   table:put transmissions ? (lput 0 table:get transmissions ?)
  ]
  foreach table:keys contaminations [
   table:put contaminations ? (lput 0 table:get contaminations ?)
```

```
  ]
end

;;;;;;;;;;;;;;;;;;;;;;
;;Grazing Procs;;
;;;;;;;;;;;;;;;;;;;;;;

to go-to-field                                  ;; FGD procedure to get to field for grazing
  if (debug > 5) [show "enter proc: go-to-field"]
  set-target-field
  travel-roads-to target-field
  graze-here ;; chk make infection possible during grazing?
  if (debug > 5) [show "exit proc: go-to-field"]
end

to set-target-field
  ;;change target if needed (otherwise do nothing)
  if (debug > 5) [show "enter proc: set-target-field"]
  if ((target-field != nobody) and (member? target-field out-of-subdistrict-patches)) [stop] ;; chk out of sim forever ...
  let %rai-needed (n-ducks * RAI-PER-DUCK)
  if (debug > 3) [show (word "rai-needed: " %rai-needed)]
  let %old-target target-field
  if ((%old-target != nobody) and ([rai] of %old-target < %rai-needed)) [
    if (debug > 3) [ show (word "rai available (" ([rai] of %old-target) ") is inadequate; change target.") ]
    ask %old-target [
      set occupant nobody
      fallow-field
    ]
    set %old-target nobody
  ]
  ifelse (%old-target = nobody) [ ; set a new target
    let %candidates access-fields with [(rai >= %rai-needed) and (occupant = nobody)]
```

```
    if (not any? %candidates) [
      show "WARNING: no unoccupied fields left with adequate rai; move flocks out of district"
      set %candidates out-of-subdistrict-patches
      show " moving out of district"
    ]
    let %new-target min-one-of %candidates [ distance myself ]
    set target-field %new-target
    ask target-field [set occupant self]
    show (word " chose target field " target-field ". ")
    if (field-only?) [
      set home-patch %new-target
      ask owner [set home-patch %new-target move-to home-patch]
    ]
    ;; update days on field
    let %who ([who] of self)
    table:put days-on-field %who (lput 0 table:get days-on-field %who)
  ][
    let %who ([who] of self)
    let %oldlist table:get days-on-field %who
    table:put days-on-field %who (lput (last %oldlist + 1) butlast %oldlist)
  ]
  if (debug > 5) [show "exit proc: set-target-field"]
end

to graze-here  ;; fgd proc
  if ((debug > 3) and (member? patch-here out-of-subdistrict-patches)) [
    show "grazing out of subdistrict"
  ]
  let consume (n-ducks * RAI-PER-DUCK)
  set rai (rai - consume)
  if (infectious? = TRUE) [risk-ground-contamination "flock-field"]
 ; expose                                        ;; FGD are not exposed at field in this model version
```

```
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;Movement Procs;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to travel-roads-to [#target]  ;; turtle proc, #target is a patch
  if (debug > 5) [show (word "enter proc: travel-roads-to with target " #target)]
  if (debug > 3) [show (word "traveling to " #target)]
  if ((debug > 3) and (member? #target out-of-subdistrict-patches)) [
    show "traveling out of subdistrict"
  ]
  if (#target = nobody) [ show "not give a target!" __error "no target" ]
  if (#target = patch-here) [show "already at target" stop]
  let intA road-access
  let intB [road-access] of #target
  let path-nodes 0
  let path-links 0
  ask intA [
    set path-nodes __network-shortest-path-nodes intB newroads
    set path-links __network-shortest-path-links intB newroads
  ]
  ifelse (length path-nodes = 0) [
    show (word "WARNING: no roads from " intA " to " intB)
    move-to intB
    set path-nodes (list intA)
  ][
    if (debug > 3) [show "traveling roads"]
  ]
  move-to intA    ;;ai this moves to the road access of the current patch
  foreach but-first path-nodes [
    let next-node ?
```

