STOCHASTIC RISK MODEL OF HIGHLY PATHOGENIC AVIAN INFLUENZA SPREAD AND IMPACT OF BIOSECURITY PROTOCOLS

by

FERNANDA C. DÓREA

(Under the direction of Charles Hofacre)

ABSTRACT

The potential spread of HPAI between commercial broiler farms in Georgia (USA) was mathematically modeled in order to evaluate the impact of different biosecurity measures in reducing the risk of disease spread. Compliance to standard biosecurity protocols by broiler growers in the state was investigated in a survey. Scenarios of biosecurity adoption were defined based on the results of the survey for two different areas of the state with different density of poultry farms, and which are facing different disease risks. Additional scenarios evaluated the impact of increasing attention to biosecurity. Off farm spread of virus for the different scenarios was estimated stochastically for periods of one day, and then modeled through days using geographical information to explicitly account for the effect of density on secondary spread. Results showed that in case of introduction of HPAI viruses in the state immediate detection is crucial, as the epidemic would start growing exponentially in the same day detection of mortality by the grower in the first affected farm is expected to occur (fifth day). The adoption of biosecurity measures was shown to delay the phase of exponential growth for the epidemic and slow spread, increasing the chances of outbreak control. Farms in North Georgia are under higher risk of disease spread due to higher farm density. The current level of biosecurity compliance in that region, where disease awareness among growers is higher in response to an ongoing outbreak of infectious laryngotracheitis, represents a small reduction in the risk of disease spread when compared to the frequency of biosecurity adoption in the South. Measures to prevent contamination spread through vehicles have the highest potential to reduce the current risk of disease outbreaks in the state.

INDEX WORDS: Highly Pathogenic Avian Influenza, Biosecurity,
Infectious Disease Model, Broiler, Poultry

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DEDICATION

To God, who traced my path;
To my parents, who made it possible for me to walk on it;
To my brother, whose example keeps driving me to the goal;
To Holger who keeps me up strong and happy no matter how rough the ground gets;
and to all the friends whose company made the journey more pleasant.

Looking back at my academic life, I can clearly identify a few key moments when my path took a whole new direction and wonderful things followed. Each one of these key moments was made possible by one key person. For that, my accomplishments are also dedicated to Dr. Wagner Fontes, Dr. Vítor Gonçalves, and Dr. Dana Cole.

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INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 HIGHLY PATHOGENIC AVIAN INFLUENZA

Highly pathogenic avian influenza (HPAI) is a viral disease that can affect all species of domestic or captive birds [3, 83]. The disease, that has become an emerging issue for world health, has caused numerous outbreaks in domestic poultry and wild bird populations around the world, and threatens human health [104]. Outbreaks of avian influenza in the poultry industry have the potential to become a worldwide pandemic both in animal and human health, making it indispensable to prevent AIV introduction into the poultry farming system [52].

VIRUS

Avian Influenza (AI) was differentiated from fowl cholera in 1880, and identified as a virus in 1901 [46].

Only influenza A viruses have been reported to cause natural infection of birds [19]. Influenza A viruses are enveloped, single stranded negative sense RNA viruses with a segmented genome, and belong to the Orthomyxoviridae family [42]. Type A influenza viruses are divided into subtypes based on the antigenic characteristics of the surface glycoproteins haemagglutinin (H) and neuraminidase (N) [19].

Influenza A viruses are highly variable as a result of molecular changes in the RNA segments that occur through several mechanisms, the most important of which are point mutation (antigenic drift) and RNA segment reassortment (antigenic shift) [42].

PATHOGENICITY

AI viruses are also divided on the basis of their ability to cause disease [19, 83]. The very virulent viruses cause HPAI, a systematic infection, in which flock mortality may be as high as 100% — the Terrestrial Animal Health Code 2007 defines a minimum of 75% flock mortality for an AI occurrence to be considered as caused by a highly pathogenic virus strain [31]. Those viruses have been restricted to subtypes H5 and H7, although not all of these subtypes cause HPAI [19, 83]. All other viruses cause a much milder disease, known as low pathogenic avian influenza (LPAI), consisting primarily of mild respiratory disease and egg production problems in laying birds [83]. Low pathogenicity strains of the H5 and H7 AI viruses are, however, associated with the potential for mutation to more highly pathogenic forms [89].

Influenza A viruses replicate in the cells of the gastrointestinal and respiratory tracts of birds and are transmitted through the fecal-oral and respiratory routes. The clinical signs vary considerably depending on the viral subtype, environmental factors, and age, health status, and species of the bird. In poultry, clinical signs of HPAI virus infection range from decreased egg production and gastrointestinal manifestations to nervous system disorders and include the more characteristic respiratory signs, such as rales, sinusitis, excessive lacrimation, edema of the head and cyanosis, congestion, and hemorrhage of the wattles and comb [42, 86]. Most often, HPAI is manifested in poultry as peracute death with very little clinical disease, which is why mortality is generally the main indicator used in HPAI detection systems [42].

The primary difference between low pathogenicity AI and high pathogenic AI viruses is local versus systemic replication, respectively [44]. Depending on the avian species, virus may be shed for days to weeks after infection, and the amount of virus present in the environment plays an important role in the likelihood of transmission to new hosts.

EPIDEMIOLOGY

The scientific evidence collected in recent years has led to the conclusion that not only must HPAI viruses be controlled in domestic poultry populations, but also LPAI viruses of the H5 and H7 subtypes, as they represent HPAI precursors [19].

Wild birds are the recognized source and reservoir for all subtypes of avian influenza viruses (AIV) [87], and the frequency with which primary infections occur in any type of bird depends on the degree of contact with reservoir bird species, such as waterfowl [3].

In domestic poultry, relatively little is known about the extent of infection and, in particular, the transmissibility of AIV between poultry farms [34]. The primary introduction occurs either through direct or indirect contact with infected birds, the latter resulting from the penetration of contaminated infectious organic material into the holding [19]. Consequently, the routes of introduction of AI viruses into domestic poultry consist mainly of movements of people (as farm owners and their staff), materials, and vehicles [91].

Other routes of transmission have been suggested and grouped as ”contiguous spread”, a comprehensive term that includes transmission from infected farms over short distances by unknown or poorly understood introduction routes [37, 91].

While some authors contend that airborne transmission plays an important role in the spread of AIV during outbreaks, even though less efficient than direct contact [97, 42], others state that this is a rare [34] and unproven [19] means of transmission for the viruses.

In any case, there is a consensus that the most important mode of transmission in domestic poultry is related to human movement. Using the data from an H5N1 outbreak in Great Britain in 2006, Sharkey *et al* (2008) [84] showed that large outbreaks cannot occur with local transmission alone. Therefore if biosecurity measures are implemented at the farm level AI infections can be prevented.

Global occurrence of HPAI Avian influenza outbreaks in the commercial poultry industry are associated with serious economic consequences as a result of bird deaths, depopulation

Table 1.1: Major highly pathogenic outbreaks in the past 50 years, based on the review of Lupiani and Reddy (2008) [46]

Year	HPAI virus type	Region
1983	H5N2	USA — Pennsylvania
1994	H5N2	Mexico
1994	H7N3	Pakistan
1999	H7N1	Italy
2002	H7N3	Chile
2003	H7N7	The Netherlands
2004	H7N3	Canada
Since 2003	H5N1	Eurasia

costs, and national and international trade restrictions. Lupiani and Reddy (2008) [46] have recently reviewed the history of major HPAI outbreaks, which are listed in Table 1.1.

Until recently it appeared that the epidemiology of AI consisted of the perpetuation of LPAI viruses of all H subtypes in wild birds where they caused little or no disease with spread to poultry from time to time. Very occasionally introductions of LPAI viruses of H5 or H7 subtype into poultry resulted in the mutation of these viruses to virulent forms that caused HPAI [3, 40]. That was the case for the outbreaks listed up to 2004 in Table 1.1 [62, 61, 88, 82, 18, 100].

In 2003, however, H5N1 AI viruses began to circulate in south-east Asia and resulted in an unprecedented expansion of the geographic range of this subtype [45, 31], coupled with a series of changes in the epidemiology and ecology of HPAI virus, namely: continuing evolution of the Asian H5N1 virus with emergence of multiple, distinct lineages that are all lethal for gallinaceous poultry; repeated transmission to humans of each lineage; transmission to large cats, civet cats, and domestic village dogs and cats in the fields; and perpetuation of HP H5N1 viruses in apparently healthy domestic waterfowl in some countries of Asia [107].

Devastating consequences for the poultry industry in Eurasian countries have been caused by this H5N1AI virus since 2003 [110, 19, 77]. By January 2009 the epidemic has affected 62 countries in Asia, Europe and Africa, 50 of which have reported cases in domestic poultry [32]. In some affected countries, the disease has become endemic. Outbreaks are now often affecting industrialized countries as this virus continues to circulate among domestic and wild birds [96].

Besides the effect on animal health, there is a fear that H5N1 could become the next pandemic influenza strain in humans [3]. Between 1997 and early 2009, a total of 399 cases of human infection in 15 countries in South-East Asia, China, central Asia, the Middle East and Africa have been reported to the World Health Organization, resulting in 251 (63%) deaths [32].

Because exposure to sick or dead poultry is a strong risk factor for human disease caused by HPAI subtype H5N1, the threat of pandemic flu can be reduced by more effectively controlling the spread of HPAI through national poultry flocks. Control programs can then be designed to reduce or avoid exposure to these risk factors, and surveillance programs can be designed to target high risk populations [102].

AI in the US HPAI has been detected three times in U.S. poultry: in 1924 (H7 virus, East Coast), 1983 (H5N2, Pennsylvania and Virginia) and 2004 (H5N2, Texas) [54, 46]. In the first occurrence it caused severe losses in bird markets in New York City, spreading rapidly to New Jersey, Pennsylvania and Connecticut. Outbreaks were also reported in Indiana, Michigan, West Virginia, Missouri and Illinois during February-April 1925. The outbreak in 1983 originated from low pathogenic forms of the virus (H5N2) that were circulating since April, and mutated into a highly pathogenic variant later that year [46].

Due to the potential for mutation of H5 and H7 AI viruses to more highly pathogenic forms, the immediate control of these outbreaks is usually a priority of state and national veterinary authorities [48]. Hence, even though North America, differently from Europe and Asia, has faced only a few sporadic outbreaks of highly pathogenic AI viruses, there is a constant worry since low pathogenic viruses persist in the U.S. especially in the live-bird markets in the Northeast [89]. Trock *et al* (2008) [94], in a study of LBM in New York, reported that 81.4% (57 of 70) of the markets were positive for LPAI H7N2 viruses either in birds, the market environment, or both.

Suarez *et al* (2003) [89] presented a review of all the isolations of H5 and H7 influenza viruses from 1997–2002. An update of their review is shown in Table 1.2.

Since 2005 there has been increased testing of animals, therefore the activity from this year on does not necessarily indicate a greater virus activity. The information is important, though, to show which strains are circulating in the country.

As seen in the table above, AI viruses are present in North America not only in live bird markets, but also in wild birds. Dugan *et al* (2008) [26] report 167 complete viral genomes in 14 bird species sampled in four locations in the USA. North American H5 wild-bird-origin AI viruses are, however, low-pathogenicity wild-bird-adapted viruses, and are antigenically and genetically distinct from the highly pathogenic Asian H5N1 virus lineage [85].

The possibility of a direct introduction of HPAI into the country is not excluded, though. The illegal trade and transport of infected poultry or exotic birds has been cited as a potential route for possible spread of H5N1 across national borders [111].

Additionally, even though the potential role of wild birds in transporting the HPAI virus over large geographical distances through migration is still debated, Peterson *et al* (2007) [59] reviewed intercontinental movements of birds, and evaluated possible entry pathways for H5N1 into North America via bird migration. They concluded that if migratory birds were capable of hosting the virus asymptomatically (which is not supported by evidence so far, but has been speculated intensively), they would have the potential to introduce the disease into North America and even South America. Currently any H5 and H7 subtypes, regardless of their pathogenicity, are reportable in the USA. The United States Department of Agriculture (USDA) has jurisdiction over avian influenza.

Table 1.2: Isolation of H5 and H7 influenza viruses in the United States since 1997. Updated from SUAREZ *et al* (2003) [89].

Year	Subtype	State(s)	Pathogen	Environment	Reference
1997–2002	H7N2	NJ,NY	LPAI	Live Bird Market (LBM)	[89]
1997	H7N2	CT	LPAI	LBM	
1997–1998	H7N2	PA	LPAI	Commercial outbreak	
1998	H5N2	NJ, NY	LPAI		
1998	H5N2	NY	LPAI	LBM	
1998	H5N2	MN	LPAI		
1998	H7N2	DE,NY	LPAI		
1999	H7N3	NY	LPAI	LBM	
2000	H7N2	DE	LPAI		
2000	H5N2	NJ,NY	LPAI	LBM	
2001	H5N2	NJ,NY	LPAI	LBM	
2001	H7N2	FL	LPAI	LBM	
2001	H7N2	PA	LPAI		
2001	H7N2	PA	LPAI	Commercial outbreak	
2001	H5N2	NY	LPAI		
2002	H7N2	MA	LPAI	LBM	
2002	H7N2	VA,NC	LPAI	Commercial outbreak	
2002	H5N2	ME	LPAI		
2002	H7N2	PA	LPAI	LBM	
2003	H5N2	CT	LPAI	Commercial outbreak	[63]
2004	H7N2	DE, NJ, PA	LPAI	LBM	[66, 67]
2004	H5N2	TX	HPAI	Commercial outbreak	[64]
2004	H7	MD	LPAI	Commercial outbreak	[68]
2004	H7N3	TX	LPAI	Routine test in layers	[65]
2005	H7N2	NY	LPAI	Duck farm	[69]
2006	H5N1	MC	LPAI	wild mute swans	[74]
2006	H5NI	MD	LPAI	Wild mallards	[72]
2006	H5N1	MT	LPAI	Wild ducks	[76]
2006	H5N1	PA	LPAI	Wild mallard ducks	[73]
2006	H5N1	IL	LPAI	Wild ducks	[75]
2006	H5N1	MI	LPAI	Wild birds	[70]
2006	H5N1	NY	LPAI	Wild ducks	[71]
2007	H5N2	WV	LPAI	Turkey farm	[78]
2007	H5N1	VA	LPAI	Turkey farm	[79]

The federal government has the regulatory authority for HPAI viruses and their eradication (and by immediate quarantine and depopulation measures in case of detection [58, 48], while the state governments regulate LPAI viruses in domestic poultry. The legal authority to conduct an emergency eradication program is shared by the state and federal governments, and the specifics are determined by both on a case-by-case basis [42].

The Animal and Plant Health Inspection Service (APHIS), part of the United Stated Department of Agriculture (USDA), defines an influenza A virus as highly pathogenic if it meets one of the following criteria: (1) kills at least six of eight experimentally inoculated susceptible four to six week old chickens; (2) any H5 or H7 subtype that kills less than six of eight chickens, but has an amino acid sequence at the HA cleavage site that is compatible with HPAI viruses; (3) any other HA subtype (nor H5 nor H7) which kills one to five chickens and grows in cell culture in the absence of trypsin [93].

1.1.2 Poultry industry in the US

LBM

Live bird markets sell poultry to the public for sale, slaughter, and/or dressing. Most of these markets operate in large cities and supply the needs of diverse populations [57].

As mentioned before, the presence of LPAI circulating in LBMs on the east coast is well known [16, 33]. Since avian influenza viruses are primarily transmitted through feces, inadequate disease control measures between the live markets and industrial poultry farms, such as insufficient hygiene measures for people and trucks, can result in the spread of influenza. Other potential routes include rendering companies and disposal of potentially contaminated materials through municipal waste [112]. Once the influenza virus invade a commercial poultry farm, it has an optimum number of susceptible poultry for rapid viral evolution and potential mutation of LPAI viruses into HPAI causing viruses [106].

Garber *et al* (2007) [33] studied the characteristics of live bird markets in the United States to identify potential risk factors for markets to be repeatedly positive for LPAI viruses.

Three risk factors identified in the study potentially relate to hygiene and ability to thoroughly clean and disinfect the market, namely: frequency of cleaning and disinfecting (odds ratio of 3.3 and 1.2 for zero and 1–2 complete cleanings in the past 30 days, respectively, when compared to three or more); disposal of dead birds and offal in the trash (odds ratio of 2.4 compared to non-adoption of this practice); and being open 7 days a week (odds ratio of 2.1 compared to markets not open every day). The results are similar to the previous study of Bulaga *et al* [15] in LBMs in New York and New Jersey.

While the previous studies pointed the risk factors for AI virus circulation, Yee *et al* (2008) [112], studying LBM in Southern California, were able to document the positive effect of compliance to preventive measures. The California LBMs have not had any detected avian influenza viruses since December 2005, which was attributed in the study to the compliance with an LPAI control program that involves: active surveillance, prevention, and rapid response measures by those involved in the LBM system; rendering services to dispose carcasses, no wholesales, and few third-party bird deliveries.

Commercial poultry

US The ‘poultry industry’ is a general term used to group three different industries: commercial layers, broilers and turkeys.

In the USA, the commercial poultry production industry is a fully integrated system of animal agriculture, in which each company has oversight over the bird husbandry and health management aspects of production [38]. In this system all or most production aspects are owned and controlled by an individual company called an ‘integrator’. The company retains ownership of the birds and expects producers to grow their flocks under very specific management programs [21]. A typical contract is one in which the grower (usually a landowner) provides the housing and growout equipment, feeders, waterers, brooders and other inputs such as water, electricity, fuel, litter and labor. The contractor (the integrator company) provides the chicks, feed, necessary medications and supervision. Company field representatives normally visit farms weekly to assist with management, but they may do so more often if necessary. Any health or production problems with the flock are reported immediately to this company representative. The integrator company also provides labor and equipment for catching and hauling the birds to market [39].

This highly integrated business structure and intensity of production of the USA poultry industry present some unique challenges for disease surveillance, outbreak control, and emergency management [20]. In such a connected network of farms, biosecurity is a critical component of disease prevention and control.

State of Georgia Poultry is the largest segment of Georgia's agriculture and agribusiness. Chickens are the largest single agricultural commodity in the state, producing over $2.4 billion in farm income annually [53]. The statewide economic impact of the industry is an estimated $13.5 billion annually. On an average day, Georgia produces 24.6 million pounds of chicken and 14 million eggs, and these are marketed around the globe [108].

The broiler production industry alone has been the number one source of agricultural income in Georgia since 1956 [39]. Major poultry processors operating in Georgia, as it is characteristic around the country, are vertically integrated companies, which are not necessarily based in the state.

Backyard

In 2004 a characterization of the US poultry industry — National Animal Health Monitoring System's (NAHMS) — was performed by the USDA [55]. The study included backyard and small production flocks in the country, as well as gamefowl and live-bird markets. The investigation was performed by drawing a 1-mile radius circle around a sample of 350 large commercial poultry operations with at least 10,000 chickens or 5,000 turkeys, in 16 participating states. These circles were then canvassed for residences with birds other than or in addition to pet birds. Therefore, while the characterization is believed to be representative of small production flocks around the country, there is no current knowledge as to the number, density or location of backyard flocks. Chickens of table-egg breeds were found in two-thirds (63.2%) of backyard flocks. On average, backyard flocks had 35.1 birds, ranging from an average of 26.1 birds in the Southeast region to 49.2 birds in the East region. Nearly 1 in 3 backyard flocks (31.8%) had fewer than 10 birds. Use of veterinarian's services was rare in backyard flocks (2.9% overall).

The most common reason identified for having birds was for fun/hobby. Overall, very few backyard flocks (3.5%) had someone in the household who worked for a commercial poultry operation, ranging from 0.9% of backyard flocks in the Southeast region to 8.5% in the Midwest region. Only 3.5% of backyard producers moved birds to locations where other birds were present. Most producers who took birds to shows, etc., and returned them to their flocks went to events that were within their county or state.

Backyard flocks, which rarely adopt biosecurity measures, can be considered a potential source of introduction of diseases into the commercial poultry system. However, given their dimension and relatively limited contact with integrated systems, they are not relevant in the amplification and spread of highly contagious viral diseases (as HPAI) once they enter the commercial poultry system.