```
    face next-node
    while [distance next-node > 0.5] [forward 0.5]
    move-to next-node
  ]
  move-to #target
  if (debug > 3) [show (word "reached target" #target)]
  if infectious? [
    let %risks patch-set [road-patches] of link-set path-links
    ask %risks [ risk-ground-contamination "flock-road" ]
  ]
  if (debug > 5) [show (word "exit proc: travel-roads-to; current location " patch-here)]
end


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;; Egg Network Go! ;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;


to pickup-eggs                          ;; Egg trader procedure to collect eggs
  ;; each day all egg traders assess if it is a pickup day for any of their contact flocks
  ;; if it is, they collect from flocks who have that pickup day.
  let %suppliers in-link-neighbors with [(not eggs-gone?) and member? pickup-days eggtrader-pickup-days]
  if (not any? %suppliers) [show (word "no pickups on " day-name)]
  foreach [self] of %suppliers [
    move-to ?
    show (word "getting eggs from " ?)
    contaminate-eggtrader
  ]
  ;;after done collecting, take eggs to usual market
  move-to target-market                 ;;currently, we do not consider market infection from eggtraders
  move-to home-patch
```

```
end

to contaminate-eggtrader  ;; eggtrader proc
  if (not influenza?) [stop] ;; exit procedure
  if not (member? self eggtraders) [__error (word self " is not an eggtrader")]
  let %risks turtles-here with [member? breed poultry-types]
  ;; eggtrader may infect poultry here
  if (contaminated?) [
     ask %risks with [not (latent? or infectious? or recovered?)] [risk-transmission "eggtrader-flock"]
  ]
  ;; contaminate egg trader at inf farm
  if (any? %risks with [infectious?]) [
    risk-human-contamination "flock-eggtrader"
  ]
end

to risk-transmission [#type]  ;; poulty proc
  if (not influenza?) [stop] ;; exit proc
  if (latent? or infectious? or recovered?) [stop] ;;exit proc  chk
  let %p 0
  if (#type = "eggtrader-flock") [
    set %p eggtrader-flock-infect-prob
  ]
  if (#type = "livetrader-flock") [
    set %p livetrader-flock-infect-prob
  ]
  if (#type = "visitor-flock") [
    show  (word "to-visit-infect-prob:" to-visit-infect-prob)
    set %p to-visit-infect-prob
  ]
  if (#type = "after-visit") [
    set %p from-visit-infect-prob
```

```
  ]
  if (#type = "road-flock") [
    if breed = bychickens [
    set %p road-infect-byc-prob
  ]
   if ((breed = byducks) or (breed = farms)) [
    set %p road-infect-pen-prob
  ]
  ]
  if (#type = "arena-fc") [
    set %p arena-FC-infect-prob
  ]
  if (#type = "fc-flock") [
    set %p FC-bkyd-infect-prob
  ]
  if (random-float 100 < %p) [
    infect-poultry
    let %oldct table:get transmissions #type
    let %newct lput (last %oldct + 1) butlast %oldct
    table:put transmissions #type %newct
  ]
end

to risk-human-contamination [#type]  ;; human proc
  if (not influenza?) [stop] ;; exit proc
  if (contaminated?) [stop]  ;; exit proc
  let %p 0
  if (#type = "flock-eggtrader") [
    set %p flock-eggtrader-contam-prob
  ]
  if (#type = "flock-livetrader") [
    set %p live-trader-contam-prob
```

```
  ]
  if (#type = "flock-visitor") [
    set %p 100
  ]
  if (random-float 100 < %p) [
    set contaminated? true
    show "is contaminated"
    let %oldct table:get contaminations #type
    let %newct lput (last %oldct + 1) butlast %oldct
    table:put contaminations #type %newct
  ]
end

to risk-ground-contamination [#type]            ;; patch procedure
  if (not influenza?) [stop]
  if (pcontaminated?) [stop]
  let %p 0
  if (#type = "flock-road") [
    set %p 100
  ]
  if (#type = "flock-field") [
    set %p 100
  ]
  if (random-float 100 < %p) [
    set pcontaminated? true
    show "is contaminated"
    let %oldct table:get contaminations #type
    let %newct lput (last %oldct + 1) butlast %oldct
    table:put contaminations #type %newct
  ]
end
```