1.1.3 Biosecurity in the Poultry Industry

Considering the constant changes observed in the epidemiology and ecology of HPAI viruses, with continuing evolution of the Asian H5N1 virus, Webster *et al* [107] opine that influenza viruses are not eradicable, and they will remain a problem for the poultry industry worldwide. Indeed, recent outbreaks in industrialized countries alarm even organized poultry industries. The H5N1 outbreak in Great Britain in 2006 showed that transmission of the virus can occur, for instance, between farms in the intensive European poultry industry [84]).

In the United States, the daily risk of introduction of HPAI into the national poultry flocks is significant considering the large traffic of commodities and people to the USA from every part of the world and the existence of circulating low pathogenic strains of the AI virus within the country. Prevention is therefore critical, and biosecurity represents the first and most important means of prevention [19].

Biosecurity consists of the measures used to prevent introductions of disease-causing organisms into a flock [51]. A routine biosecurity protocol includes security practices that should be implemented as standard operating procedure for any infectious disease [42].The adoption of these measures can not only significantly reduce the risk of disease introduction, but also reduce the magnitude of the financial losses that may occur following infection [51].

Dolberg *et al* (2005) [24] classified poultry production systems into four sectors based on the typical level of biosecurity and marketing practices, both of which are very important factors influencing the spread of HPAI. The minimal biosecurity in nonindustrial production and live bird markets is frequently identified by animal health agencies as a risk to the commercial poultry industry, even in developed regions where nonindustrial production and live bird markets account for a negligible share of poultry outputs and sales, respectively [8]. Gifford *et al* (1987) [35] concluded, by means of a cost-benefit analysis, that in the case of the most pathogenic infections [as HPAI], investment in biosecurity is justified even if there is a 0.01 probability of an outbreak.

TYPES OF BIOSECURITY

Farm security Inside the integrated poultry system, the contact structure can be viewed as a social network of potentially infectious links amongst premises, which are defined by associations via one or more potential routes of interaction. Dent *et al* (2008) [23] studied these potential routes of disease transmission between farms, and categorized them in four categories:

- transmission through vehicles — litter disposal, catching companies, disposal and replacement, cleaning, dead bird collection, imports, hatching egg collection, feed delivery, visiting, vaccination, farmer;

- transmission through people — catchers and thinners, drivers, cleaning teams, artificial insemination teams, area managers, farm staff, dead bird collectors and vets;
- transmission through fomites — catching equipment, containers, pallets, culling equipment, workman's clothes, dead bird collecting, holding stations and raw feed material;
- transmission through the environment — wildfowl, water and feed, airborne (dust), flying insects and game birds.

Considering the listed routes of transmission, biosecurity can be applied at several points: controlling the source of birds; minimizing access to the flock; implementing disinfection stations and worker disinfection practices; limiting visitors to the farm; disposing dead birds and waste appropriately; all-in and all-out flock management with disinfection between flocks; and preventing contact with other animals [51].

To control the access of people and visitors to the flock, access to the poultry house must be limited to authorized personnel, and the control over visitors is an obligation usually set in the contract by the integrator company. Most company protocols require that producers avoid visiting other poultry farms and eliminate any contact with other poultry, especially hobby, exhibit and backyard flocks [22]. For people allowed to enter a poultry producing premise, an additional precautionary measure is to require that the vehicles park offsite when possible. If entry into the facility is necessary, proper disinfection of tires and undercarriages of vehicles is recommended before entry [42].

Barriers are an integral part of biosecurity because they can restrict the movement of microbes and the animals and humans that harbor them. Simple barriers such as outerwear, gloves, and footwear worn during day-to-day activities are part of general biosecurity. They protect the wearer and, if properly cleaned or discarded, prevent transfer of organisms [109].

Additionally, facilities should consider influenza A viruses in their routine disinfection protocols. Influenza A viruses are relatively unstable in the environment and are inactivated by high temperatures, extreme pH changes, ultraviolet light, and desiccation. After removal of organic material, several classes of disinfectants will inactivate influenza A viruses: phenolics, quarternary ammonia compounds, and oxidizing agents (e.g., bleach) [42].

Personnel security Many infectious diseases, including avian influenza viruses, may be transmitted as fomites on clothes, shoes, or hands [42]. Personnel security, as routine cleaning and disinfection of clothing and hands, and the use of personnel protective wear, intends not only to prevent the iatrogenic dissemination of disease-causing organisms [109], but also to protect workers from the potential exposure to these agents. Due to the seriousness of H5N1 avian influenza outbreaks worldwide, epidemiologists are concerned about the development of a new kind of deadly influenza for which humans would have no resistance. Staff should be provided with information regarding human health precautions and trained for proper use of personal protective equipment [42].

It is recommended that employees, regardless of facility type, change from street clothes to specific facility clothing and footwear immediately upon entering the workplace. Specific facility clothing should never be worn outside of the facility and ideally should be laundered on site daily. Only footwear that can be disinfected easily should be allowed; alternatively, disposable plastic over-the-shoe booties can be worn [42].

Disease detection/Infection control Infected countries have implemented varying degrees of control measures such as restrictions on poultry movement, cleaning and disinfecting, and vaccinating poultry. No control measure alone has been successful in eradicating H5N1, and the measure of success of any one activity is largely based on overcoming the obstacles that can prevent it from being carried out [111], depending on the type of institution and birds [42].

Therefore, understanding the dynamics of disease spread within and between farms (how the disease would spread if introduced in a country) is essential for the adoption of preventive and control measures that can prevent disease introduction and minimize the serious economic consequences that would result from the introduction of a highly contagious poultry disease [104].

While much is known from experimental studies on the course of infection in individual birds, estimates of the generation time at the farm-level are more difficult to obtain. Although it is perhaps surprising that the generation time differs between outbreaks, it is plausible that such differences could arise because of variation in farming practices or in the contact patterns between farms [34].

How HPAI infection is detected in a flock will depend on how rapidly the disease kills birds with clinically apparent illness. If death is sudden with little or no clinical signs, then rapidly rising mortality may be the first indication that something is amiss. If clinical signs are more apparent, then decreased food and water intake or reduced egg production in layers may be the first indication. However, deciding whether something is wrong with a flock is made difficult by the day-to-day variation in food and water usage and egg production. For that reason, mortality thresholds have been recommended to guide the decision of whether to consult veterinary practitioners and inform the authorities. In the Netherlands, the recommended threshold for floor-reared broilers chickens is 0.5% mortality over two consecutive days. An additional weekly mortality detection threshold of 3% was instigated by the Dutch authorities during and after the 2003 outbreak [29].

Savill *et al* (2008) [81] showed, by modeling different parameters for virus spread within a flock, that time to detection of clinical signs takes much longer than detection of mortality thresholds, for both H5N1, which causes a short symptomatic period, and H7N7, which infection was assumed to cause clinical signs in most of the birds. Time to detection is, however, less variable for H5N1 due to its higher estimated reproductive number (number of secondary infections from an infected animal). In their model, although a detection threshold for clinical signs was included, clinical signs were never detected before rising mortality in the simulations. However, the observation of clinical signs within a flock should trigger closer scrutiny of the flock in order to respond rapidly to rising mortality if it occurs.

ROLE OF BIOSECURITY IN PREVIOUS OUTBREAKS OF AI

Previous HPAI outbreaks have been studied by different authors in an attempt to identify risk factors for the infection, comparing case and non-case farms.

In 2002 the USDA in conjunction with the Virginia Department of Agriculture and Consumer Services organized an AI task force to eradicate an outbreak of H7N2 low pathogenic AI as a measure to protect the Virginia poultry industry, given the concern over the virus's ability to mutate to a highly pathogenic strain. Transmission from the index farm occurred first to farms from the same company that shared common truck routes, which highlighted the need for vigilant biosecurity in the commercial poultry industry [48]. In addition, infected farms were more likely to have non-family member caretakers and an owner or family member working off site. Similarly, spread by personnel and fomites were most strongly implicated in the widespread LPAI outbreak in Pennsylvania during 1983–1984 [12]; and the international spread of HPAI from the Netherlands in 2003 was also associated with increased number of contacts between farms [91]. Breaches in on-farm biosecurity were also recognized as playing a significant role in the spread of HPAI in the H7N3 outbreak in Canada in 2004 [60].

Kung *et al* (2007) [43], studying the Hong Kong outbreak in 2002, documented as factors positively associated with case farms: sale of chickens directly to retail markets ($OR = 11.15, p < 0.01$); automatic manure scrapers ($OR = 4.55, p = 0.02$); visits of persons from retail markets during the two previous months ($OR = 10.00, p = 0.01$); and visitor entry in the shed during the same period $OR = 3.94, p = 0.04$). Farm owner living on the farm was a significant protective factor ($OR = 0.05, p < 0.01$).

Nishiguchi *et al* (2007) [52] performed a similar case-control evaluation for the H5N2 outbreak in Japan. Lack of hygiene measures of visitors was identified as one of the risk factors. The main visitors who entered the farm areas were farm service crews for procedures such as debeaking or vaccination, catching crews for shipment of spent hens, official and private veterinarians, farm consultants, and the owners of company farms. These visitors also have opportunities to enter other farms. Even in farms which applied complete hygiene protocols to employees, the same strict measures were not always applied to the visitors who entered the farm area. And in some farms hygiene procedures were entrusted to the contractors and farm managers did not know the actual degree of implementation. Sharing of farm equipment was also associated with case farms (OR= 29.4, 95% CI= 4.2–207.5).

1.1.4 Modeling HPAI

Disease spread modeling

WWhile documenting sources of disease exposure is relatively straightforward, quantification is needed when it comes to determine the importance of a given exposure [36]. Biosecurity practices are necessarily based on risk assessments, whether formal or informal, such that the use of resources is balanced against the threat. On the one hand, insufficient biosecurity may result in more widespread disease. On the other hand, excessively restrictive protocols and expenditure of resources in the presence of minimal risk may lead to unnecessary stress, inefficient use of time, and depletion of limited resources [109].

In quantifying the risk of disease, not only characteristics of the disease agent must be taken into account, but also the likelihood of disease transmission and the consequences of the disease [36].

Mathematical models of the transmission of infectious agents are valuable tools to integrate epidemiological and biological data to give quantitative insights into patterns of disease spread and the effect of interventions. Therefore, models can be a powerful tool to optimize the use of limited resources or simply to target control measures more efficiently. Several forms of control measures exist, all of which operate by reducing the average amount of transmission between infectious and susceptible individuals. Which control strategy (or mixture of strategies) is used will depend on the disease, the host, and the scale of the epidemics [41].

In this context, it is important to describe in detail the contact structures within the industry over which transmission could occur. It is unclear, however, to what extent transmission parameters from other countries can be applied to models that include detailed industry structure [23].

By considering associations among sub-populations defined by their interactions, e.g. associations with the same catching companies, slaughterhouses, common ownership, etc., we determine the extent to which industry structures might influence the demographic and geographic extent of a potential AIV epidemic in the poultry industry. Improvement of our understanding of how poultry premises are potentially connected can also identify where further data collection is necessary [23].

In addition to the information that can help understanding spread off-farm, it has been suggested that a better understanding of the transmission dynamics of HPAI virus within flocks could help improve the effectiveness of control measures. In fact, quantification of the within-flock transmission could be useful for: determination of the time of introduction of the virus into a flock; estimation of the proportion of animals requiring vaccination; and assessment of the effect of intervention strategies [92].

Transmission of a contagious disease is, by definition, through the medium of contact. In order to model transmission of a contagious disease it is therefore necessary to quantify the following key factors:

- The duration of the latent and infectious period;
- How many contacts will a diseased unit make per unit of time and hence during the whole infectious period;
- How many of those contacts could potentially lead to infection of a susceptible unit, i.e. how many ‘effective contacts’ there will be.

TThe average number of effective contacts made by each infected unit would be the number of secondary infections to be expected when a single primary case of infection is introduced into a fully susceptible population. This number is called the basic reproductive ratio (otherwise known as basic reproductive rate or number), R_o [5].R_o has a threshold value that indicates whether an infection will spread or fade out: if $R_o > 1$, a disease can spread; if $R_o < 1$, chains of transmission will inevitably fade out [92].

Two types of model are useful in the study of infectious diseases at the population scale: stochastic and deterministic models.

Deterministic Deterministic models attempt to describe and explain what happens on the average at the population scale, and therefore fit well in large populations in which the value of random variation has little impact. These models categorize individuals into different subgroups (compartments), and are therefore known as compartmental models. The models specify the transition rates between the compartments as susceptibles may become exposed, exposed infectious, and so on [95].

Stochastic Stochastic models rely on among-individual chance variation in risks of exposure, disease, and other factors. Therefore, they allow follow-up of each individual in the population on a chance basis. They are used when chance fluctuations or known heterogeneities are important [41].

The output of a model built to study the dynamics of an infectious disease in a population can be the number of subjects (animals, premises, herds, etc) in each infectious stage in a given time; the total number of subjects exposed, infected, recovered, dead or remaining susceptible at the equilibrium (when the disease becomes endemic, dies out, or is eradicated); the time for the epidemic to die out or be eradicated; or even the variation in all the aforementioned when specific measures are applied. In any case, the output of the deterministic models will be a point value (a single number estimate), while stochastic model outputs are represented by a range of possible values, each associated with a specific probability of occurrence.

Models of AI

The process of modeling in epidemiology attempts to understand the prevalence and distribution of the pathogen, together with factors that determine incidence, spread and persistence. In the case of viral diseases the focus is not in predicting the number of viral particles in a population, but instead on the simpler tasking of categorizing individuals (or any other unit, as premises for instance) in the host population according to their infectious status [41].

Infections such as influenza, foot-and-mouth disease and classical swine fever, characterized by 'fast' infections, in which the pathogen causes illness for a short period of time followed by immunity or death, are mathematically best studied by 'SIR' models, which categorize hosts within a population as Susceptible (if previously unexposed to the pathogen), Infected (if currently colonized by the pathogen), and Recovered (in they have successfully cleared the infection). Refinements of the model can include particular characteristics of these diseases for improved realism. For instance, the hosts can die instead of recovering, or have different probabilities of going either way. In the case of some pathogens, as is the case for HPAI, following infection the pathogen reproduces rapidly within the host, relatively unchallenged by its immune system. During this latent stage, pathogen abundance is too low for active transmission to other susceptible hosts, and yet the pathogen is present. Since the host cannot be categorized as susceptible, infected or recovered, a fourth category is included to account for the individuals who are infected but not yet infectious — the Exposed individuals, giving rise to a particular model referred as 'SEIR' model [41].

In this model, key parameters are: the transmission rate, which determines the rate at which susceptible individuals are exposed to the virus by infected ones; the latent period, during which infected individuals are not yet infectious; the infectious period, during which infected individuals shed virus; and the rate of recovery (or death rate, depending on the outcome) of infected individuals.

The epidemiological factors that influence the HPAI reproductive number include infectiousness, susceptibility, the amount of virus transferred during contact, the duration of the infectious period, the contact rate between an infectious source and susceptible populations, and the number of flocks that make contact with each other [8]. Therefore, the dynamics of AI virus spread is expected to vary among the various types of flocks, because of the contact-structure differences between them, due to age, flock size, breed, management, and possibility of contact. Moreover, the differences in the density at which the chickens are housed will have an impact on transmission. Avian influenza kills chickens of all ages and will increase the death rate in older age groups. In addition, chickens in this age group are more likely to be visited by the stock agents, catchers, or farmers before being sent to the markets.

Data sources and outcomes Capua *et al* (2009) [18]reported the HPAI outbreak in Italy in 1997, caused by the H5N2 strain, to cause death in chickens within three to five days after contact, with mortality ranging from 11.4 to 100%. Van der Goot *et al* (2003) [98], working experimentally with the H5N2 virus, reported similarly high pathogenicity, with fully susceptible animals being invariably infected when confronted with HPAI virus and dying within six days after infection.

MMore recent work, focusing mainly on the H7N7 subtype, reported an extended infectious period, that can last up to eight days (six in average) [100, 99, 88, 80]. All the previously mentioned references agree on a latent period of one to most likely two days.

Savill *et al* (2008) [81] pointed out the differences in transmission and pathogenicity between the H7N7 subtype and the currently circulating H5N1, the latter one having faster dynamics with shorter latent (mean of 36 hours) and infectious (mean of 39 hours) periods, and consequently smaller reproductive number (2.5), even though the expected daily transmission rate is higher.

In accordance with the work of Savill *et al* [81], Tiensin *et al* [92], using data from a H5N1 outbreak in Thailand in 2004, showed a much faster epidemic when compared to the cited values for H7 HPAI viruses, but with lower R_o: flocks showed 100% mortality within 6 days, with the estimated transmission rate varying from 2.26 to 0.66/day depending on

Table 1.3: Virus transmission parameters for different strains of the Avian Influenza Virus (Highly Pathogenic) found in literature review.

Reference	HPAI virus type	INCUBATION Period	INFECTIOUS Period	R_o *or* β^*
Stegeman *et al* [88] (The Netherlands outbreak)	H7N7	2 days	2–6 days Based on Van de Goot 2003 [98]	Modeled farm transmission, not individual animals
Savill *et al* [80]	H5N1	2days minimum	5d minimum (1 asymptomatic)	$R_o = 66$
Van der Goot *et al* [98]	H5N2	1–2 days	6–8 days	$R_o = 31.7$
Van der Goot *et al* [99]	H7N7	2 days	Average 6.3 95% CI = 3.9–8.7	$\beta = 33d^{-1}$ $R_o = 208$
Capua *et al* [18] (Italy outbreak)	H5N2	Total 3–5 days		—
SAVIL *et al* [81] (Great Britain outbreak)	H5N1	36h	39h	$R_o = 2.5$
WARD *et al* [103] (Romania outbreak)	H5N1			$R_o = 1.95 - 2.21$

*β = number of exposed birds per infectious bird in a single day

Ro= number of exposed birds per infectious bird throughout the whole infectious period.

the assumed infectious period (1 to 4 days). The model assumed that infected chickens shed virus 1–2 days after infection and die within 2–6 days.

The parameters available in the literature are reviewed in Table 1.3.

Additionally, Spickler *et al* (2008) [86] reviewed the effects post inoculation in chickens of 34 HPAI viruses, including the subtypes H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N3 and H7N7. Their work summarizes a large range of morbidity, mortality and disease and virus shedding onset. The review indicates that, considering all subtypes listed, the morbidity and mortality associated with HPAI viruses is in the majority of the cases 100%. Mean time to onset of disease may vary from 1 to 11.2 days, mean disease time from 1 to 16.4 days, and time to death from 1 to 13 days. Shedding of virus in feces or respiratory secretions usually occurs on the second day, but can often vary from 1 to 4 days, or even the 8^{th} day when viruses of fowl origin are inoculated in chickens.

While these parameters allow the construction of models for prediction of within-flock dynamics, in the face of the threat of introduction of a major infectious disease, such as H5N1 HPAI, policymakers and animal disease control agencies need locally specific answers to the following questions: What is the most likely size, duration and geographical distribution of the epidemic? What are the critical components that exacerbate the risk of spread? What are the most effective control strategies? Such questions can only be addressed through detailed consideration of the specific features of the population at risk and potential transmission routes within this population [84].

To answer these questions, parameters are not as easily available for the transmission of HPAI between farms. Little is known regarding the types and frequencies of contact that exist between farms and which of these may act as pathogen transmission routes; however it is likely that farms demonstrate considerable heterogeneity in such contacts [14]. For this reason, extrapolation of data from outbreaks to other regions is problematic due to regional differences in the poultry industries and in the characteristics of the viruses.

Attempting to gain this needed information on the dynamics of the virus in field conditions, many authors used epidemic data to estimate transmission parameters, and develop disease spread models [88, 47, 50, 49, 11, 10].

Boender *et al* (2007) [11, 10] used data from the H7N7 epidemics in the Netherlands in 2003 to fit a spatial model designed to detect higher risk areas for the disease based on the spatial distribution of poultry farms. The results of the model are used to discuss the effectiveness of control measures in these different density areas. Le Menach *et al* [49] used an extended SEIR model in which the units are individual farms, therefore the final category represents depopulated premises, and the infectious class is split to represent detected and undetected cases. Again data from the 2003 epidemics in the Netherlands was used to fit the model, therefore estimating disease transmission parameters and higher risk areas. After fitting the data to the model the estimated time from infection of a farm to detection of the disease was an average of 4 days (range: 0–6), and the transmission rates between farms initially varied from 0.076 to 0.336 depending on the distance radius from the infected farm, and decreased to 0.016 to 0.13 as the epidemic continued and awareness of the disease was increased among growers.

Garske *et al* (2007) [34] analyzed published data from three outbreaks of HPAI in commercial poultry in industrialized countries to estimate the farm-to-farm reproductive number of HPAI to explore the extent to which different intervention measures implemented during these outbreaks reduce the reproductive number. The outbreaks analyzed were: an outbreak of H7N1 in Italy in 1999–2000, an outbreak of H7N7 in the Netherlands in 2003, and an outbreak of H7N3 in Canada in 2004. For all outbreaks the estimates of the mean reproductive number (the expected number of secondary infections from each infected subject) prior to the controls being implemented are between 1.1 and 2.4 with upper 95% bounds in the range 1.5–3.6. The impact of control measures on the effective reproductive number can be clearly seen in all four outbreaks.

Conclusions Despite the large amount of experimental research on HPAI viruses, including some laboratory-based transmission studies, knowledge of the transmission of HPAI viruses in field conditions still has considerable gaps, which complicates epidemic control [12]. At present, there is little quantitative understanding of how long it takes to detect the effects of HPAI infection in commercial poultry flocks, and how such time to detection is affected by various factors such as flock size, species, age and housing [81].

Models previously developed used a mechanistic approach, meaning that transmission is modeled as a single process, and data is used to fit probabilities of farm infection that explain the observed epidemics. To the best of our knowledge, human movement data was analyzed before only by Sharkey *et al* [84], using parameters from a H5N1 outbreak in Great Britain. The size of the epidemic was limited to the initial infected premises (IPs) in the majority of simulated outbreaks (73%). Geographical distribution of risk was not a trivial function of farm density. The chance of an outbreak being large was shown to be dependent on the sector of the poultry industry in which it is seeded and the characteristics of the production system. Therefore, extrapolation of the results to commercial poultry systems in other countries is very limited.

1.2 Project Outline

1.2.1 Antecedents

In 2005 a stochastic risk model estimating the potential spread of Highly Pathogenic Avian Influenza (HPAI) within and between commercial farms in Georgia was developed [25]. Virus spread among birds on the index farm was assumed to follow an SEIR epidemic model [41] and spread between farms was estimated by the probability and nature of human movement between farms. Input data was derived from a scientific review of the literature, the results of a producer survey that assessed the number and type of farm visitors in seven days [101], and input from a panel of scientific experts regarding the likelihood of each farm visitor contacting infectious materials. The model estimated the number of farms potentially exposed to virus from the index farm during the time period between initial infection of the index farm and the day the bird grower noted serious illness in his flock. The probability of farm exposure to HPAI virus was dependent upon the daily likelihood of a farm visitor having contact with the index farm during the model time period, and the probability that the visit resulted in contact with infectious materials on the index farm. It was assumed that a visit to an infected farm that resulted in a high probability of exposure to virus-contaminated materials was likely to result in spread of the virus when another farm was contacted by this same visitor within 24hrs. Consequently the potential role of biosecurity measures was not taken into account in this model.

In an effort to improve the model's predictiveness and to estimate the role of biosecurity practices in preventing virus spread, this study is designed to gain information on biosecurity practices used by commercial poultry growers in Georgia, and include these data in a stochastic risk model of HPAI spread among commercial poultry. By including biosecurity information in the model this study made it be possible to study current strengths and weakness in the Georgia poultry production system and the potential impact of biosecurity on reducing the risk of disease spread between farms.

1.2.2 Purpose of the Study

This study aims at investigating the impact of biosecurity measures in reducing the risk of spread of Highly Pathogenic Avian Influenza virus among commercial poultry farms through human movement, using the specific scenario of the Georgia poultry industry to reproduce the disease spread dynamics in a hypothetical outbreak.

Specifically, this study seeks to answer the following questions:

- What biosecurity protocols are common among commercial poultry companies?
- How well are biosecurity protocols followed among growers?
- How does farm biosecurity modify the risk of HPAI spread between farms in a predictive model? How well does on farm biosecurity protect poultry from disease introduction?
- Which measures have the highest estimated impact on reducing the probability of spread between farms?
- Following secondary spread, would the infection of one farm evolve to a major outbreak? Can we predict the number of farms involved in a potential HPAI epidemic by the time of detection, and the effect of biosecurity measures on reducing it?

To answer these questions, the following steps will be accomplished:

- Gain information about biosecurity standards set by the poultry industry and the level of compliance by growers through contact with the companies and an individual survey of growers;

- Use mathematical modeling to combine the acquired information with information from other studies and simulate the daily risk of contamination spread, and evaluate the impact of prevention measures in reducing this risk;
- Simulate the dynamics of an HPAI outbreak in order to evaluate the impact of prevention measures in reducing silent spread of HPAIv before detection.

By following these steps, this study will be able to characterize the contact structure among commercial poultry operations in Georgia, determine the patterns of transmission of highly virulent diseases within this network, and assess the efficacy of biosecurity measures in preventing virus introductions from becoming fast spreading epidemics, taking into account the particular conditions of this scenario. This will allow companies and growers to assess their current level of preparedness against poultry disease epidemics, in particular HPAI, and implement efficient biosecurity programs.

CHAPTER 2

SURVEY OF BIOSECURITY PROTOCOLS AND PRACTICES ON COMMERCIAL POULTRY FARMS IN GEORGIA

2.1 INTRODUCTION

Biosecurity consists of the measures used to prevent introductions of disease-causing organisms into a flock or herd. The adoption of these measures can not only significantly reduce the risk of disease introduction, but also reduce the magnitude of the financial losses that may occur following infection in a flock [51]. Therefore, a routine biosecurity protocol should be implemented as standard operating procedures in animal production systems to protect , against any infectious disease [42]. This is especially important when the economic impact of an introduction could be devastating.

In the United States the combined value of production from broilers, eggs, turkeys, and the value of sales from chickens in 2008 was \$35.9 billion. The value of broilers alone was \$23.1 billion. The state of Georgia is the largest producer of broilers in the country, producing 1.4 billion birds in 2008 [53]. Poultry production is the number one agricultural enterprise in the state, accounting for approximately 50% of the value of farm products produced. More than 4,000 family-owned poultry farms produce more than \$5 billion annually in farm gate value [22].

Close to 98% of the commercial poultry farms in the state belong to company integrators (Dr. Louise Dufour-Zavala, personal communication). This is expected to be a closed system, where biosecurity measures in place are supposed to make the whole system an isolated compartment in relation to non-commercial or non-integrated poultry premises. Routine activities common to all commercial farms, however, keep the farms in constant contact inside the network and increase the risk of disease transmission. In the case of Avian Influenza, for example, the most important mode of transmission in domestic poultry is related to the movement of humans, materials, and vehicles [48, 91, 12, 43, 52]. Studies of previous outbreaks have shown that breaches in on-farm biosecurity, such as a lack of hygiene measures for visitors, played a significant role in the spread of the virus [60, 43, 52].

Other diseases of high impact in the commercial poultry industry, such as Newcastle disease virus (NDV), and Salmonella and Campylobacter species, are also mainly transmitted by the movement of people and equipment between farms [23], and a failure in the biosecurity barrier constitutes a risk for the introduction of any of these diseases in the system. The rapid spread of AI in outbreaks of Low Pathogenic Avian Influenza in the USA, and the outbreak of Infectious Laryngotracheitis in North Carolina and Georgia in 2007 illustrate that the biosecurity of many farms may be inadequate [89].

To prevent opportunities for disease introduction, and protect this vital business for Georgia farmers, it is expected that poultry companies establish biosecurity standards among their contract growers. It's probable that the companies set similar standards, but it is hypothesized that the level of implementation on individual farms may vary significantly, impacting the risk of infectious disease spread. With an assessment of the level of implementation by individual growers, quantitative studies can then evaluate the role of individual biosecurity measures in reducing the risk of disease spread at the farm level.

A survey was designed and distributed to poultry growers in Georgia in order to assess specific information concerning their implementation of biosecurity measures. The survey focused specifically on the risk of disease spread associated with human movement between farms, and therefore on biosecurity measures that impact this particular risk.

The first objective of the study was to obtain the company-level general biosecurity protocols established by poultry integrators in Georgia; and the second was to assess the individual implementation by growers using an anonymous individual survey. The goal of the first objective was to determine whether there were different levels of biosecurity awareness among different companies, and the goal of the second was to assess variability in individual grower compliance. It was hypothesized that awareness and compliance to biosecurity measures is not homogeneous among all poultry growers, with variation existing at both the individual grower and integrator levels. It was anticipated that variation would be noted in farming practices (use of equipments, routine cleaning, etc), use of protective personnel equipment by farm personnel, and hygiene requirements enforced for visitors entering the premises.

We also intended to assess the variation between two geographical regions of the state, North and South Georgia, since these two areas have different poultry farm densities, and the work of Vieira *et al.* (2009) [101] showed that this results in different patterns of contact between farms. Moreover, in 2007 an outbreak of infectious laryngotracheitis (ILT) affected North Carolina, and later spread to Georgia. Many counties in North Georgia were included in the control zone for the epidemic. The incident raised the disease awareness level among growers in the control areas, which may have enhanced their adoption of biosecurity measures, making the results of the survey potentially biased if trying to assess routine practices. For this reason, variation was also evaluated between the areas in the state inside (North Georgia) and outside (South Georgia) the control zone for the ILT outbreak.

Based on an assumption that human movement would be the most important means of disease transmission, an anonymous questionnaire was designed and distributed to commercial broiler growers in Georgia. The questionnaire intended to investigate a number of farming characteristics and assesses compliance with biosecurity measures that could reduce the risk of virus transmission between farms by human movement. Variation was expected to be associated with regions, companies, individual growers, farm size, and number of farms owned by the same grower.

The integrated poultry system in Georgia is composed of 12 broiler integrators and 4 commercial layer companies, in addition to 4 hatching egg and 3 primary breeder farms (Dr. Louise Dufour-Zavala, personal communication).

2.2 Materials and Methods

2.2.1 Survey of company protocols

All 12 broiler integrators in the state were contacted and invited to participate in the study, 8 of which agreed to collaborate. The collaborating companies were asked about the availability of written biosecurity guidelines distributed to the integrated growers, in order to evaluate the level of biosecurity expected to be adopted by individual farms, and also the level of awareness the companies maintain among their contract growers.

2.2.2 Growers survey

Target population

The two regions of Georgia selected for comparative study represent poultry industries with different challenges, as the density of poultry farms is higher in the North, as shown in Figure 2.1, and growers in this region were still included in the control zone for an outbreak of ILT initiated in 2007at the time the questionnaires were distributed in 2008.

Questionnaires were distributed to all growers in the companies that agreed to participate.

Questionnaire preparation

To functionally characterize biosecurity practices described in the scientific literature [27, 28, 48, 51], an expert elicitation of poultry veterinary scientists representing the University of Georgia (UGA) and the United States Department of Agriculture (USDA), was done. All individual biosecurity practices considered relevant for study were grouped as: biosecurity practices adopted by on-farm personnel, biosecurity practices required from visitors and their vehicles, bird disposal and house cleaning, and equipment sharing and cleaning. Characterization of the farm size (number of houses and number of birds) was included to investigate the possible correlation of farm size with the compliance to biosecurity measures. Additional questions investigated factors that could constitute risk for disease introduction,

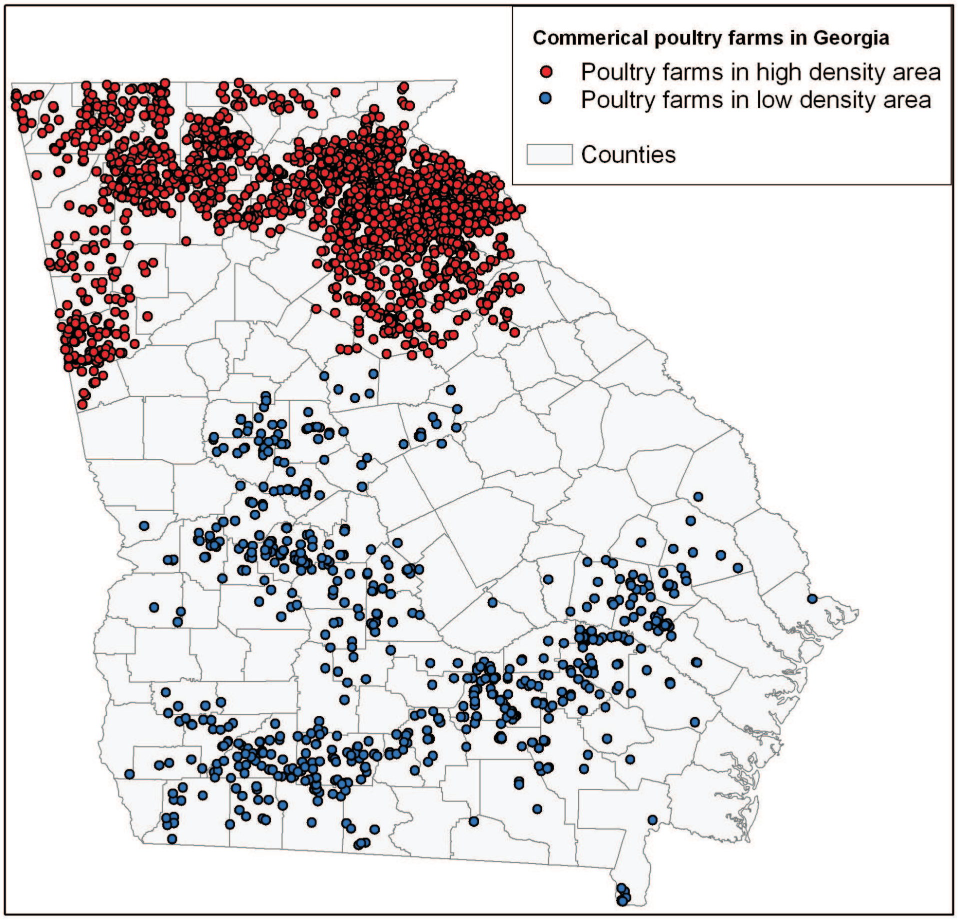

Figure 2.1: State of Georgia and the location of commercial poultry farms, according to information provided by the Georgia Department of Agriculture. The subsets shown in the map correspond to the two regions of different poultry farm density in the state, which we refer to as North and South Georgia.

such as ownership of other farms by the owner or relatives, and the type of visitors allowed inside the houses during growout.

The questionnaire was piloted in January 2008, with the distribution of 80 questionnaires in a single poultry company in the state. Thirty-one (38.8% response rate) growers returned the questionnaires. Following review of the answers, the questionnaire was modified to increase clarity, and to include some options that were considered more relevant. A summary of the questions in the final questionnaire is given in Table 2.1. A copy of the full questionnaire is attached in the Appendix.

Questionnaire distribution

Of the eight companies that agreed to participate, 6 operated in the North of the state (including the company in which the questionnaire was piloted), and 2 in South Georgia. Whenever possible, an investigator visited the collaborating companies to explain to the servicemen the study goals and potential benefits of the results to the industry. Servicemen received the survey packets to distribute among the poultry producers that they regularly visited. Packets contained the questionnaire and a letter explaining the purpose of the study placed in a pre-addressed stamped envelope, allowing growers to mail completed surveys directly to study investigators at the University of Georgia. Visit of an investigator was not possible for the two companies in South Georgia, in which case the packets were mailed to the field manager. In two companies in the North, even though the company was visited, meeting with the field staff was not possible, and all the packets were handed to the manager for further distribution.

No information was collected that could allow identification of the grower, and because the questionnaires were sent directly back to investigators, companies did not review grower's answers. Company names were also not documented, but the differentiation among companies was kept by color coding the questionnaires. This was considered important in order to

Table 2.1: Summary of the questions addressed to broiler growers in Georgia in a survey of the adoption of biosecurity measures.

Question	Answer type/options	Variable type
Farm characterization		
Number of farms owned	Open	Integer
Number of poultry houses operated		
Total one-bird capacity of all houses		
Person in the extended family owning a poultry farm	Yes–No	Binary
Biosecurity practices adopted by PERSONNEL		
Use of plastic boots or shoe covers	5 points Likert scale: Never, Rarely, Sometimes, Usually, Always	Rank 0–4
Stepping on a footbath		
Use or hairnets		
Use of coveralls		
Shower/change when entering and exiting farm		
Hands washing after handling birds		
When are coveralls removed	Non-exclusive options: after exiting each house; prior to leaving the farm; at the end of the day	Binary for each option
Biosecurity practices required from VISITORS		
Requirement of vehicles to park outside	5 points Likert scale: Never, Rarely, Sometimes, Usually, Always	Rank 0–4
Vehicles tires disinfected when entering the farm		
Vehicles tires disinfected when exiting the farm		
Distance of parking to the houses	Multiple choice: within 10ft; 10-30ft; 30-50ft; over 50ft	Rank 1–4
Plus all the questions used for personnel		
Birds disposal and cleaning		
Frequency of dead birds removal	Multiple choice: more than twice a day, twice a day, once a day, every other day, once or twice a week, less than once a week	Categorical
Mode of bird disposal	Non-exclusive options: composting on-farm, composting off-farm, dead pit/burial on-farm; dead-pit/burial off-farm; other (please list)	Binary for each option
Distance of disposal site to houses	Multiple choice: Within 10ft; 10-20ft; more than 20ft	Rank 1–3
Distance of disposal site to driveway		
Frequency of litter cleaning	Multiple choice: every flock, every 2nd flock; 3rd, 4th, 5th, 6th, after more than 6 flocks	Categorical
All houses cleaned out at a time	Yes–No	Binary
If no, how many houses cleaned at each time	Open	Integer
Equipments		
Use of contracted services	5 points Likert scale: Never, Rarely, Sometimes, Usually, Always	Rank 0–4
Share if equipments for decaking or clean out		
Number of growers sharing the same equipment	Multiple choice: 1, 2, 3, 4, 5, more than five (please specify)	Integer
Cleaning of equipment after use	Non-exclusive options: Disassembled and pieces cleaned separately; Disinfectant used to clean all washable surfaces; Visible debris removed with water; Power washer used on all washable surfaces; Not cleaned.	Binary for each option
Visitors allowed to enter a house during flock grow out		
Growers asked to check all that apply from a list including: Shaving suppliers; LP Gas delivery personnel; Feed truck drivers; Egg truck drivers; Chick bus drivers; Veterinarians; Meter readers		Binary for each option
Company service persons, managers; Repairmen; Live haul service personnel; Vaccination crews; Clean-out service personnel; Decaking service personnel; Pit inspectors; Non service-related visitors; Agricultural extension agents		

control, in the statistical analysis, for variables that could be associated with the company of origin.

The color coding of the questionnaires also allowed recognition of the area of origin — North or South Georgia, while keeping the farms and companies anonymous.

DATA ANALYSIS

Data was entered into an Excel spreadsheet (Microsoft® Office Excel® 2007). Binary variables were recorded as “0” or “1”, and percentages reported refer to the proportion of ‘1’ (Yes). For the rank variables in the Likert scale used for the frequency of adoption of listed biosecurity measures, a scale of 0 to 4 was used to translate the ordinal ranks “never”, “rarely”, “sometimes”, “usually” and “always” (0 being never, and 4 being always). Other rank variables (such as distance to parking or to disposal, for instance) were also recorded as numbers.

Data were stratified according to the state region (North and South), the company of origin, the number of houses (farms with up to four houses in one group, versus another group for farms with more than four houses) and the number of farms owned by the same grower (growers owning one single farm in one group, versus another group for growers owning multiple poultry premises).

Using Intercooled Stata® 9.2 for Windows (Stata Corp LP), integer and rank variables were compared between geographic region, company, farm size and number of farms owned by the grower groups using the Kruskal-Wallis non-parametric test of equality of medians. Binary variables were tabulated and the result of the Fisher’s exact test reported for comparisons between two groups, and the Pearson chi-square for comparisons among three or more groups.

To summarize the overall level of biosecurity adopted by a farm and compare this among strata a scoring system was developed. Only the practices associated with daily activities were included in the biosecurity score. All assigned scores for frequency of adoption of each measure (from 0 to 4 for each item referring to never, rarely, sometimes, usually and always, in this order) were summed, giving higher scores for farms adopting more practices and more often. For the use of coveralls, 1 was summed for every listed time when coveralls are removed (after leaving each poultry house, prior to leaving the farm or at the end of the day). The rank scores for distance were also kept as ranks, from 1 to 4, and summed. The maximum biosecurity score obtainable on a farm was 70. Again, groups were compared using the Kruskal-Wallis test of equality of medians. Since this scoring system would count non-answered items as zero, incomplete questionnaires would count as a lack of biosecurity. To correct this the assigned score was weighted according to the percentage of valid answers in all the items accounted for in the sum (the total sum obtained was multiplied by 70 — the highest score if all questions were answered — and divided by the maximum score that could be attainable with the questions actually answered). This was considered as an "adjusted score".