```
to infect-poultry
  ifelse (latent? or infectious? or recovered?) [show "WARNING: wrong initial state for new infection"]
  [
  set latent? TRUE
  set infection-time 0
  show (word "is infected")
  if breed = fcowners [set own-infected-fc? TRUE]
  record-infection
  ]
end

to record-infection
  file-open location-datafile
  file-show patch-here
  file-close
end

to deliver-eggs  ;;flock owner proc
  ;; deliver eggs to market
  if egg-delivery?
  [
    set target-market min-one-of markets [ distance myself ]
    move-to target-market
    move-to home-patch
  ]
end

to fgd-deliver-eggs                    ;; FGD-owner procedure
  if (debug > 5) [show "enter proc: fgd-deliver-eggs"]
  if egg-delivery? [
    ask my-fgd-links [untie show (word "fgd-links untied for egg delivery")]
    ifelse co-op?
```

```
    [
      set target-market one-of cooperatives
    ]
    [
      set target-market min-one-of markets [ distance myself ]    ;;chk move to set up?
    ]
    move-to target-market
    move-to home-patch
    ask my-fgd-links [tie show (word "fgd-links retied after egg delivery")]
  ]
  if (debug > 5) [show "exit proc: fgd-deliver-eggs"]
end


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;; Live Poultry Trading Network Go! ;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

;; Each day of the week, the live poultry traders are either delivering or not, based on the number of days per week they trade (1, 2 or 3)
;; There is no poultry trading network made at setup, because, unlike the egg trading network, it will change daily based on who they have already collected birds from
;; On the Go command, each trader that trades on the present day will form a network of the 5 nearest houses that have not yet sold birds during the 2-month iteration

to set-livebird-availability                                        ;; live poultry supplier procedure
  if (livebirds-gone? and ticks - livebird-selldate > 60) [
    set livebirds-gone? FALSE                                   ;; livebirds available again after 2 months
    if (debug > 3) [show "livebirds-gone reset"]
  ]
end

to livetrader-pickup
```

```
  ;; each day each livetrader assesses if it is a pickup day for any of their contacts
  ;; if it is, collect live birds from flocks
  ;; comment: in this subdistrict, livetraders slaughter birds at home (live birds don't go to market)
  if (member? n-bird-pickup-days livetrader-pickup-days)[
    make-livetrader-pickup-network
    pickup-livebirds
  ]
end

to make-livetrader-pickup-network ;; livetrader proc
                                                ;; called by each trader, so it may be called 1, 2 or 3 times each week,
            depending on the trader's pickup schedule
  if (debug > 5) [show "enter proc: make-livetrader-pickup-network"]
  let %livebird-suppliers (turtle-set byduckowners byowners)
  set %livebird-suppliers (%livebird-suppliers with [not livebirds-gone?])
  let %ct min (list 4 count %livebird-suppliers)
  if (%ct < 4) [show (word "WARNING: not enough live birds available, run number " behaviorspace-run-number)]
  create-livebird-links-to min-n-of %ct %livebird-suppliers [distance myself]
  ask livebird-links [ set color blue ]
  if (debug > 5) [show "exit proc: make-livetrader-pickup-network"]
end

to pickup-livebirds   ;; livetrader proc
  let %collectingbirds? TRUE
  while [ %collectingbirds? ] [
    ifelse all? out-link-neighbors [livebirds-gone?] [
      set %collectingbirds? FALSE                ;; when they are done collecting for the day,
      move-to home-patch                         ;; they go home, where they will slaughter the birds
    ] [
      let %livebird-supplier min-one-of out-link-neighbors with [not livebirds-gone?] [ distance myself ]
      ;print (word "Live bird trader " who " chose pickup" ([pxcor] of %livebird-supplier) ([pycor] of %livebird-supplier))
      move-to %livebird-supplier
```