2.3 Results

2.3.1 Survey of company protocols

Five of the eight companies responded the request for biosecurity protocols. Two declared they did not have any written standards concerning biosecurity measures, but stated that all growers are instructed not to allow non-company personnel inside the houses, and that field managers visit the farms weekly and give growers further advice concerning biosecurity. Three companies shared written material, but none of them consisted of an explicit set of guidelines or procedures expected from the growers: one contained a list of the only people who should be allowed inside the poultry houses, and statements to be signed by visitors to ensure that they did not contact non-company birds in the previous 3 days; the second consisted of educational material regarding the benefits of biosecurity, and situations in which the company could enforce additional levels of biosecurity; and the third company's material consisted of a list of biosecurity items that are checked periodically by the company servicemen upon visits to the farms.

2.3.2 GROWERS SURVEY

PARTICIPATING COMPANIES AND GROWERS RESPONSE RATE

Table 2.2 shows the number of surveys distributed and mailed back in each company. The company in which the survey was piloted is listed as company "0". In one of the participating companies all growers were interviewed by the servicemen, but since anonymity was not preserved, the 135 questionnaires were not included in the results. Therefore, only 7 companies are listed in Table 2.2.

Table 2.2: Response rate in the survey addressed to broiler growers in Georgia.

Geographical region	North Georgia					South Georgia		
Company	0	1	2	3	4	5	6	Total
Number distributed	80	300	170	100	120	91	171	1032
Number received back	31	88	34	15	33	20	46	267
Response rate	38.8%	29.3%	20.0%	15.0%	27.5%	22.0%	26.9%	25.9%

Response rates were compared between companies in North Georgia (0 to 4) and South Georgia (5 and 6) and no significant difference was found (Fisher's exact = 0.86). However, companies in which an investigator was given the chance to talk directly to the servicemen (companies 0,1 and 4) showed an overall higher response rate (28.8%) when compared to the companies in which the envelopes were handed (companies 2 and 3) or mailed (5 and 6) to the field manager for further distribution (21.6%; $P = 0.01$).

STRATIFIED ANALYSES

Table 2.3 summarizes the farm characteristics, and shows that there are no significant differences in the profiles of surveyed farms by region, size or number of farms owned by the same grower. Surveyed broiler growers typically own just one farm, growing out around

96,000 birds at a time distributed in four houses. It was not uncommon for growers to have members in the immediate family also owning/working for poultry farms (35.2%).

Table 2.4 summarizes the results concerning the adoption of biosecurity measures by farm personnel, while Table 2.5 shows the requirements of similar practices from visitors, with the addition of biosecurity measures applied to their vehicles. The median frequency of adoption for a large number of biosecurity practices was zero, as a large number of growers reported never wearing personal protective equipment themselves or requiring these practices from visitors.

Again, no significant differences in the frequency of adoption of biosecurity measures were found between growers owning one or more farms, or farms of different sizes, except for a more frequent adoption of the practice of showering or changing clothes before leaving the farm in small farms (median of two for farms with four houses or less, representing a reported frequency of “Sometimes”, versus a median of one, “Rarely”, for farms with more than four houses, $P = 0.019$). Even though growers owning farms of any farm size most often do not request visitors to park outside the farm, farms with more than four houses were more likely to have a greater distance between parking and the houses ($P = 0.003$).

As to the differences by region, Tables 2.4 and 2.5 show a higher level of biosecurity in North Georgia, where there is a higher frequency of personnel that shower or change clothes before leaving the farm ($P = 0.001$) or of visitors that wear coveralls ($P < 0.001$), When coveralls are used by either personnel or visitors, they are most frequently taken off prior to exiting the farm, in both regions.

In North Georgia there is also a higher frequency of use of footbaths by personnel and visitors ($P < 0.001$). Use of plastic boots or shoecovers by personnel is low in both areas (median frequency of zero in North Georgia and of one in the South, which was not found to be a significant difference), but the frequency of use by visitors is high (median $=$ 4, “Always”). Washing hands after handling birds is the measure with highest adoption in both regions.

In neither region were visitors likely to be asked to park outside the farm, nor wash their tires before entering or exiting the farm, but a few growers in North Georgia did state that they always required tires to be washed, making the profile in the region significantly different from the South ($P < 0.001$).

Tables 2.6 and 2.7 focus on farming practices. Table 2.6 shows information concerning bird disposal and house cleaning, while Table 2.7 shows information on equipment use and sharing. Growers usually enter the houses to pick up dead birds once a day, and these are almost always disposed on farm, with variations in the method according to the region of the state — growers in the North dispose birds mainly in pits, while in the South having incinerators on the farm is a common practice ($P < 0.001$). Growers in farms with more houses seem to use composting as the method of disposal more often than in smaller farms ($P = 0.003$). The median distance between the site of disposal on-farm and the poultry houses or any roads is always 20–30 feet, for any farm size and state area.

The median number of flocks kept on the same litter was three in North Georgia for any size farm and number of farms owned, while the median in South Georgia was one flock ($P = 0.054$). When poultry houses are subjected to a complete change of litter, the cleaning is more likely to be carried out in all houses on the farm in South Georgia when compared to North ($P = 0.001$), and in smaller farms ($P < 0.001$). When not all houses are cleaned out at the same time, all growers in the state are most likely to clean half of the houses at a time.

The frequency of contracting services for cleaning out or house decaking is higher in the North (median frequency of two, "Sometimes", versus a median of zero, "Never", in the South), but the difference was not found to be significant ($P = 0.086$). Sharing of equipment does not seem to be a frequent practice, and the number of growers sharing equipment is generally low (the upper limit of the range in Table 2.7, 20 growers sharing the same equipment, corresponds to the answer of two questionnaires, the largest number below that being 5 growers). But when equipment is shared, it's usually cleaned with a power washer, disinfectants were used in only 17% of the responding farms , while only 3% of the growers reported cleaning parts separately (no differences between areas of the state of farm profiles for any of these items).

Growers were also asked what type of visitors are allowed (either sporadically or routinely), to enter the poultry house during growout. Table 8 shows the proportion of growers that answered yes for each visitor listed in the questionnaire. The type of visitors allowed inside the houses was similar regardless of region or farm profile, with the exception that the percentage of growers allowing repairmen to enter the house and using vaccination crews is higher in the South ($P = 0.037$ and $P = 0.002$, respectively). Veterinarians come to the poultry houses more frequently on bigger farms ($P = 0.044$).

Table 2.3: Summary of characteristics of farms surveyed about their biosecurity activities in two regions (North and South) of Georgia. Median (range) or percentages are reported as appropriate.

		Response	Region			Houses/Farm			No Farms Owned			
	n	rate	South	North	†P	1–4	5+	†P	1	2+	†P	Total
Number of farms owned	266	99.6%	1(1–6)	1(1–6)	0.055	1(1–6)	1(1–6)	0.55	–*	–*	–*	1(1-6)
Houses/farm	266	99.6%	4(1–10)	4(1–12)	0.991	–*	–*	–*	4(1–12)	3(1–8)	0.786	4(1-12)
Birds/house x(1,000)	261	97.8%	26.7 (9–27)	23.7 (5.9–37.5)	0.093	23 (6.7–37.5)	24.8 (5.9–35)	0.274	24.1 (5.9–37.5)	23 (9-31.7)	0.301	24 (5.9-37.5)
Relatives also have a poultry farm	236	88.4%	37.9%	34.1%	0.346	33.1%	39.7%	0.377	33.7%	44.1%	0.249	35.2%

*Variable was used to define groups, therefore comparisons are not applicable

†Comparison P-value

Table 2.4: Frequency of adoption of specific biosecurity measures by personnel, as assessed in the survey of broiler growers in Georgia. Median (range) or percentages are reported as appropriate.

		Response	Region			Houses/Farm			No Farms Owned			
	n	rate	South	North	†P	1–4	5+	†P	1	2+	†P	Total
Plastic boots/shoe covers	260	97.4%	1(0–4)	0(0–4)	0.086	0(0–4)	0(0–4)	0.737	0(0–4)	0(0–4)	0.98	0(0–4)
Footbath	259	97.0%	0(0–2)	3(0–4)	< 0.001	1(0–4)	1(0–4)	0.873	1(0–4)	0(0–4)	0.366	1(0–4)
Hairnets	254	95.1%	0(0–4)	0(0–4)	0.007	0(0–4)	0(0–4)	0.484	0(0–4)	0(0–4)	0.357	0(0-4)
Shower and change clothes	258	96.6%	0(0–4)	2(0–4)	0.001	2(0–4)	1(0–4)	0.019	2(0–4)	2(0–4)	0.388	2(0–3)
Coveralls	248	92.9%	0(0–2)	0(0–4)	0.236	0(0–4)	0(0–4)	0.569	0(0–4)	0(0–4)	0.408	0(0–4)
Coveralls are taken off:												
Exiting every house			16.7%	5.7%	0.227	11.1%	0.0%	0.313	7.0%	12.5%	0.493	7.7%
Before leaving farm			75.0%	79.3%	0.506	80.0%	75.0%	0.747	77.2%	87.5%	0.675	78.5%
At the end of the day			16.7%	26.4%	0.383	22.2%	30.0%	0.542	22.8%	37.5%	0.395	14.6%
Wash hands	267	100.0%	4(0–4)	4(0–4)	.**	4(0–4)	4(0–4)	.**	4(0–4)	4(0–4)	.**	4(0–4)

*Total for the question (24) was considered to be the number of growers that reported using coveralls.

**Not enough observations under the median value to allow comparisons.

†Comparison P-value

Table 2.5: Frequency of adoption of specific biosecurity measures by visitors, as assessed in the survey of broiler growers in Georgia. Median (range) or percentages are reported as appropriate.

		Response	Region			Houses/Farm			No Farms Owned			
	n	rate	South	North	†P	1–4	5+	†P	1	2+	†P	Total
Parking outside	242	90.6%	0(0–4)	0(0–4)	0.845	0(0–4)	0(0–4)	0.796	0(0–4)	0(0–4)	0.528	0(0–4)
distance to parking***			3(1–4)	3(1–4)	0.299	2(1–4)	4(1–4)	0.003	3(1–4)	3(1–4)	0.262	3(1–4)
Wash tires before entering	247	92.5%	0(0–2)	0(0–4)	< 0.001	0(0–4)	0(0–4)	0.392	0(0–4)	0(0–3)	1	0(0–4)
Wash tires prior to leaving	247	92.5%	0(0–3)	0(0–4)	< 0.001	0(0–4)	0(0–4)	0.622	0(0–4)	0(0–3)	0.4	0(0–4)
Plastic boots/shoe covers	245	91.8%	4(0–4)	4(0–4)	.**	4(0–4)	4(0–4)	.**	4(0–4)	4(1–4)	.**	4(0–4)
Footbath	229	85.8%	0(0–3)	4(0–4)	< 0.001	2(0–4)	2(0–4)	0.898	2(0–4)	2(0–4)	0.887	2(0–4)
Hairnets	229	85.8%	0(0-4)	3(0-4)	< 0.001	2(0–4)	2(0–4)	0.621	2(0–4)	2(0–4)	0.858	2(0–4)
Shower and change clothes	222	83.1%	0(0–4)	0(0–4)	0.009	0(0–4)	0(0–4)	0.543	0(0–4)	0(0–4)	0.689	0(0–4)
Coveralls	242	90.6%	0(0–4)	4(0–4)	< 0.001	3(0–4)	2(0–4)	0.325	3(0–4)	2(0–4)	0.551	3(0–4)
Coveralls are taken off:												
Exiting every house			15.6%	9.9%	0.252	12.1%	8.3%	0.614	12.3%	3.5%	0.208	1.9%
Before leaving farm			84.4%	85.5%	0.525	82.3%	91.7%	0.12	90.7%	93.4%	0.62	85.3%
At the end of the day			0.0%	9.2%	0.062	8.1%	6.7%	1	5.8%	17.2%	0.049	7.6%
Wash hands	243	91.0%	4(0–4)	4(0–4)	.**	4(0–4)	4(0–4)	.**	4(0–4)	4(0–4)	.**	4(0–4)

*Total for the question (136) was considered to be the number of growers that reported using coveralls.

**Not enough observations under the median value to allow comparisons.

***Numbers refer to the distance rank used in the survey: 1=within 10ft; 2=10–30ft; 3=30–50ft; 4=over 50ft.

†Comparison P-value

Table 2.6: Disposal and cleaning practices adopted by the growers as assessed in the survey of broiler growers in Georgia. Median (range) or percentages are reported as appropriate.

		Response	Region			Houses/Farm			No Farms Owned			
	n	rate	South	North	†*P*	1–4	5+	†*P*	1	2+	†*P*	Total
Frequency of dead bird removal(times a week)	267	100.0%	7(2–14)	7(4–21)	0.353	7(2–21)	7(7–14)	0.291	7(2–21)	7(7–2)	0.589	7(2–21)
Disposal of birds	266	99.6%										
Disposal on farm			98.5%	99.5%	0.431	99.5%	98.8%	0.519	99.1%	100.0%	1.00	99.3%
Disposal off-farm			1.5%	0.5%	0.435	0.5%	1.2%	0.522	0.9%	0.0%	1.00	0.8%
Composting			9.1%	15.0%	0.156	9.2%	23.2%	0.003	14.5%	7.7%	0.318	13.5%
Dead pit			12.1%	83.5%	< 0.001	67.4%	62.6%	0.484	66.1%	64.1%	0.856	65.8%
Incinerator			80.3%	6.5%	< 0.001	26.1%	22.0%	0.54	23.8%	30.8%	0.422	24.8%
Distance disposal to houses***	229	85.8%	3(1–3)	3(1–3)	.**	3(1–3)	3(2–3	.**	3(1–3)	3(2–3)	.**	3(1–3)
Distance disposal to road***	163	61.0%	3(1–3)	3(1–3)	.**	3(1–3)	3(1–3)	.**	3(1–3)	3(1–3)	.**	3(1–3)
Litter change	263	98.5%										
growers reporting use of litter for more than 6 flocks			10.6%	9.6%	7.1%	16.5%	10.3%	7.7%	9.9%			
Median when use litter for 1-6 flocks			1(1–5)	3(1–6)	0.054	3(1–6)	3(1–6)	0.191	3(1–6)	3(1–6)	0.431	3(1–6)
Houses are cleaned all at a time	232	86.9%	98.4%	84.5%	0.001	89.3%	69.9%	< 0.001	89.0%	84.4%	0.55	88.4%
If not, houses cleaned			8	2(1–4)	0.092	2(1–4)	3(2–8)	0.062	2(1–4)	4(2–8)	0.022	2(1–8)
% of houses cleaned at a time			0.54	0.5 (0.17–0.67)	0.203	0.5 (0.25–0.67)	0.5 (0.17–0.60)	0.924	0.5 (0.17–0.67)	0.5 (0.5–0.67)	0.231	0.5 (0.17–0.67)

*Numbers refer to the distance rank used in the survey: 1=within 10ft; 2=10–20ft; 3=more than 20ft.

**Not enough observations under the median value to allow comparisons.

*** Only two growers reported the number of houses cleaned at a time, and both reported 8.

†Comparison *P*-value

Table 2.7: Equipment source and sharing as assessed in the survey of broiler growers in Georgia. Median (range) or percentages are reported as appropriate.

		Response	Region			Houses/Farm			No Farms Owned			
	n	rate	South	North	†P	1–4	5+	†P	1	2+	†P	Total
Use of contracted services	233	87.3%	0(0–4)	2(0–4)	0.086	2(0–4)	0(0–4)	0.15	2(0–4)	3(0–4)	0.487	2(0–4)
Share of equipments	261	97.8%	0.50(0–4)	0(0–4)	0.151	0(0–4)	1(0–4)	0.063	0(0–4)	0(0–4)	0.878	0(0–4)
Number of growers sharing			1(1–5)	1(1–20)	0.446	1(1–20)	1(1–5)	0.389	1(1–20)	1(1–4)	0.765	1(1–20)
Cleaning procedures	213	79.8%										
Disassemble and clean parts			0.0%	3.8%	0.175	2.1%	4.2%	0.409	2.7%	3.5%	0.589	2.8%
Disinfectant used			9.4%	20.0%	0.056	17.0%	18.1%	0.85	17.4%	17.2%	1.00	17.4%
Water wash only			35.9%	33.8%	0.452	32.6%	37.5%	0.542	35.9%	14.1%	0.293	34.3%
power washer			60.4%	64.4%	0.357	63.1%	63.9%	1.00	63.0%	65.5%	0.839	63.4%
Not cleaned			1.9%	3.5%	0.487	0.8%	7.6%	0.017	3.0%	3.6%	1.00	3.1%

†Comparison P-value

Table 2.8: Percentage of growers reporting each specific visitor to be allowed (either sporadically or routinely) to enter the poultry house during growout, in the broiler grower survey in Georgia.

		Response	Region			Houses/Farm			No Farms Owned			
Visitor	n	rate	South	North	†P	1–4	5+	†P	1	2+	†P	Total
Shaving suppliers	262	98.1%	21.5%	26.4%	0.511	27.5%	20.0%	0.219	24.1%	31.6%	0.319	25.2%
Feed truck	262	98.1%	9.2%	15.7%	0.223	12.6%	17.5%	0.337	1518.0%	7.9%	0.317	14.1%
Chick bus	262	98.1%	55.4%	57.9%	0.773	55.0%	62.5%	0.28	54.5%	73.7%	0.033	57.3%
Meter readers	262	98.1%	7.7%	6.1%	0.771	5.5%	8.8%	0.414	6.7%	5.3%	1.00	6.5%
Repairmen	262	98.1%	75.4%	60.9%	0.037	63.7%	66.3%	0.78	63.8%	68.4%	0.714	64.5%
Vaccination crews	262	98.1%	89.2%	70.1%	0.002	73.6%	77.5%	0.54	73.7%	81.6%	0.419	74.8%
Decaking	262	98.1%	15.4%	7.1%	0.079	9.9%	7.5%	0.646	8.0%	15.8%	0.132	9.2%
Visitor	262	98.1%	15.4%	17.8%	0.709	16.5%	18.8%	0.723	18.3%	10.5%	0.351	17.2%
Agricultural extension agents	262	98.1%	9.2%	7.1%	0.593	6.0%	11.3%	0.204	7.1%	10.5%	0.506	7.6%
Gas delivery	262	98.1%	12.3%	15.2%	0.686	14.3%	15.0%	0.851	14.3%	15.8%	0.804	14.5%
Egg truck	262	98.1%	0.0%	3.55%	0.198	3.9%	0.0%	0.105	3.1%	0.0%	0.598	2.7%
Veterinarians	262	98.1%	35.4%	47.2%	0.114	40.1%	53.8%	0.044	42.9%	52.6%	0.292	44.3%
Company service persons	262	98.1%	90.8%	93.4%	0.581	90.7%	97.5%	0.068	93.3%	89.5%	0.494	92.8%
Live hauk	262	98.1%	52.3%	47.7%	0.568	45.1%	57.5%	0.081	46.9%	60.5%	0.16	48.9%
Clean-out	262	98.1%	24.6%	19.3%	0.379	21.4%	18.8%	0.741	18.8%	31.6%	0.093	20.6%
Pit inspectors	262	98.1%	10.8%	10.1%	1.00	9.9%	11.3%	0.826	11.2%	5.3%	0.39	10.3%

†Comparison P-value

The comparison of the overall level of biosecurity by a single score is presented in Table 2.9. The table shows both the raw sum of the reported frequency of adoption of daily biosecurity measures, and the score adjusted for the number of answered questions. Both the raw and adjusted biosecurity scores were significantly higher in the North than in the South ($P < 0.001$). Biosecurity did not differ by farm size or number of farms owned.

Table 2.9: General biosecurity score by groups, as assessed in the broiler growers survey in Georgia. Median (range) is reported (a score of 70 is the maximum possible). Adjusted scores correct for unanswered questions.

		Region			Houses/Farm			No Farms Owned		
	Total	South	North	†P	1–4	5+	†P	1	2+	†P
Biosecurity Score	27 (0–62)	17.5 (1–38)	30 (1–62)	< 0.001	28 (0–62)	25 (1–61)	0.157	17 (0–62)	28.5 (10–47)	0.749
Adjusted score	30.3 (0–70)	20.7 (0–42.9)	34.4 (1–70)	< 0.001	33 (3.5–70)	29.3 (1–61)	0.218	31.2 (1–70)	34.4 (11.7–57.8)	0.670

†Comparison P-value

2.4 Conclusions and Discussion

The highly integrated business structure and intensity of production of the USA poultry industry present some unique challenges for disease surveillance, outbreak control, and emergency management [20]. In such a connected network of farms, biosecurity is a critical component of disease prevention and control, and therefore in avoiding economic losses. The effectiveness of biosecurity plans designed by integrator companies, however, depends on the compliance of individual growers. We surveyed poultry growers in two areas of the state of Georgia facing different disease risks to assess the level of compliance to biosecurity measures that can reduce the risk of disease spread associated with human movement between farms. Variability due to farm size and number of farms owned by the same growers was also assessed. The results showed that disease threats can increase awareness of poultry growers and compliance to biosecurity measures, as growers in North Georgia, responding to an outbreak of ILT, reported a higher frequency of adoption of such measures. Variability due to farm size or number of farms owned was low.

The sample size was almost three times larger in North Georgia, since the number of farms in that region is almost three times higher than the total number of farms in South Georgia. The survey distribution reflects this difference, as we distributed surveys to almost three times more farms in North Georgia (770 farms in North Georgia versus 262 in the South), and in 5 different companies compared to only 2 in the South. Therefore caution should be taken when looking at the totals for variables in which a significant difference was observed between the two geographical regions, as the samples from North Georgia will have a higher weight in the totals for the state.

The participation rate in the survey was below 30% except for the company in which it was piloted. That company, however, has a long history of collaboration with the University of Georgia, and servicemen and growers may have a higher awareness of how the results of research can benefit them directly. Besides the high response rates, growers answering the pilot survey were very informative in their answers, adding comments that were fundamental to improving the quality of the questionnaires and by consequence the quality and validity of the data. For the other companies participation was significantly higher when servicemen had a chance to hear about the goals of the project, and therefore may have participated in the project more actively, stimulating growers to return the questionnaires, rather than just distributing the envelopes. Overall grower participation was lower than attained by previous studies — 62% by Vieira *et al.* (2009) [101], 38% by Tablante *et al.* (2002) [90], and 29.4% by Bates *et al.* (2001) [7], but considered satisfactory since there was not active interview or contact with the growers.

As the survey was designed to assess compliance, response bias is an important concern, especially at such a low participation rate, as non-participant growers may be those with the poorest level of biosecurity compliance. However, since companies do not seem to be enforcing a standardized biosecurity program, growers implementing poor biosecurity measures may not be aware of it, nor feel noncompliant. Moreover, the results of the survey show a low level of adoption of biosecurity measures, as can be seen by the number of biosecurity practices that should be adopted by personnel and required from visitors to which the median frequency is zero (never adopted). The assurance of confidentially was considered critical for the honesty of answers. This low frequency of adoption of biosecurity measures by personnel and visitors creates the opportunity for disease transmission, confirming the findings of previous studies of poultry disease outbreaks that have demonstrated the central role of human and equipment movement among poultry facilities in the spread of virus between operations [2, 48, 91, 12, 27, 43, 52].

Vieira *et al.* (2009) [101] showed that 31% of the growers surveyed in two counties in Georgia had employees from outside the household working with their poultry. Their results also showed that broiler growers are likely to personally interact with other poultry growers, as 49% of them reported interacting with another grower in the previous week. We showed that it is not rare for growers to have members in the immediate family also owning/working for poultry farms (35.2%), increasing the chances of contact with other flocks, directly or indirectly. Therefore, adoption of biosecurity measures by the grower and farm personnel may have an important impact in the risk of disease transmission, especially since they contact birds every day. Our results showed that except for washing hands after handling birds, and the use of footbaths in North Georgia, there is an overall low frequency of adoption of biosecurity measures by farm personnel in the state. Shoecovers may be sometimes used in South Georgia, and clothes changed in North Georgia, but the median frequency for all other items regarding use of personnel protective equipment is zero (reported frequency is 'never'). The implication of this low compliance by personnel on the risk of disease spread is corroborated by the findings of McQuiston *et al.* (2005) [48] during the H7N2 avian influenza outbreak in Virginia in 2002, which demonstrated that infected farms were more likely to have non-family member caretakers and an owner or family member working off site.

Visitors frequently have a lower degree of contact with birds, but may visit several farms in the same day. Their role on the spread of disease has also been demonstrated. The international spread of HPAI from the Netherlands in 2003 was associated with increased number of contacts between farms [30, 88, 91]. Our results showed that biosecurity measures are more frequently enforced on farm visitors, when compared to the adoption by farm personnel. The median score for use of shoecovers by visitors is four (always used) in both areas of the state. While in the South the median frequency of adoption of all other measures concerning the adoption of biosecurity measures by visitors is zero (never adopted), growers in the North in general always require the use of coveralls and footbaths. In neither area, however, did growers seem to be aware of the potential for contamination spread through vehicles, as visitors are in general never asked to park outside or wash tires before entering the farm or prior to leaving the premises.

In the outbreak of highly pathogenic avian influenza in Hong Kong in 2002, a case-control study documented as a factor positively associated with case farms whether a visitor went inside the shed [43]. Our results showed that all visitors can be allowed inside the poultry houses, more or less frequently, increasing the vulnerability of farms in both areas of the state to disease introductions, and especially if visitors are not required to wear personal protective equipment.

Poultry farm density in North Georgia is much higher than in South Georgia (1.45 farms/5 mi^2 and 0.49 farms/5 mi^2, respectively). Outbreak experiences have shown that diseased farms tended to cluster geographically, as showed by the work of Boender *et al.* (2007) [11] with the HPAI outbreak in the Netherlands in 2003, and the Australian seroprevalence study reported by East *et al.* (2007) [27], suggesting a higher risk of disease spread in areas of higher farm density. In Georgia, the results of the survey of Vieira *et al.* (2009) [101] showed that broiler growers reporting the highest number of personal contacts with other growers were in the high-density county. However, we expected awareness of disease prevention to be higher in North Georgia, at least at the time of our survey, due to the recent ILT outbreak. We expected this to translate in a higher level of adoption of biosecurity measures, as in fact the results showed. The biosecurity score calculated is intended to give a general perception of the level of awareness of the grower, and his commitment on the adoption of biosecurity measures. We assumed that the more items a grower adopts on their farm and enforces with higher frequency, the more complete their biosecurity. But the score was not intended to be a measure of the risk of spread, since it considers with equal relevance all items listed — for instance, using hairnets gives the same points as using coveralls, washing hands or wearing disposable boots. These items likely do not contribute equally to the risk of spread, hence adoption of each does not have the same impact in the overall efficiency of the biosecurity program in preventing disease introduction or release. Moreover, not all items are independent. If shoe covers and coveralls are used, for instance, but hands are not washed, contamination from protective equipment is likely to be transferred to personnel/visitors hands, reducing their intended efficacy.

Differences in compliance between the two regions in Georgia may be temporary, and the profile of adoption of biosecurity protocols seen in South Georgia is likely to be closer to the general profile for the state in times when there is no recognized circulation of disease between farms. For farming practices, such as use of contracted services and disposal of birds and litter, however, the differences likely reflect peculiarities of the poultry industry in the two areas of the state. Vieira *et al.* (2009) [101] surveyed growers in two counties of different poultry farm density in Georgia and showed, for example, that broiler growers in the county with low farm density were less likely to use borrowed equipment or contracted litter services compared with those in the high-density county. These results were confirmed in our survey.

During the control of the ILT outbreak that affected the North region of Georgia, growers in the control zones were not allowed to transport used litter off their farms. Therefore, the median number of flocks using the same litter was expected to be higher in that region. Many growers wrote in their questionnaires that they would normally change litter after every flock, if there were no restrictions imposed. This is consistent with the median observed in the South, region not subject to the same restrictions. It is worth noting that in the work of Vieira *et al.* (2009) [101] on-farm bird disposal was reported by 100% of the producers. While differences in the method of disposal were found between the two geographical regions in our work, a small percentage of growers (0.5% in North Georgia and 1.5% in the South) did report disposal off-farm. Because some dead birds are transported off-farm, in the case of infection the practice of disposing birds off-farm may pose a high risk of pathogen dissemination. Akey *et al.* (2003) [2] reported daily transportation of dead birds to rendering facilities off farm as having the highest association with infected premises in the H7N2 avian influenza outbreak in Virginia.

Busani *et al.* (2008) [17] showed that size of farm was a risk factor in the H7N1 HPAI outbreak in Italy in 1999–2000. In our study no significant association was observed between the size of farms or the number of farms owned by the grower and the adoption of biosecurity measures, therefore regional differences seem to be more relevant than differences related to farm profile. Farms with more than four houses were more likely to have a greater distance between parking and the houses, but that could be an effect of the bigger farm size. If larger farms do have a higher risk of disease introduction, as the work of Busani *et al.* (2008) [17] showed, attention to biosecurity protocols on these farms may be more critical, and if compliance is not increased these farms may be under more risk of disease introduction. Thomas *et al.* (2005) [91]found the higher risk of disease on larger farms to be a confounder in their study of the HPAI outbreak in The Netherlands in 2003.

We also tried to stratify the data based on the integrator company, but no significant differences were found, showing that variability among growers is more relevant than among companies, and again pointing out for a more relevant stratification based on the geographical region of the state, which may be a result of inherent differences in the poultry industries in these two areas of different farm density, and especially of the different disease risks at the time of the survey.

Our results showed that the overall frequency of adoption of biosecurity measures intended to reduce the risk of contamination spread associated with human movement is low in the state of Georgia. Growers seem to respond to a disease threat by requiring visitors to wear protective personal equipment, but they don't seem aware of the need to adopt the same measures by farm personnel, nor to direct biosecurity practices also to vehicles. We believe that this work can guide integrated companies in identifying which points must be strengthened to fully implement biosecurity plans to protect their flocks. As we identified which breaches may persist and be responsible for disease dissemination, companies can now identify which practices to focus on. The compliance by individual growers, however, will depend on implementing an awareness program, and regular inspections of compliance.

In the face of the threat of introduction of a major infectious disease, such as H5N1 HPAI, Sharkey *et al.* (2008) [84] state, among other questions, the need to know what are the critical components that exacerbate the risk of spread, and what are the most effective control strategies. While our work showed which measures may need an immediate focus to increase compliance, establishing which of them is more efficient would depend on experiments that determine the efficacy of each biosecurity measure in reducing the potential for contamination of people and vehicles, coupled with quantitative studies of the whole of each contamination pathway in the mechanisms of disease transmission.

CHAPTER 3

ASSESSING THE IMPACT OF BIOSECURITY ADOPTION IN REDUCING THE POTENTIAL OF DISEASE SPREAD AMONG COMMERCIAL POULTRY OPERATIONS IN GEORGIA USING PROBABILISTIC MODELING

3.1 INTRODUCTION

Poultry production is the number one agricultural enterprise in the state of Georgia, accounting for approximately 50% of the value of farm products produced. More than 4,000 family-owned poultry farms produce more than $5 billion annually in farm gate value [22]. In 2008, 1.4 billion birds were produced in the state, which is the largest producer of broilers in the country [53].

The introduction of diseases, whether clinical or subclinical, significantly reduces the productivity, profitability and long term financial viability of poultry operations [56] Biosecurity consists of the measures used to prevent such introductions of disease-causing organisms into a flock and their spread [51]. Biosecurity is, therefore, an integral part of any successful poultry production system.

Infectious agents are introduced or transmitted to poultry in a variety of ways. Biosecurity measures are primarily directed at preventing the indirect spread of disease, which occurs when birds are infected through contact with contaminated media. For example, humans can carry infectious organisms on their feet, hands and clothing; and feathers or manure on equipment or vehicles can transport disease causing organisms from one location to another [51].

Movement of people and equipment between farms within the commercial poultry industry has been identified as the primary mode of transmission for a number of important pathogens such as avian influenza viruses, Newcastle disease virus (NDV), and Salmonella and Campylobacter species [23]. The important role of human movement in spreading highly pathogenic avian influenza viruses (HPAIv) among operations, for instance, has been demonstrated by different authors through the study of previous outbreaks. Infected farms were more likely to share equipment [12, 52], have non-family member caretakers, or have an owner or family member working or living off site [48, 43].

For diseases transmitted in such a way (mainly through the movement of people and vehicles), information about the types and frequencies of horizontal contact are critical in modeling transmission details, in order to simulate the potential for disease spread in any particular network and assess the impact of prevention and control strategies. Considering that production characteristics — and therefore the horizontal contact patterns — are likely highly variable between companies, poultry production type, and region [14], it is unclear to what extent transmission parameters from other countries or regions can be applied to models that include detailed industry structure.

Vieira *et al.* (2009) [101] collected information on the frequency and patterns of horizontal contact among commercial poultry growers in Georgia as well as farm management practices. Building on that work, Dorea *et al.* (in peer review) [25] developed a model to assess the daily risk of HPAI spread associated with human movement in the network formed by the commercial poultry industry in the state.

Several studies of HPAI outbreaks shared the conclusion that breaches in on-farm biosecurity, as well as a lack of hygiene measures adopted by visitors, played a significant role in the spread of HPAIv [60, 43, 52]. While the model of Dorea *et al.* (in peer review) [25] considered the role of visitors in between-farm spread, it did not evaluate the mitigation of transmission that might be achieved through various biosecurity measures. To address this deficiency, an anonymous survey was carried out in 2008 (Chapter 2) to assess the biosecurity compliance among growers in Georgia. Growers were questioned about the frequency of adoption of biosecurity measures among farm personnel and requirements for visitors. A general conclusion from this survey was that compliance with standard biosecurity protocols in the state should be increased. It was also concluded, however, that a more quantitative evaluation was needed to determine the level of biosecurity that provides an adequate balance between disease prevention and use of resources for this particular network. While insufficient biosecurity could result in more widespread disease, excessively restrictive protocols and expenditure of resources in the presence of minimal risk, on the other hand, may lead to unnecessary stress, inefficient use of time, and depletion of limited resources [109].

Aiming to assess and compare the impact of different biosecurity measures on the risk of disease transmission associated with human movement, a model of disease transmission between farms in the contact network formed by commercial poultry operations in the state of Georgia was developed. The model explicitly took into account how individual biosecurity measures can impact transmission of contaminated material by people or vehicles when visiting farms, considering that birds and litter would be the main sources of contamination. Therefore, the results illustrate the risk of spread for any pathogen transmitted through feces, to which contact between birds is mainly indirect, making human movement between premises the most important mode of transmission. It was hypothesized that the impact of different biosecurity measures in reducing the daily risk of disease spread would depend on specific characteristics of the network, such as the frequency and patterns of visits.

This hypothesis was evaluated by assessing the impact of different scenarios of biosecurity compliance on the expected number of farms exposed to potentially contaminated material daily, by each infected source. The scenarios were based on the 2008 survey of growers in the state (Chapter 2). In that survey, a higher frequency of biosecurity adoption was found among growers in North Georgia than in South Georgia. This was interpreted as a shift towards increased compliance with biosecurity measures in the northern part of the state in response to a regional outbreak of infectious laryngotracheitis (ILT). We therefore assumed that the results from the South and North of Georgia indicate biosecurity compliance in the state under typical conditions, and after increased awareness in response to a disease threat, respectively. Both were compared to a scenario of no biosecurity, in order to evaluate the impact of biosecurity adoption predicted by the model. Additional theoretical scenarios were modeled to assess how further increases in biosecurity compliance might impact the risk of disease transmission.

3.2 Materials and Methods

3.2.1 Data sources

The model of Dorea *et al.* (in peer review) [25] was used to represent the network structure among commercial poultry farms in Georgia, as it incorporates the frequency and patterns of human movement among these operations.

To explicitly incorporate scenarios of biosecurity adoption, information from the 2008 survey of Georgia poultry growers (Chapter 2) was used to inform the model. Data from the survey provided estimates on the frequency of adoption of different biosecurity measures by growers, frequency of equipment sharing and disposal of birds and litter off-farm, and information on visitors entering the poultry houses during growout. The reported results for the South and North areas of the state were stratified to represent different scenarios of biosecurity adoption: South Georgia was assumed to represent a 'typical' level of biosecurity compliance in the state, while the North represents a higher level of biosecurity attention by growers in response to an ongoing infectious disease threat.

Information on the efficiency of the biosecurity measures included in the model was collected through the experimental work of Johnson and El Gazzar (unpublished). The efficiency experiments tracked contamination spread from a poultry house by simulating the presence of infective material using Glo-Germ^{TM}, an odorless powder which glows brightly when exposed to ultraviolet light. Detection of the powder on visitors and vehicles that had contact with litter, and its presence in the environment following human movement, allowed the estimation of: 1) the percentage of visitors getting contaminated after entering a poultry house and performing different activities; 2) the distribution of contamination over their feet, hands and clothes; 3) the frequency that contamination was found on subjects after removal of protective personnel equipment; 4) the spread of organic matter originating from the litter to specified distances from the poultry house; and 5) the effect of washing in removing organic matter from vehicle tires.

3.2.2 Stochastic model of off-farm spread

In the stochastic off-farm model developed by Dorea *et al.* (in peer review) [25] the transmission pathways were based upon the daily likelihood of contact to the infected farm through each of fifteen listed visitors, an estimated probability that the farm activity was associated with direct contact with infectious materials, and the daily number of farms visited by each visitor. In order to include the role of biosecurity in model estimates of disease spread, the results of the biosecurity efficiency experiments (unpublished) and the grower survey (Chapter 2) were used to calculate the specific probability associated with visitor infection upon contact, and the probability that biosecurity practices are not adopted or are not efficient in removing contamination from the visitor. Figure 3.1 schematically shows the events in the model, and details of the calculations follow.

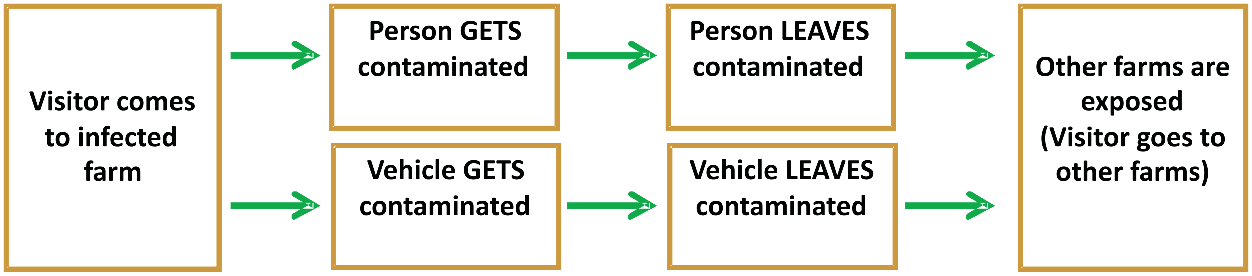

Figure 3.1: Process flow of the stochastic off-farm model.

Visitor comes to infected farm

The chance of a visitor coming to the farm was calculated as in Dorea *et al.* (in peer review) [25], where who estimated the daily frequency of farm visitors independently for regions of low and high densities of poultry farm were estimated from a survey of Georgia poultry growers [101]. These frequencies were then used as the probability of success in a Bernoulli trial representing the event that a ”visitor comes to the infected farm” on a particular day.

Visitor (person or vehicle) gets contaminated on the farm

The model considers contamination of the visitor as a ‘success-failure’ event; consequently different degrees of contamination are not considered. The parameters used to simulate each of the model events (Figure 3.1) were assumed to be dependent on the degree of contact the visitor has with infectious materials. Potential visitors were separated into three general contamination profiles:

1. Visitor comes to the infected farm, but does not enter a poultry house;
2. Visitor enters a poultry house, but does not handle birds or litter;
3. Visitor enters a poultry house and handles birds or litter.

The probability that each visitor will enter a poultry house was estimated from the proportion of growers reporting whether each visitor was allowed to enter the houses (Chapter 2). Visitors that enter a house were assumed to touch birds if the nature of their activity on the infected farm requires or allows this to occur, as in the case of company service people. Table visitors shows the contamination profile assigned to different visitors.