```
      ask %livebird-supplier [
        set livebirds-gone? TRUE
        set livebird-selldate ticks
      ]
      let %risks turtles-here with [member? breed poultry-types]
      ;; live trader may infect susceptible farm
      if (contaminated?) [
        ask %risks [risk-transmission "livetrader-flock"]
      ]
      ;; infected farm may contaminate live trader
      if (any? %risks with [infectious?]) [
        risk-human-contamination "flock-livetrader"
      ]
    ]
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;   Fighting Cocks  ;;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to fight-cocks ;;observer proc (called in ``go``)
  if (debug > 3) [show "enter proc: fight-cocks"]
  if ((day-name != "Wednesday") and (day-name != "Sunday")) [
    type (word "No fighting cock activity today. ")
  ]
  if (day-name = "Wednesday") [
    ask practice-arenas [ask n-of 20 (fcowners with [target-practice-arena = myself]) [move-to myself]]
    ask practice-arenas [show (word "There are " count fcowners with [target-practice-arena = patch-here ] " fc owners at this practice arena.")]
    ask fcowners with [target-practice-arena = patch-here ] [ fight-here set practice-fight-week? TRUE]
    ask fcowners with [practice-fight-week? != TRUE] [ set practice-fight-week? FALSE]
    ask fcowners [ move-to home-patch infect-backyard set practice-fight-week? FALSE]
```

```
  ]
  if (day-name = "Sunday") [
    ;; Only those who compete go to the matches. (Simplification.)
    ;; Each fc-owner brings a FC to the match arena about twice yearly;
    ;; thus we average N-FCOWNERS/26 cocks fighting per week.
    let my-owners n-of round (N-FCOWNERS / 26) fcowners
    ask my-owners [
      move-to target-match-arena
      fight-here
    ]
    ask my-owners [
      move-to home-patch
      infect-backyard
    ]
  ]
  if (debug > 3) [show "exit proc: fight-cocks"]
end

to fight-here
  if influenza? [
    if ((any? turtles-here with [(breed = fcowners) and (infectious? = true)]) or (infected-outsider-FC-here? = TRUE)) [
      risk-transmission "arena-fc"
    ]
  ]
end

;;;;;;;;;;;;;;;;
;; Visiting ;;
;;;;;;;;;;;;;;;;

to visit ;;  poultry owner procedure
```

```
                                        ;; all poultry owners can move to a few neighbors daily for a visit (number of                                visits
per day determined by a binomial at initialization)
  if not member? self poultry-owners [__error "should be a poultry owner"]
  if member? n-visit-days-per-week visit-days  [
    if (any? turtles-here with [member? breed poultry-types and infectious?])
    [ set contaminated? TRUE]                    ;; contaminate owner at their house/barn
    foreach ([self] of visit-list) [
      move-to ?
      if (contaminated? = TRUE)
      [let %risks turtles-here with [member? breed poultry-types]        ;; expose flock at neighbor's house
        ask %risks [ risk-transmission "visitor-flock" ]
      ]
      if ((contaminated? = FALSE) and (any? turtles-here with [member? breed poultry-types and infectious?])) [
        risk-human-contamination "flock-visitor"
      ]
    ]

    move-to home-patch                           ;; after visit, backyard owner may infect own flock
    let %risks turtles-here with [member? breed poultry-types]
    if (contaminated?) [
 ask %risks [
        risk-transmission "after-visit"] ]
    set contaminated? FALSE
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;Contamination and Infection;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to infect-initial                        ;; infect an initial flock at model setup
  if influenza? [
```