Visitors involved in clean out services, decaking, disposal of litter off-farm, disposal of birds off-farm, or live haul activities which involve direct removal of infective material from the farm (birds or litter), are always assumed to have a high probability of contamination as shown in Table 3.2. For each visitor listed in Table 3.1 the probability of contamination was assigned based on the field experiments that simulated contamination using the UV fluorescent powder (unpublished). For these same visitors the contamination of vehicles was

Table 3.1: Contamination profile assigned to each visitor and their probability of entering a poultry house as assessed in the growers survey.

Visitor	Profile	Probability of activity
Shaving Suppliers	3-Enters the house and touch litter	100%
Repairmen	1-Does not enter the house	35%
	2-Enters the house but does not touch birds	65%
Gas Delivery	1-Does not enter the house	85%
	2-Enters the house but does not touch birds	15%
Feed Trucks	1-Does not enter the house	85%
	2-Enters the house but does not touch birds	15%
Chick Bus	1-Does not enter the house	45%
	3-Enters the house and touch birds	55%
Service Persons, Managers, Vets	3-Enters the house and touch birds	100%
Utilities, Meter readers,	1-Does not enter the house	92%
Pit inspect.	2-Enters the house but does not touch birds	8%
Unauthorized Visitors	1-Does not enter the house	82%
	3-Enters the house and touch birds	18%
Vaccination Crews	3-Enters the house and touch birds	100%
Grower, Hired Help	1-Does not enter the house	82%
	3-Enters the house and touch birds	18%

considered to pose an additional risk of disease spread, which was modeled independently of personal contamination.

Table 3.2: Probability of a visitor (person or vehicle) getting contaminated on the farm.

Contamination profiles	Probability of getting contaminated during visit to an infected farm	
	Risk category	Risk values
Cleaning and disposal activities	High	Uniform (0.8 – 1.0)
1-Visitor does not enter the poultry house	Moderate	Uniform (0.4 – 0.6)
2-Visitor enters a poultry house, but does not handle birds or litter	High	Uniform (0.8 – 1.0)
3-Visitor handles birds or litter	High	Uniform (0.8 – 1.0)
Vehicles	Low	Uniform (0.2 – 0.4)

The probabilities of contamination associated with each visitor (Table 3.2) were used as the probability of success in independent Bernoulli trials. For all visitors listed in Table 3.1 (shaving suppliers, repairmen, gas delivery, feed trucks, chick bus, company people, utilities, unauthorized visitors, vaccination crews and hired help), the person and their vehicle are evaluated for contamination independently.

VISITOR (PERSON OR VEHICLE) LEAVES THE FARM CONTAMINATED

For the cleaning activities, in which contamination spread is associated with equipment, contamination leaving the farm was considered to be certain (given that the equipment was contaminated in the previous step) when borrowed/shared or contracted equipment is used, and zero when the equipment is owned by the grower. Similarly, disposal of litter or birds off-farm was considered to result in contamination spread, while no risk was assigned to disposal on-farm. Live haul is always associated with contamination leaving the farm if birds are infected.

For all other visitors the probability of leaving the farm contaminated was assumed to depend on whether biosecurity measures are adopted, and the efficiency of the specific biosecurity measures. The use of shoecovers, washing hands (or wearing gloves) and wearing coveralls were the biosecurity measures modeled to account for contamination removal from feet, hands and clothes, respectively.

The frequency of adoption of each measure was estimated from the results of the grower survey (Chapter 2), based on the proportion of growers reporting adoption and their declared frequency. Therefore, listed percentages refer to the proportion of times each measure is expected to be adopted (a 50% frequency of adoption, for instance, could indicate that 50% of the growers adopt a measure 100% of the time when visitors come to the farm, or that 100% of the growers adopt the measure 50% of the time). The efficacy of each biosecurity measure was calculated as the percentage of trials in which contamination of a visitor was prevented by the use of PPE (Personal protective equipment) in the field experiments (unpublished).

Each body part was assigned a proportion of the visitor's total contamination based on their contamination profile and the observed contamination in the Glo-Germ^{TM} field experiments, and this percentage was used to derive the final probability of contamination as a single value for each visitor.

Table 3.3 shows the body part proportions, as well as the efficiency parameters estimated from the field experiments, the frequency of adoption of each measure, and a summary of the calculations. Calculations were performed independently for the levels of compliance considered as the ‘typical’ scenario in the state, and compliance after ’increased awareness’ due to a disease threat.

Table 3.3: Final probability of leaving the farm contaminated for each visitor profile.

	A-Probability of decontamination if biosecurity adopted	B-Frequency of adoption of biosecurity measures**	C-final probability of contamination $= 1 - (A * B)$	Percentage of total contamination in each visitor that can be attributed to each body part:		
				Visitor profile1	Visitor profile2	Visitor profile3
Feet	90%	IA = 87% T = 83%	IA = 22% T = 25%	100%*C	85%*C	45%*C
Hands	90%	IA = 82% T = 82%	IA = 26% T = 26%	0%*C	10%*C	35%*C
Torso/ Legs	90%	IA = 68% T = 30%	IA = 32% T = 70%	0%*C	5%*C	20%*C
Final probability a visitor leaves contaminated (sum):				IA = 22% T = 25%	IA = 23% T = 27%	IA = 26% T = 35%

*See Table 3.2 for a description of the profiles

Level of compliance reported in the growers survey (Chapter 2). T=Typical scenario of biosecurity adoption in the state; IA = scenario of increased awareness in case of outbreak.

For vehicles, two biosecurity measures were considered: washing the tires prior to leaving the farm, and parking outside the farm. Again, their effectiveness is considered to be the percentage of times contamination can be prevented, not the amount of contamination removed. The different vehicle biosecurity measures were assumed not to overlap. That is, a grower would either require visitors to park outside the farm or request them to wash tires before/after entering the farm, but would not require both. Consequently, it was also assumed that the sum of adoption percentages for the different vehicle biosecurity measures would not exceed 100%. Because levels of compliance in North and South Georgia were not significantly different, only one value for this parameter was estimated, as shown on Table 3.4.

The final probabilities for the event “visitor leaves the farm contaminated” (Tables 3.3 and 3.4) are once again used as the probability of success in Bernoulli trials, run independently for each visitor and their vehicle.

The results of the two events related to visitor contamination — getting contaminated and leaving the farm contaminated — are multiplied, giving a non-zero result only if both trials

Table 3.4: Final probability of a vehicle leaving the farm contaminated.

		Distance from house to parking*		
	Tire washing	$> 50ft$ from poultry house	$10 - -50ft$	$< 10ft$
A-Probability of contamination reduction	75%	76%	68%	0%
B-Adoption in Georgia	20%	10%	14%	56%
C-Final reduction by method = $(A * B)$	15%	10%	8%	0%
Final probability of a vehicle carrying contamination out of a contaminated farm in GA	$= 1 - (15\% + 10\% + 8\% + 0\%)$ $= 67\%$			

Growers that did not report asking visitors to park outside were assumed to allow visitors to park very close to the poultry houses.

were successful, and 0 if any of the two trials resulted in failure. If the result is 1 for the person, the vehicle, or both, the model considers that a visitor will leave the farm contaminated, and assigns a non-zero result. For the cases in which different profiles are associated with the same visitor — different possibilities for equipment usage, different methods of disposal, or different visitor profiles (entering the house or not entering the house) — a random number between zero and one is generated to choose which of the profiles ‘happened’, based on their relative prevalence. The described algorithm assumes that vehicle and visitor can be contaminated independently, and in either case contamination will leave the farm and potentially expose other farms to the infectious agent.

Finally, the results of the contamination steps are multiplied by the results of the first step, “visitor comes to the farm”, giving a non-zero result only if the visitor came to the farm, became contaminated, and left contaminated.

Visitor goes to other farms

Once the model determines whether a contaminated visitor left the farm (non-zero result after all previous steps), the number of farms exposed to contaminated material through this visitor is calculated as reported by Dorea *et al.* (in peer review) [25]. The total number of farms exposed is calculated by summing the outcomes for all activities in a day. The final outcome — the total number of farms exposed daily from an infectious source — is a probability distribution generated after 30,000 iterations of the model.

3.2.3 BIOSECURITY SCENARIOS EXPLORED

For all parameters corresponding to visitor frequency, number of farms visited, and farming practices, information provided by experts and poultry companies referring to South Georgia was used to inform the model for areas of low poultry farm density, and information from North Georgia was considered to represent the area of high farm density. The proportion of growers using owned equipment for decaking and cleaning out, and disposing litter or birds off-farm was also considered to be region dependent, and the results of the grower survey (Chapter 2) in North and South Georgia were used to characterize the areas of high and low density of poultry farms respectively.

When including the frequency of biosecurity adoption by visitors, however, we assumed that the results of the grower survey (Chapter 2) in the North of the state were an indication of higher biosecurity compliance that can be readily achievable when growers' awareness is increased, as in the case of a disease threat. The impact of increased disease awareness was assessed to determine how this would affect disease transmission in areas of both high and low density of poultry farms, when compared to the lower level of biosecurity observed in the South, which was considered to represent the typical conditions in both regions of the state.

The different biosecurity adoption scenarios were compared to one in which no measures were adopted in order to estimate the impact of the current level of biosecurity compliance in reducing the potential for disease spread.

Additionally, to assess the impact of further increasing the adoption of different biosecurity measures in each region, five additional scenarios were modeled. Four scenarios evaluated the effect of increasing the biosecurity measures required from visitors, and one extra scenario evaluated the effect of combining the previous with reducing the risk associated with cleaning activities and disposal of litter and birds. Starting from the typical scenario of biosecurity in the state, the frequency of adoption was changed in order to make each scenario represent the effects of particular biosecurity measures, as follows:

- Scenario 1 (Vehicle biosecurity) — adoption of biosecurity measures towards vehicles increased to an overall 90% (45% for tire washing and 45% for parking outside the farm), as this was considered the best case scenario (100% compliance was not considered likely to be achieved).
- Scenario 2 (Use of personal protective equipment - PPE) — compliance with the use of PPE by visitors increased to 80% use of coveralls (since the current adoption is very low, this was considered to be the best achievable scenario), 90% use of shoecovers (this was considered achievable since compliance is already high) and 90% of visitors washing hands after handling birds (again, best scenario considered achievable).
- Scenario 3 (Controlled access) — no unauthorized visitors allowed inside the poultry houses.
- Scenario 4 (Visitors biosecurity) — all previous together.
- Scenario 5 (Complete farm biosecurity) — all biosecurity measures required from visitors listed before, plus 100% of the growers adopting farm practices that reduce the risk of off-farm spread: disposal of birds and litter on-farm only, and use of grower's own equipment for decaking and cleaning out.

The parameters for compliance with measures that refer to visitor biosecurity, in each scenario, are shown in Table 3.5. According to the grower survey (Chapter 2), 50% of the growers in North Georgia (high density of poultry farms) use their own equipment to clean out, and 90% for decaking. The proportion of growers disposing off-farm was 30% for litter disposal, and 0.05% for bird disposal. In the South (low density of poultry farms) 90% of the growers use their own equipment for cleaning out, and 95% for decaking. The proportion of disposal off-farm in the South was 25% for litter and 1.5% for birds. These parameters were used for each density model in all scenarios but the fifth (complete farm biosecurity), in which all growers were assumed to use their own equipment and dispose litter and birds on-farm.

Table 3.5: Biosecurity parameters used in the scenarios set up to model the impact of different frequencies of biosecurity adoption on the potential for disease spread.

	Frequency growers require visitors to:					
	park vehicles outside the farm	Wash tires	Wear coveralls	Wear shoecovers	Wash hands	Not enter the house
No biosecurity	0%	0%	0%	0%	0%	0%
Typical compliance	24%	20%	30%	83%	82%	82%
Increased awareness	24%	20%	68%	87%	82%	82%
1-vehicles	**45%**	**45%**	30%	83%	82%	82%
2-PPE	24%	20%	**80%**	**90%**	**90%**	82%
3-Controlled access	24%	20%	30%	83%	82%	**100%**
4-visitors bios.	**45%**	**45%**	**80%**	**90%**	**90%**	**100%**
5-complete bios.	**45%**	**45%**	**80%**	**90%**	**90%**	**100%**

3.2.4 SENSITIVITY ANALYSES

The independent contribution of each biosecurity measure to the total reduction in the risk of disease spread was evaluated. To assess which biosecurity measure had the greatest impact, compliance with individual measures was increased from 0 to 100%, and compared to a scenario in which no biosecurity measures were required from visitors, and no farming practices that can reduce the risk of spread were in place (no growers disposing litter or birds on-farm, or using their own equipment for cleaning).

Additionally, the sensitivity analysis tool of Crystal Ball® (Oracle) was used to evaluate the contribution of the assumptions to the total variance in the expected number of farms exposed daily. The software attributes ranks to each assumption in the model based on their contribution to the total variance of the outcome. These contributions were summarized by visitor type, in order to determine their individual contribution to the outcome.

3.3 Results

3.3.1 Daily off-farm spread

In any scenario considered, including no adoption of biosecurity measures, the most likely number of farms exposed in one day of spread from an infected source is zero. As shown in Table 3.6, even when no biosecurity measures are in place there is a 60% chance that no other farm will be exposed to contamination from one infected source in a single day of spread. As compliance with biosecurity measures increases. In the scenario of highest adoption of biosecurity modeled, for instance, there is a 87% chance that infection is limited to the first infected farm in its first day of off-farm transmission. The maximum number of farms that can be exposed is similar among scenarios in the same density model. However, as the chance of no farm being exposed increases, this worst case becomes more improbable when biosecurity measures are in place.

Table 3.6: Details of the probability distribution of the expected number of farms exposed to contaminated material from an infected source in 24hs, under different scenarios of adoption of biosecurity measures with 30,000 iterations.

	Low density of poultry farms		High density of poultry farms	
	Chance of no farm being exposed in 24hs (95% CI)	Maximum number of farms exposed	Chance of no farm being exposed in 24hs (95% CI)	Maximum number of farms exposed
No biosecurity	60.0% (59.4,60.5)	14	57.8% (57.2,58.3)	19
Typical bios.	78.8% (78.3,79.3)	12	76.7% (76.2,77.2)	18
Increased bios.	80.3% (79.8,80.7)	13	78.3% (77.8,78.7)	18
1-vehicles	83.4% (83.0,83.8)	12	81.5% (81.1,82.0)	18
2-PPE	81.1% (80.7,81.6)	13	79.1% (78.7,79.6)	18
3-Controlled access	79.1% (78.7,79.6)	12	77.2% (76.8,77.7)	18
4-visitors bios.	86.5% (86.1,86.9)	13	84.8% (84.3,85.2)	18
5-complete bios.	87.0% (86.6,87.3)	13	85.8% (85.4,86.2)	18

In either density model the typical scenario of adoption of biosecurity measures in the state represents an increase of almost 20% in the probability of no farm being exposed in the first day of spread from an infected source compared to the no biosecurity scenario, but the further reduction when disease awareness among growers is increased is low (around 2%). Increasing adoption to all biosecurity measures required from visitors would increase the chance of no farm being exposed by another 6%. Reducing sharing of equipment or disposal off-farm would add little to that (0.5% in the low density model and 1.0% in areas of high density of farms).

The means of the outcome distributions for the number of exposed farms in 24hours are shown in Table 3.7. The different scenarios of biosecurity have very similar impact in reducing the risk of disease spread in each density model, but the final means are always higher in the model for areas of high density of poultry farms.

Table 3.7: Mean expected number of farms exposed to contaminated material from an infected source in 24 hours under different scenarios of adoption of biosecurity measures (scenarios are described in Table 3.5), in the models for areas of low and high density of poultry farms.

	Low density of poultry farms		High density of poultry farms	
	Mean no. exposed farms (95% CI)†	Median no. exposed farms (95% PI)‡	Mean no. exposed farms (95% CI)†	Median no. exposed farms (95% PI)‡
No biosecurity	1.00 (0.99, 1.02)	0 (0, 5)	1.18 (1.16, 1.20)	0 (0, 6)
Typical bios.	0.47 (0.46, 0.49)	0 (0, 4)	0.57 (0.56, 0.59)	0 (0, 4)
Increased bios.	0.44 (0.43, 0.45)	0 (0, 4)	0.53 (0.52, 0.55)	0 (0, 4)
1-vehicles	0.36 (0.35, 0.37)	0 (0, 3)	0.45 (0.43, 0.46)	0 (0, 4)
2-PPE	0.42 (0.41, 0.43)	0 (0, 4)	0.51 (0.49, 0.52)	0 (0, 4)
3-house control	0.47 (0.46, 0.48)	0 (0, 4)	0.57 (0.55, 0.58)	0 (0, 4)
4-visitors bios.	0.29 (0.28, 0.30)	0 (0, 3)	0.36 (0.35, 0.38)	0 (0, 4)
5-complete bios.	0.29 (0.28, 0.30)	0 (0, 3)	0.35 (0.34, 0.36)	0 (0, 4)

†95% confidence interval for the mean

‡95% probability interval (2.5th and 97.5th percentiles)

Compared to a scenario of no adoption of biosecurity measures, the typical frequency of biosecurity adoption in Georgia can reduce the expected number of farms exposed daily by more than 50% in both farm density models. The impact of increased biosecurity awareness only represents a further reduction of 6–7% in the number of farms exposed daily.

In the low density model, increasing biosecurity from the typical scenario to that of increased awareness has greater impact in reducing the expected number of exposed farms than if control over the entry of visitors into the poultry houses alone is increased. Increased control over the movement of vehicles is the factor that yielded the single greatest impact, followed by the use of personal protective equipment. When all biosecurity requirements for visitors are increased, however, the expected number of exposed farms is reduced by approximately 70%. Reducing off-farm disposal and sharing of equipment had little impact when applied in addition to the enhanced visitor biosecurity measures. Results were similar in the high density model.

3.3.2 Sensitivity analyses

We also evaluated, independently, which biosecurity measures had the most impact in reducing the number of farms exposed daily as the frequency of adoption is increased. Figure 3.2 shows the reduction in the mean number of farms exposed when the frequency of adoption of each specific biosecurity measure is increased from 0 to 100%, in comparison to a base scenario in which compliance with all other measures (including farming practices that could reduce the risk of disease transmission, such as use of owned equipment and disposal on farm) is zero. Lines are solid up to the frequency of adoption corresponding to the typical scenario of biosecurity in the state. Dotted lines indicate simulations of the impact of further increases in the frequency of adoption. Because the results were similar for the models of low and high density regions, only the simulations for the low density model are shown.

The greater impact in the number of farms exposed daily for each 10% increase in the frequency of adoption is observed for the use of shoecovers, which has the potential to reduce the mean number of farms exposed daily by 0.40 (33% reduction), if compliance were increased from 0 to 100%. Restricting disposal of birds and litter to on-farm is the second most efficient measure, followed by washing hands. Vehicle biosecurity and control over visitors that enter the poultry houses (company people only allowed) have a similar effect, with fluctuations observed in the graph being due to stochastic variation. Full compliance of visitors to the use of coveralls could decrease the mean number of farms exposed daily by only 0.07 in comparison to a scenario of zero adoption, and full compliance with use of owned equipment would only have an impact of 0.04 units.

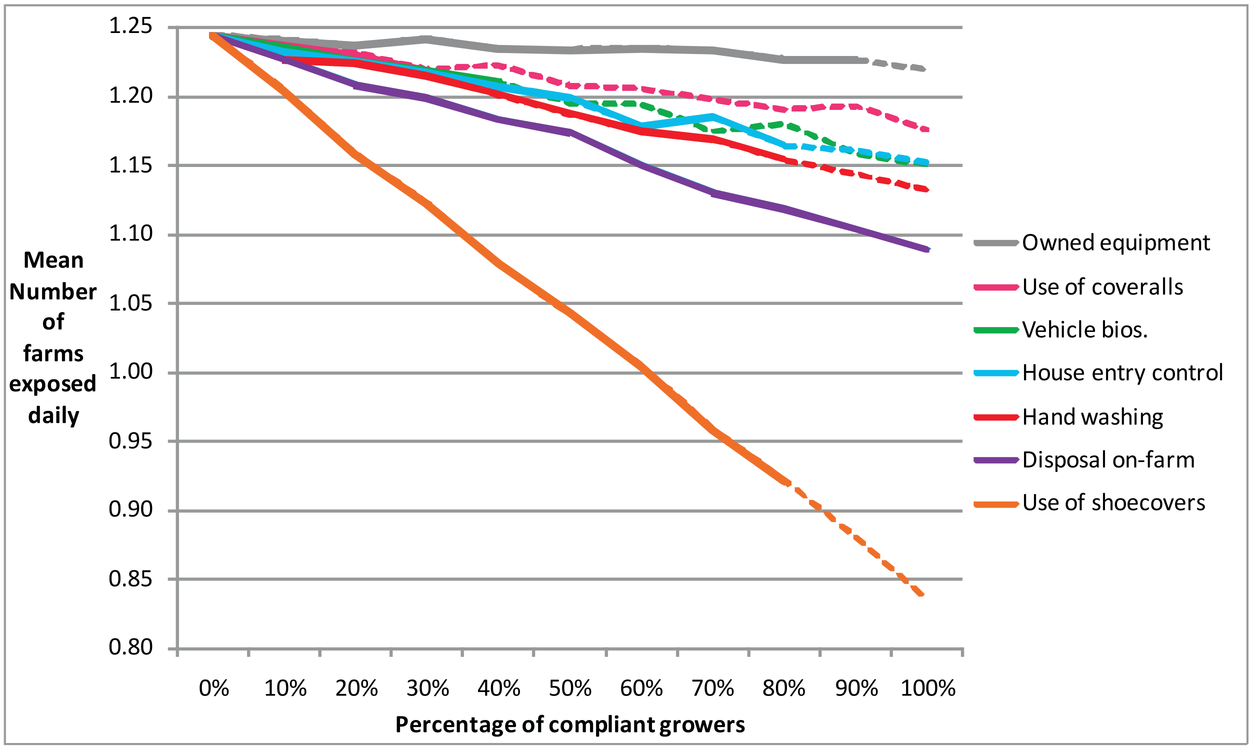

Figure 3.2: Mean number of farms exposed to contaminated material from an infected farm when frequency of adoption of specific biosecurity measures is improved from 0 to 100%, while all other measures remain at a 0%. Points in the graph correspond to the mean of 10,000 iterations. Solid lines correspond to the frequency of compliance among growers in the typical scenario of biosecurity adoption in Georgia, and the dotted extensions refer to simulated increases in frequency.

We also assessed the contributions of each visitor to the total variability of the outcome, for the low and high density poultry farm models. The results showed that the most relevant differences in the contribution of different visitors to the total variance of the outcome are observed when the scenario of no adoption of biosecurity measures is compared to any scenario in which biosecurity measures are in place. The increased compliance with individual biosecurity measures does not affect the sources of outcome variance as much as the scenarios in which the overall level of biosecurity is higher. We present the results for selected scenarios in Table 3.8.

Table 3.8: Contribution of the assumptions related to each visitor to the total variance of the output (number of farms exposed in 24hs) in different scenarios of adoption of biosecurity measures.

	Low density of poultry farms			High density of poultry farms		
Visitor	No biosecurity	Typical biosecurity	Scenario 5 - complete bios.	No biosecurity	Typical biosecurity	Scenario 5 - complete bios.
Feed trucks	41%	36%	34%	35%	31%	28%
Servicemen	25%	25%	20%	29%	22%	19%
Hired help	18%	20%	20%	15%	18%	15%
Utilitity persons	5%	3%	4%	5%	3%	3%
Unauthorized visitors	2%	3%	3%	4%	4%	4%
Gas Delivery	2%	2%	3%	4%	3%	3%
Chick Bus	3%	3%	3%	3%	2%	2%
Live Haul	1%	5%	12%	3%	13%	24%
Vaccination crews	1%	1%	1%	1%	1%	1%
Repairmen	0%	0%	1%	0%	0%	0%
Clean Out	0%	0%	0%	0%	2%	0%
Decaking	0%	0%	0%	0%	0%	0%
Litter disposal	0%	0%	0%	0%	0%	0%
Shaving Suppliers	0%	0%	0%	0%	0%	0%
Birds disposal	0%	0%	0%	0%	0%	0%

Feed trucks, company servicemen and hired help are together responsible for 84% of all the outcome variation when no biosecurity measures are in place in the low density model, and 79% in the model for high density of poultry farms. The contribution of each of them to total output variance decreases as more biosecurity measures are in place, in the low density model summing up to 81% in the typical scenario of biosecurity compliance, and 74% in the most complete biosecurity scenario modeled. Similarly, in the model for high density of poultry farms, their summed contribution to output variance decreases to 71% and 62% through the same scenarios.

Live haul workers, on the other hand, have a low contribution to the output variance when no biosecurity measures are in place, and its contribution increases as compliance is also increased. Through the three scenarios of progressive biosecurity compliance by growers shown in Table 3.8, the percentage of variation in the outcome explained by live haul worker visits increases from 1% to 5% and then 12% for the areas of low density of poultry farms, and from 3% to 13% and then 24% in the case of high density.

3.4 Discussion

Information gathered from companies, growers and poultry experts was used in order to capture specific characteristics of the commercial poultry system in Georgia that could determine the potential for indirect disease spread in this contact network. The goal was to evaluate the potential spread associated specifically with human movement, and the impact of different scenarios of biosecurity adoption in reducing the risk of transmission. These scenarios included observed data in areas inside and outside the control zone for a recent outbreak of ILT in the state of Georgia, and also theoretical scenarios of increased biosecurity compliance. Because the two areas of the state represent different frequencies and patterns of horizontal contact [101, 25], it was also of interest to capture the differences in the potential for disease spread within these two areas. By comparing different biosecurity adoption scenarios, it was hoped that the most effective measures could be identified and adopted by companies and growers to protect their flocks. In this sense, the results of these simulations may also be valuable for other commercial poultry networks.

The expected daily number of farms exposed by each infected source was greater for areas of high poultry farm density, under any scenario, as a result of the higher frequency and range of visitors in the high density area [101, 25]. The work of Boender *et al* (2007) [11, 10] studying the H7N7 HPAI outbreak in the Netherlands in 2003 showed that short distance between farms is expected to translate to a higher probability of disease spread, and that when the density of farms is high control by implementation of local measures may have low success. This was also observed in the current study, as even under a higher level of biosecurity adoption by growers the risk of disease spread in the North of the state (high density) was higher than the risk in the South, where biosecurity adoption and density are lower.

The higher attention to biosecurity measures in the North of the state was assumed to be a response to the recent ILT outbreak in the region (Chapter 2). Therefore, comparisons in each density model between the scenarios of biosecurity corresponding to the results of the survey in the South and North of the state can be considered as representing, respectively, the general compliance by growers under typical conditions, and the level readily achievable when awareness is increased in response to an epidemic threat. The results show that the reduction in the expected number of farms exposed daily by an infected source is reduced by only 6% in the low density model and 5% in the high density model between different scenarios, indicating that even though growers in the North seem to have responded to the disease threat, the compliance increase was either low, or not effective, as it may not have been directed to the measures with the highest impact in reducing the risk of disease spread.

Compliance with biosecurity measures was then modeled using levels even higher than those observed. Increases in the use of personal protective equipment (PPE) by visitors, restrictions to visitors entering the poultry houses, use of owned equipment or disposal on-farm are not associated with very high marginal increases because the compliance with these measures is already high in the state. The higher adoption of biosecurity measures aimed at preventing the spread of contaminated material through vehicles is associated with the greater decrease in risk, when compared to the current adoption of biosecurity measures, which highlights this as the current main gap in biosecurity in the state.

If compliance with biosecurity requirements for visitors is increased as a whole — including use of PPE, control of vehicles and control of visitors allowed inside the house — the current risk of disease spread in both density models can be decreased by approximately 30%, but further restriction on disposal of birds and litter off farms and on equipment sharing would not have a great impact on spread, even if all are adopted together. Because compliance with requirements for visitors was not modeled up to a frequency of 100%, and target levels were kept lower for the measures with current low adoption, we believe that the scenarios modeled are feasible targets of adoption.

The scenarios of increased adoption can only be evaluated in comparison to the typical scenario of biosecurity adoption in the state, on which they were based. An independent assessment of the impact of each biosecurity measure in reducing the number of exposed farms was performed using sensitivity analysis methods. The results showed that the use of shoecovers is the measure of greatest impact in the risk of disease spread. This is a result of the higher contribution of personal contamination on the risk of disease spread. Case control studies of previous outbreaks showed that the frequency of visitors coming to the farm and entering the poultry houses were both positively correlated with a higher odds of disease infections [43, 52]. Contamination experiments (unpublished) demonstrated that every visitor that enters the house will get contamination on their shoes, and they can also get contamination on their feet by walking close to the houses. Measures that only reduce personal contamination in the small group of the visitors who enter the poultry houses — such as wearing coveralls or reducing their frequency of contact with birds — do not have as important a role, when compared to the use of shoecovers.

These results show that the efficacy of each biosecurity measure in reducing the potential for disease spread depend on the importance of each contamination pathway in the overall risk, such as: which type of visitors can come to the farm, their relative frequency, the nature of their visit and the level of contact of each visitor with birds, the relative importance of persons, vehicles and equipment in contamination transmission, etc. Biosecurity measures will be more efficient if they target more important contamination pathways.

Moreover, the impact of different biosecurity measures cannot be evaluated independently. To develop a plan for biosecurity improvement in the state, the current level of biosecurity compliance must be taken into account. Evaluated independently, increasing attention to vehicle biosecurity, for instance, seems to have the same impact in reducing the potential for disease spread as increasing control over which visitors can enter the poultry houses, and less impact than asking visitors to wash hands, or disposing litter and birds on-farm. Given the current adoption of biosecurity measures in the state, however, increasing the frequency of adoption of these measures showed to have a small impact in reducing the risk of disease spread, and to be less important than the urgent need to improve biosecurity attention to vehicles.

We also evaluated the contribution of all inputs, grouped by visitor, in the total variability of the outcome. In any scenario only four visitors are responsible for around 80% of the total variance of the outcome. These are the visitors with the highest frequencies, coupled with a high level of contact with birds — company servicemen, hired help and live haul workers — or the greatest number of farm contacts — feed trucks. This indicates that these visitors are the most important determinants of the number of farms that will be exposed from a source farm, which agrees with field experience — feed vehicles and farm machinery had an important role in HPAI transmission in the H7N1 outbreak in Italy in 1999–2000 [113].

When we compare the scenario of no adoption of biosecurity measures to the one that included all visitor biosecurity measures considered (scenario four), we see that if measures are taken to prevent visitors from leaving the farm contaminated, visitors that have high frequency but low contact with birds become less important, and activities that involve the direct removal of infective material, or a high level of personal contact with birds become more important. The latter are activities in which the prevention of contamination carriage by adopting biosecurity measures is more difficult. This point is illustrated by the reduction in the contribution of utility persons and feed trucks to the total outcome variance as we compare typical and complete biosecurity scenarios to the scenario of no adoption of biosecurity measures. There is little difference between the variance profiles between the typical and complete scenarios, as well as among other scenarios of biosecurity adoption.

Live haul workers have a very low chance of going to multiple farms in the same day, but their probability of leaving the farm infected is very high, and it is not reduced by any biosecurity measure considered. It is important to note, however, that because commercial poultry operations in Georgia always place all birds at the same time, and birds are also hauled from all houses for slaughter at the same time ('all-in all-out' system), the farms visited by live haul workers are always being depopulated, and therefore were included in the model as farms in downtime. These farms were considered to be exposed in the model, but are unlikely to become infectious. In the British poultry industry for instance, where total depopulation of the farm at the same time only occurs in 50% of the times, Sharkey *et al* (2008) [84] and Dent *et al* (2008) [23] found the effect of live haul to be an important component of the risk for HPAI transmission.

Bird disposal has the highest frequency among all activities considered, as dead birds are removed from the houses by the grower daily. This will be considered as a potential contact with other farms if birds are disposed off-farm. Because infected birds will be carried out if a pathogen is spreading in the farm, this activity is associated with a very high chance of contamination spread. Indeed, in the H7N2 outbreak in Virginia the disposal of dead birds by rendering was found to be the most significant factor for AI infection of farms [48]. However, the results of the grower survey (Chapter 2) show that in Georgia taking birds to rendering facilities off of the farm is not a common practice, and very few growers reported any type of disposal off-farm in the survey. As a consequence, the results of the model did not show a high risk of disease spread associated with this activity in Georgia.

3.5 Conclusion

The daily risk of spread is always greater in the region of the state with a higher concentration of farms, due to a higher frequency of contacts and number of farms visited, even in scenarios of higher compliance with biosecurity measures. Therefore, even under a higher level of biosecurity adoption following an ILT outbreak, the higher farm density in the North of the state still places it at a higher risk of disease spread when compared to the South.

The evaluation of different biosecurity measures showed that requiring visitors to wear shoecovers is the measure with the highest impact in reducing the risk of disease transmission among commercial poultry operations. However, attention to biosecurity measures towards vehicles is the most important gap in biosecurity in the state currently, and increasing awareness of growers to the benefit of requesting visitors to park outside the farm or wash tires has the greatest potential to reduce the current risk of disease spread in the state.

Because the particular details of the contact structure in the North and South of the state were captured, as well as details of grower awareness and compliance with biosecurity measures, the results regarding the daily risk of spread are applicable to any diseases transmitted by the movement of people and equipment between farms within the commercial poultry industry. By focusing on the daily risk of spread, we were able to capture the impact of biosecurity measures in reducing the risk of disease introduction and transmission. In order to explicitly model the dynamics of outbreaks, however, and study how these measures can prevent pathogen introductions from becoming major outbreaks, specific information about particular diseases needs to be considered. Coupled with information about latent and infectious period, for instance, the daily transmission rates presented in this work can be used to model the potential for specific disease outbreaks.

CHAPTER 4

PROBABILISTIC MODEL OF THE POTENTIAL SECONDARY SPREAD OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS AMONG COMMERCIAL POULTRY OPERATIONS IN GEORGIA

4.1 INTRODUCTION

Avian influenza viruses (AIV) remain a threat to the bird population worldwide [107]. Outbreaks of avian influenza (AI) in the poultry industry have the potential to become a worldwide pandemic both in animal and human population, making it critical to prevent AIV introduction into the poultry farming system [52].

UUntil recently it appeared that the epidemiology of AI consisted primarily of the perpetuation of low pathogenic avian influenza (LPAI) viruses in wild birds where they caused little or no disease with spread to poultry from time to time. Very occasionally introductions of LPAI viruses of H5 or H7 subtypes into poultry resulted in the mutation of these viruses to virulent forms that caused highly pathogenic avian influenza (HPAI) [4, 40]. In 2003, however, H5N1 AI viruses began to circulate in south-east Asia and resulted in an unprecedented expansion of the geographic range of this subtype [45]. Outbreaks are now often affecting industrialized countries as this virus continues to circulate among domestic and wild birds [96], making it crucial that commercial poultry systems worldwide implement measures to prevent the introduction of the disease.

The highly pathogenic H5N1 strain of current global concern has not reached the United States, but the possibility of a direct introduction of HPAI into the country is possible. The illegal trade and transport of infected poultry or exotic birds has been cited as a potential route for possible spread of H5N1 across national borders [111]. In 2008, the combined value of production from broilers, eggs, turkeys, and the value of sales from chickens in the United States was \$35.9 billion [53]. An introduction of avian influenza viruses in the commercial poultry industry, especially if not controlled properly, would be associated with serious economic consequences as a result of bird deaths, depopulation costs, and national and international trade restrictions.

Prevention is therefore critical, and farm biosecurity represents the first and most important means of prevention [19]. Biosecurity consists of the measures used to prevent introductions of disease-causing organisms into a flock [51]. A routine biosecurity protocol includes security practices that should be implemented as standard operating procedure for the prevention of any infectious disease [42]. The adoption of these measures can not only significantly reduce the risk of disease introduction, but also reduce the magnitude of the financial losses that may occur following infection [51].

A number of studies used epidemic data from outbreaks to estimate transmission parameters and develop disease spread models of avian influenza transmission. They aimed at using the generated information to better understand and prevent outbreak spread [49, 11, 10, 47, 50]. HHowever, to the best of our knowledge, none of these authors explicitly modeled the impact of the adoption of biosecurity measures.

BBecause the routes of introduction of avian influenza (AI) viruses into domestic poultry flocks consist mainly of inter-farm spread of virus-contaminated media by people, fomites and vehicles prior to the detection of disease and isolation of an infected farm [91, 47], information about the types and frequencies of horizontal contact are critical in modeling transmission details. Considering that these production characteristics - and therefore the horizontal contact patterns - are likely heterogeneous between companies, poultry production type, and region [14], it is unclear to what extent transmission parameters from other countries can be applied to models that include detailed industry structure.

In the state of Georgia, the largest producer of broilers in the United States [53], specific characteristics of the commercial poultry system that could determine the potential for disease spread through human movement were captured by the work of Dorea *et al.* (Chapter 3), who explicitly modeled the impact of biosecurity measures in reducing the daily risk of pathogen transmission. The authors estimated, for two areas of the state with different density of poultry farms, the daily probability of off-farm spread from an infected source. In order to do so, the authors used information concerning the frequency and patterns of horizontal contact among commercial poultry growers in the state gathered by Vieira *et al.* (2009) [101], the results of a survey of broiler growers which determined the frequency of adoption of biosecurity measures (Chapter 2), and the results of an assessment of the effectivity of specific biosecurity measures carried Johnson and El Gazzar (unpublished).

In order to understand how the daily risk of transmission translates into the potential for outbreak development for a particular disease, specific information about the disease can be used to model transmission during the infectious period. The model in the current study was used to estimate the daily transmission rate and spread of highly pathogenic avian influenza (HPAI) among commercial poultry operations in Georgia in the absence of control measures (such as farm isolation and depopulation). The results of the on-farm model of HPAI published by Dorea *et al.* (in peer review) [25] were used as estimates for the latent period of the disease on the farm and the time to grower detection. Spatial information was used to explicitly incorporate farm distance and to model secondary spread.

By simulating the potential for HPAI transmission in the current scenario of biosecurity adoption in the state of Georgia, this study intended to evaluate the dynamics of a potential outbreak resulting from virus introduction, determine the critical times for disease reporting and control enforcement. By incorporating different scenarios of biosecurity adoption, we also intended to identify how biosecurity measures might reduce the potential for disease spread before detection on the index farm.

4.2 Materials and Methods

4.2.1 Data sources

TThe dynamics of virus spread within a broiler flock following introduction of HPAI virus was estimated based on the model of Dorea *et al.* (in peer review) [25], which simulated on-farm dynamics under different scenarios of virulence - variable daily transmission rate, latent period and infectious period.Dorea *et al.* (Chapter 3) ddescribed calculations to estimate the daily rate of exposure to contaminated material from one infected farm, based on the chance of movement between commercial operations for fifteen listed visitors. This daily rate was calculated independently for each of the visitors listed, under different biosecurity scenariaos for two areas of the state of Georgia with different densities of poultry farms.

SSpatial information was included in the model using the geographical coordinates of poultry farms provided by the Georgia Department of Agriculture. Coordinates were separated into two databases to represent the area of high poultry farm density (North of the state) and the area of low density (South of the state), as shown in Figure 4.1.