```
    set infectious? TRUE
    set color pink
    set shedding-time 0
    ;record-infection chk current record separately
  ]
end

to road-infect-backyard                ;; BYC procedure to infect backyard flocks from road contamination
  if (pcontaminated? = true) [
    risk-transmission "road-flock"
  ]
end

to road-infect-penned ;;byduck and farm proc          ;; procedure to infect penned flocks from road
            contamination
  if (pcontaminated? = true) [
   risk-transmission "road-flock"
  ]
end

to infect-backyard              ;; FC infected at arena may infect the BYC flock at home
  if influenza?                 ;; if BYC flock is not infected by the FC on same day, his infection stops
  [
    if ((own-infected-fc? = TRUE) and (not any? turtles-here with [member? breed poultry-types] with [(infectious? = true) or (latent? = TRUE) or (recovered? =
TRUE)]))
    [ ask turtles-here with [member? breed poultry-types] [risk-transmission "fc-flock"]]
    set own-infected-fc? FALSE
  ]
end

to contaminate-field        ;; future versions will have a continuous contamination value to the rice fields so                                    that other flocks
can be infected from the field
```

```
  set field-contaminant (field-infection-prob / 100)
  set plabel "c"
  set plabel-color blue - 2
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;Transmission Procedures ;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to change-state                          ;; both ducks and chickens have a 1 day latent period.
  if latent? [
    if infection-time > latent-period [
      set infectious? TRUE
      set latent? FALSE
      set color pink
      set shedding-time 1
    ] ]

  if (breed = bychickens) or ((breed = farms) and (egg-type = "chicken"))[
    if infectious?  [
        if shedding-time > shedding-period-chicken [
        set recovered? TRUE
        set death? TRUE
        set infectious? FALSE
        set color gray
      ] ] ]

  if ((breed = byowners) and (own-fightingcocks? = TRUE ))[
    if infectious?  [
      if shedding-time > shedding-period-chicken [
        set recovered? TRUE
        set infectious? FALSE
```

```
      set color gray
      ] ] ]

  if ((breed = farms) and (egg-type = "duck")) or (breed = byducks) or (breed = fgds) [
    if infectious?  [
          if shedding-time > shedding-period-duck [
      set recovered? TRUE
      set infectious? FALSE
      set color gray
     ] ] ]
end

to add-influenza-day                                    ;; keep transmission time moving forward
  if latent? [
    set infection-time infection-time + 1 ]
  if infectious? [
    set shedding-time shedding-time + 1 ]
  set eggs-gone? FALSE
end


to set-trader-contam-time
  if contaminated? = TRUE [ set trader-contam-time trader-contam-time + 1 ]
end
to set-road-contam-time
  if pcontaminated? = TRUE [ set road-contam-time road-contam-time + 1 ]
end
to set-field-contam-time
  if pcontaminated? = TRUE [ set field-contam-time field-contam-time + 1 ]
end

to decontaminate-traders
```

```
    if trader-contam-time >= trader-contam-period
      [
        set contaminated? FALSE
        set trader-contam-time 0
      ]
end
to decontaminate-roads
  if (road-contam-time >= road-contam-period) [
    set pcontaminated? FALSE
    set road-contam-time 0
  ]
end

to decontaminate-fields
  if (field-contam-time >= field-contam-period) [
    set pcontaminated? FALSE
    set field-contam-time 0
  ]
end

to-report basic-info
  report (word
  "fcowners: " (count fcowners)
  "\neggtraders: " (count eggtraders)
  "\nrice fields: " (count patches with [patch-type = "ricefield"])
  )
end

to test-for-endsim
  if (
      (count turtles with [member? breed poultry-types and latent? ])
      + (count turtles with [member? breed poultry-types and infectious? ])
```

```
      + (count byowners with [latent? ])
      + (count byowners with [infectious? ])
      + (count turtles with [member? breed human-types and contaminated? ])
      + (count barns with [ pcontaminated? ])
      + (count practice-arenas with [ pcontaminated? ])
      = 0) [
    set stop-sim-reason (word "Outbreak is over " ticks " ticks.")
    set runsim? false
  ]
end

to do-plots
  set-current-plot "Contamination"
  set-current-plot-pen "Barns Near Road"
  plot count barns with [pcontaminated? = TRUE]
  set-current-plot-pen "Egg Traders"
  plot count eggtraders with [contaminated? = TRUE]
  set-current-plot-pen "Live Traders"
  plot count livetraders with [contaminated? = TRUE]

  set-current-plot "FGD Infection State"
  set-current-plot-pen "Latent Flocks"
  plot count fgds with [ latent? ]
  set-current-plot-pen "Infectious Flocks"
  plot count fgds with [ infectious? ]
  set-current-plot-pen "Recovered Flocks"
  plot count fgds with [ recovered? ]

  set-current-plot "Backyard Chickens Infection State"
  set-current-plot-pen "Latent Flocks"
  plot count bychickens with [ latent? ]
  set-current-plot-pen "Infectious Flocks"
```

```
plot count bychickens with [ infectious? ]
set-current-plot-pen "Flock Death"
plot count bychickens with [ death? ]

set-current-plot "Small Duck Flocks Infection State"
set-current-plot-pen "Latent Flocks"
plot count byducks with [ latent? ]
set-current-plot-pen "Infectious Flocks"
plot count byducks with [ infectious? ]
set-current-plot-pen "Recovered Flocks"
plot count byducks with [ recovered? ]

set-current-plot "Farm Infection State"
set-current-plot-pen "Latent Flocks"
plot count farms with [ latent? ]
set-current-plot-pen "Infectious Flocks"
plot count farms with [ infectious? ]
set-current-plot-pen "Flock Death/ Recovery"
plot count farms with [ death? or recovered?]

set-current-plot "Proportion of Flocks Affected (Post-shedding)"
set-current-plot-pen "Backyard Flocks"
plot ((count bychickens with [death?]) / N-BYOWNERS)
set-current-plot-pen "Small Duck Flocks"
plot ((count byducks with [recovered?]) / N-BYDUCKOWNERS)
set-current-plot-pen "FGD Flocks"
plot ((count fgds with [recovered?]) / N-FGD)
set-current-plot-pen "Farms"
plot ((count farms with [recovered? or death?]) / N-FARMOWNERS)

set-current-plot "rai available"
clear-plot
```

```
  set-current-plot-pen "rai01"
  set-plot-pen-mode 0
  ;;make cumulative sum
  let %rai reduce [lput (?2 + last ?1) ?1] fput [0] (sort [rai] of access-fields)
  let %xvals n-values (count access-fields + 1) [?]
  ;;if (last %rai < 0.5 * plot-y-max) [set-plot-y-range 0 last %rai]
  (foreach %xvals %rai [plotxy ?1 ?2])
  set-plot-y-range 0 7500

end

;;;;;;;;;;;;;;;;;;;;;;; tests ;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to test-setup
  show "enter proc: test-setup"
  test-setup-patches
  test-setup-agents
  show "exit proc: test-setup"
end

to test-setup-patches
  ;;;;;;  check that setup-patches meets expectations, ow raise error


  ;; ensure that only ricefields shd have a non-empty field-state
  ask patches [
    if (patch-type = "ricefield") and (not member? field-state ["growing" "harvested" "fallow"]) [
      __error "ricefields should have field-state"
    ]
    if (patch-type != "ricefield" and field-state != "") [
      __error "non-ricefields should not have field-state"
    ]
```

```
  ]

  ask access-fields [
    ask field-patches [
      if (field-num != [field-num] of myself)
      or (field-state != [field-state] of myself) [
        __error "problem in field setup"
      ]
    ]
  ]
end

to test-setup-agents
  ask fgds [
    if (not is-turtle? owner) [__error "problem setting owner of fgds"]
  ]

  if not (count livetraders = N-LIVETRADERS) [__error "wrong number of livetraders"]
end

to test-go
  ask fgds with [field-only?] [
    let %patch patch-here
    let %owner-patch nobody
    ask owner [set %owner-patch patch-here]
    if not (%owner-patch = %patch) [
      show (word "I'm on " %patch " but my owner is on " %owner-patch)
      __error "fgds should be tied to owner"
    ]
  ]

end
```