4.2.2 Parameters input in the model

Assuming that the HPAI virus is introduced to a single broiler farm in the state of Georgia, the dynamics of spread from this infected source during the infectious period was modeled accounting explicitly for: the dynamics of the virus on-farm, which determines the time between virus introduction and off-farm spread; secondary spread once other farms are infected; the small potential for secondary spread if contact farms are in downtime; and the effect of farm density in two different areas of the state (North and South). The following parameters were used to account for these details:

Geographical location: The geographic coordinates of all poultry farms in the state were separated into two subsets, as shown in Figure 4.1. In the North of the state the density of farms varies from 0.45 to 1.22 farms/mi^2, depending on the county. In the South the average

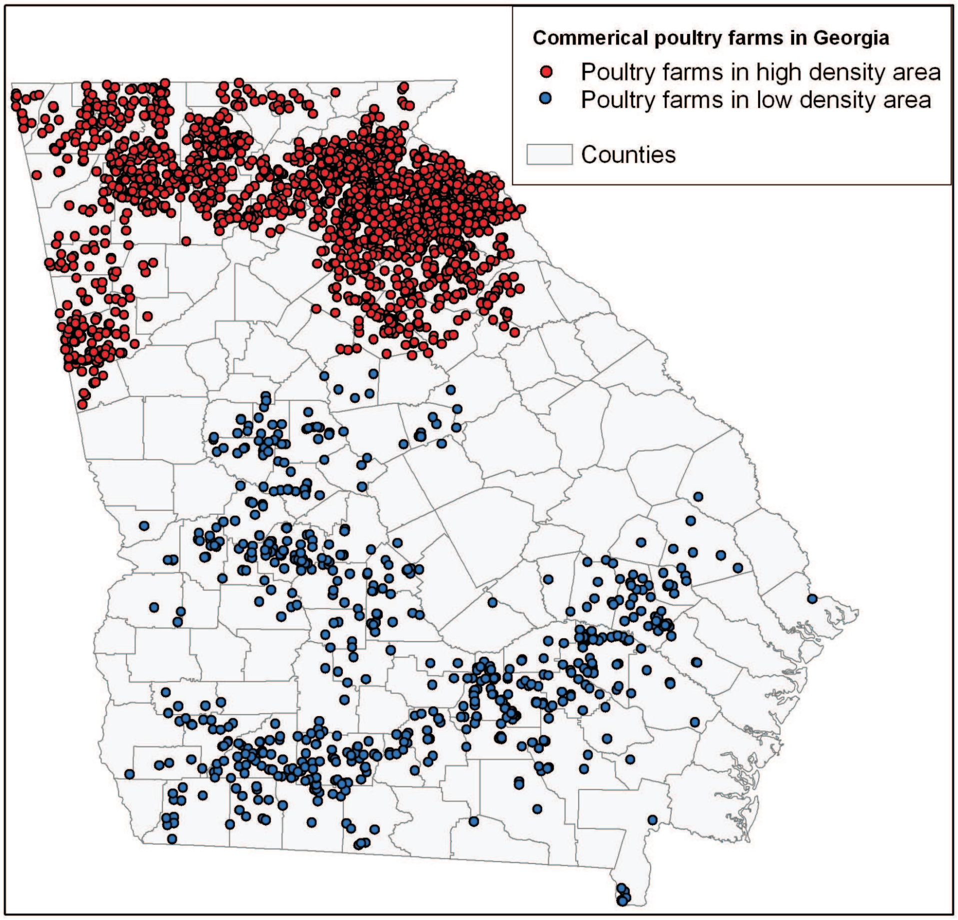

Figure 4.1: Location of commercial poultry farms in the state of Georgia according to information provided by the Georgia Department of Agriculture. The subsets of farms shown for an area of high poultry farm density (farms in red on the map) and another of low density (blue) correspond to the point locations used in independent models of HPAI spread

farm density is 0.20 farms/mi^2. The model assumed that all point locations refer to broiler farms.

Distance between every pair of farms: A matrix of the Euclidean distances ($\sqrt{(x_{distance})^2 + (y_{distance})^2)}$) between every pair of farms was calculated by transforming the latitude and longitude differences into miles, using the following ratios: 1 degree of latitude = 68.89miles; and 1 degree of longitude = 59.34 miles [1].

Contact radius: Based on statistics provided by a poultry expert (Dr. John Smith) regarding company feed trucks, we used 35 miles as the contact radius in the high density model, and 30 miles in the low density model. Every farm within a set distance from an infected farm was considered a potential contact. There is therefore a defined set of possible contacts for each farm.

Daily contact rate: the daily contact rate was calculated individually for each visitor as described in Dorea*et al.* (Chapter 3). For each visitor, a contact rate was established based on the daily probability of coming to the farm, the probability of getting contaminated according to the nature of the activity performed on farm, the probability of contamination being removed by specific biosecurity measures, and the daily number of farms visited. This contact rate represents the number of farms expected to be exposed to contamination from the infected source through the movement of that specific visitor. The daily contact rate depends on the scenario of biosecurity adoption considered. Dorea*et al.* (Chapter 3) described the scenarios representing the typical compliance in the state of Georgia, and increased compliance reported by growers responding to a disease outbreak. Five additional scenarios simulated targets for increased biosecurity. All these scenarios were also compared to a scenario of no adoption of biosecurity measures.

The fifteen visitors considered, as well as the daily contact rate for each of them under different biosecurity conditions, are shown in Table 4.1.

Clean out services, decaking services, litter disposal trucks, live haul and shaving suppliers were assumed to only visit farms during downtime or when farms are being depopulated.

Table 4.1: Mean number (after 30,000 iterations) of farms exposed to contaminated material from an infected farm through movement of each listed visitor under different scenarios of biosecurity adoption

	Scenario of biosecurity adoption*							
	No biosecurity	Typical biosecurity	Increased awareness	Vehicle biosecurity	Use of PPE	Controlled Access	All visitor biosecurity	Complete biosecurity
	Model for areas of low density of poultry farms							
Clean Out	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000
Decaking	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Litter disposal	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000
Birds disposal	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000
Live Haul	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
Shaving Suppliers	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.000
Repairmen	0.004	0.002	0.002	0.001	0.002	0.002	0.001	0.001
Gas Delivery	0.025	0.011	0.011	0.007	0.010	0.011	0.006	0.007
Feed trucks	0.415	0.186	0.182	0.136	0.174	0.191	0.115	0.118
Chick Bus	0.032	0.016	0.015	0.011	0.013	0.015	0.010	0.010
Servicemen	0.202	0.097	0.082	0.068	0.073	0.097	0.055	0.055
Utilitity persons	0.067	0.030	0.029	0.022	0.027	0.030	0.020	0.020
Unauthorized visitors	0.030	0.015	0.013	0.011	0.012	0.014	0.009	0.009
Vaccination crews	0.015	0.008	0.007	0.004	0.006	0.008	0.004	0.004
Hired help	0.201	0.098	0.092	0.068	0.087	0.090	0.054	0.054
Sum**	1.005	0.478	0.447	0.343	0.419	0.471	0.29	0.288
	Model for areas of high density of poultry farms							
Clean Out	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000
Decaking	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000
Litter disposal	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Birds disposal	0.007	0.008	0.007	0.008	0.008	0.007	0.008	0.000
Live Haul	0.027	0.027	0.027	0.027	0.027	0.027	0.027	0.027
Shaving Suppliers	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.000
Repairmen	0.004	0.002	0.002	0.001	0.002	0.002	0.001	0.001
Gas Delivery	0.046	0.021	0.019	0.014	0.019	0.021	0.013	0.014
Feed trucks	0.417	0.188	0.182	0.135	0.175	0.190	0.117	0.115
Chick Bus	0.031	0.014	0.015	0.012	0.013	0.015	0.011	0.010
Servicemen	0.269	0.129	0.110	0.109	0.098	0.129	0.074	0.074
Utilitity persons	0.103	0.046	0.044	0.037	0.043	0.046	0.032	0.031
Unauthorized visitors	0.060	0.028	0.026	0.021	0.025	0.027	0.016	0.016
Vaccination crews	0.015	0.008	0.007	0.006	0.006	0.007	0.004	0.004
Hired help	0.202	0.099	0.092	0.070	0.087	0.090	0.054	0.054
Sum**	1.186	0.575	0.535	0.445	0.507	0.566	0.362	0.346

*Scenarios as described by Dorea *et al.* (Chapter 3): 'No biosecurity' = no adoption of biosecurity required from visitors; 'Typical' = typical compliance to biosecurity in the state of Georgia; 'Increased Awareness' = higher frequency of biosecurity adoption reported by growers respondind to a disease threat; 'Vehicle biosecurity' = frequency of vehicle biosecurity increased to 90% (45% for all visitors required to wash vehicle tires and 45% required to park outside the farm); 'Use of PPE' = increasing the use of personal protective equipments (PPE) to 80% of visitors wearing coveralls, 90% wearing shoecovers and 90% washing hands after handling birds; 'Controlled access' = not allowing unauthorized visitors to enter the poultry houses; 'All visitor biosecurity' = combining all previous measures together; and 'Complete biosecurity' = in addition to the previous, all users cleaning out houses with owned equipment and disposing litter and birds on-farm.

**The sum of the expected number of farms exposed to contaminated material through each of the visitors listed corresponds to the mean daily contact rate reported by Dorea *et al.* (Chapter 3).

Repairmen, gas delivery, utility persons, unauthorized visitors and hired help were assumed to come to the farm during any flock period. All other visitors (birds disposal, feed trucks, chick bus, company servicemen and vaccination crews) were assumed to always come to the farm during growout.

Flock period of the farm: Based on information provided by poultry experts, the probability of a farm being on downtime was set as 2/9 (two weeks of rest after a seven weeks growout), and a status of "growout" or "downtime" was assigned randomly to each farm based on this probability. Farms on downtime can be exposed, but they will not become infectious, since virus amplification will not occur in the absence of birds. Considering however the litter conditions (temperature around 34^o C [6], and humidity between 18 and 21% [9]) and the survivability of the HPAI virus [105], there is a chance that the virus may still be viable when chickens are placed again on the contaminated litter.

Latent and infectious period for farm as a unit: We set the latent period of each farm to two days (no off-farm spread until the third day after exposure) [25]. Once off-farm virus spread begins, the farm will remain infective until the grower detects and reports increased mortality, and contingency measures (movement restrictions and depopulation) are put in place. Based on the results of Dorea *et al.* (in peer review) [25] we determined that detection by the grower can occur any day from the fifth day on, and these farms will be labeled as reaching the mortality detection threshold, but no assumption was made concerning the time until reporting and depopulation of farms. Because the goal was to study the dynamics of disease spread until detection of the first case, the removal of infected farms by any control measure was not included. Infected farms therefore have the potential to start spreading on day three and remain infectious for as long as the model runs, but those farms that already have reached the mortality threshold for grower detection, which could lead to reporting and enforcement of control measures, are identified. This way, daily scenarios can be evaluated under the assumption that reporting did not yet occur, and the effect of delayed detection/notification can also be assessed.

Disease status of each farm: To keep track of the infectious state of each farm, the model assigns a status number to each farm, which represents the count of days since exposure. This number is used to identify those farms that are still susceptible (status = 0), farms that are exposed but not infectious (status = 1 or 2), infected and infectious farms (status = 3, 4 or 5), and farms that reached the mortality threshold for detection, which will still be considered infectious (status > 6).

4.2.3 ALGORITHM

Each iteration of the model begins with all farms labeled as susceptible (status=0). The low density area model includes a total of 906 farms, and the high density area 2,810 farms. Independently in the two density areas, one farm is randomly assigned to be the introduction case, by setting its status value to three (infected farm), and from then on the model algorithm proceeds as follows:

1. For each time step (each day), the model determines all possible contacts (farms within the contact radius) for each infective unit and identifies in which flock period they are (growout or downtime).

2. The model decides, randomly, whether each visitor will expose another farm to contaminated material, based on the daily contact rate.

3. Based on whether the visitor type goes to farms during downtime, growout, or any flock period the exposed farm is randomly selected among the contact group.

4. If the farms exposed by each visitor have a status of zero (susceptible), the model assigns them the status of one, to indicate that they are now exposed. Farms already exposed or infected in a previous step are not influenced by additional exposures. At the end of the step, all previously exposed farms have their status updated to reflect the addition of one day, with the exception of farms in downtime, which always remain exposed.

The secondary spread was modeled over 40 days, and repeated through 100 iterations.

Because farm density influences the rate of secondary spread, the average daily transmission rate is not expected to reflect the summed average reported in Table 4.1 throughout the entire time interval modeled. We calculated the effective daily transmission rate as the number of farms exposed in a single day divided by the number of infectious farms when the day time step started. The actual reproductive rate (R_o, total number of farms exposed by each infective unit during its entire infectious period) was not calculated because we did not set a fixed infectious period, as infected farms were considered to remain infectious for as long as the model runs.

4.3 Results

Farm infectious states, averaged over 100 iterations, were assumed to follow the dynamics of an Susceptible-Exposed-Infectious-Recovered (SEIR) model [41], with the exception that farms were not considered to be ”recovered” or removed, but rather to reach the mortality threshold for grower detection. Figure 4.2 shows the distribution of the farm infectious states under the scenario of no biosecurity adoption.

The number of susceptible farms decreases as farms become exposed, infectious, or exceed the mortality detection threshold. Because farms in downtime can be exposed, but cannot become infectious, the number of exposed farms in downtime accumulates over time. In general, fewer than 10% of the farms exposed daily are in downtime. The dynamics for farms in growout, which become infectious two days after exposure and reach the mortality threshold for detection three days after that, more closely follows the theoretical predictions for an SEIR model. After reaching the threshold for mortality detection, farms accumulate in this compartment until reporting and control measures can be put into place.

The scenarios of biosecurity adoption that simulated the typical compliance in Georgia, and increased grower awareness, are shown for both density models in Figure 4.3.

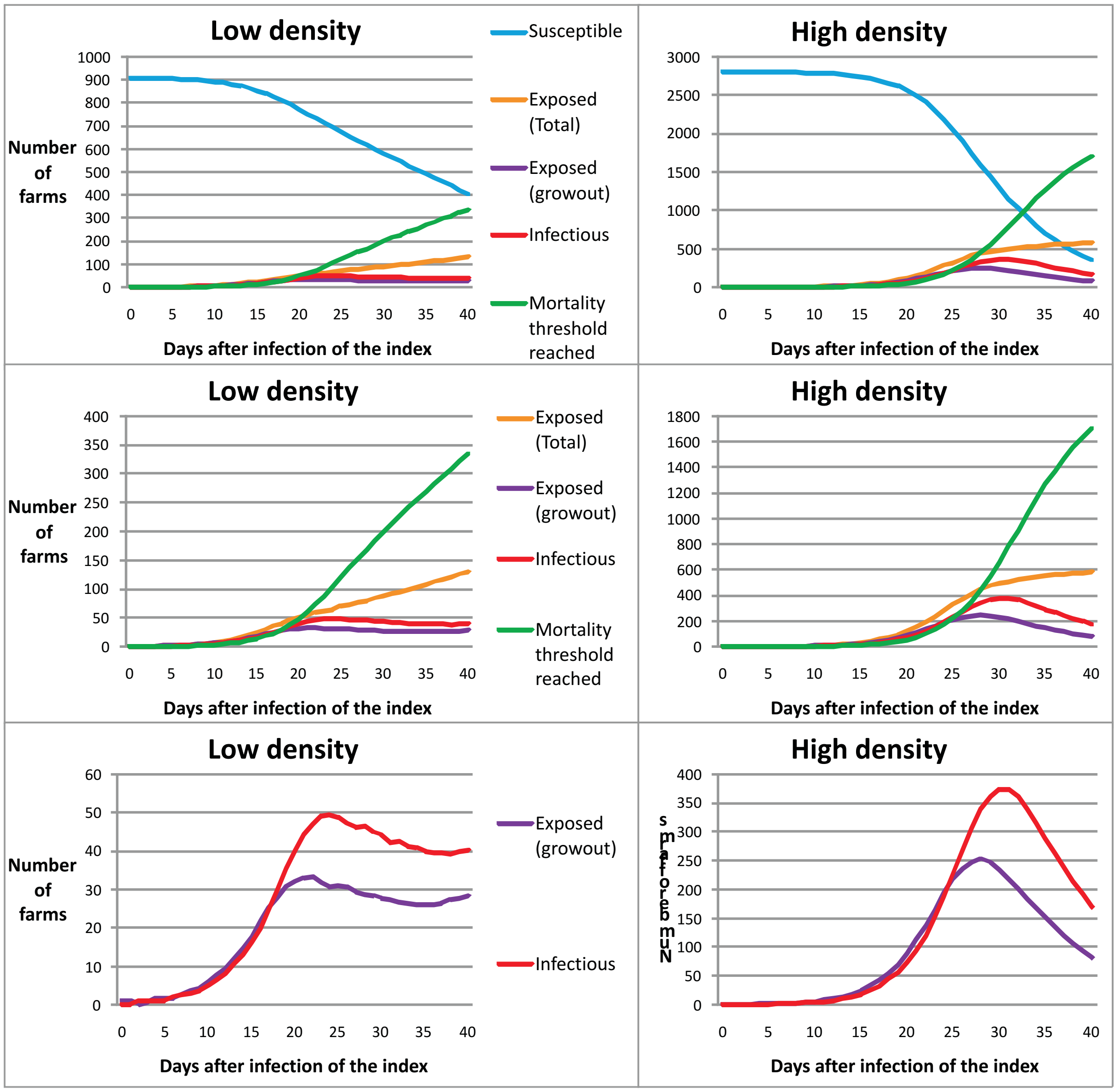

Figure 4.2: Dynamics of the infection for the scenario of no adoption of biosecurity measures in the models for areas with low and high farm densities. Curves represent the average of 100 iterations. Different panels in each figure adjust the scale to focus on different infectious states. “Mortality threshold reached” refers to farms in which daily mortality of birds is expected to be high enough for detection by the grower

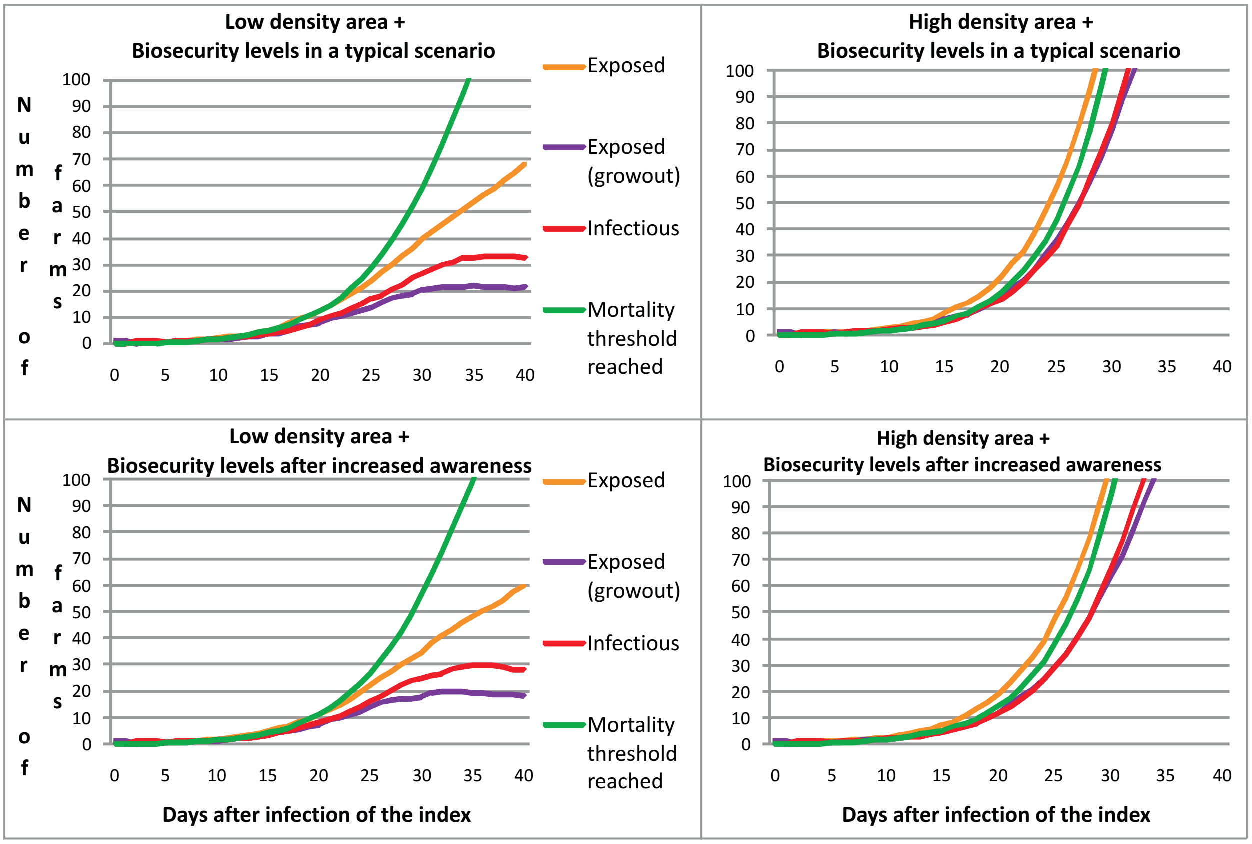

Figure 4.3: Dynamics of the infectious states for low and high density models, for the typical scenario of biosecurity adoption in Georgia and following increased disease awareness. Curves represent the average of 100 iterations. "Mortality threshold reached" refers to farms in which daily mortality of birds is expected to be high enough for detection by the grower. The vertical axes have been adjusted to focus on the number of infectious units.

The scenario of biosecurity adoption following increased disease awareness, when compared to the typical compliance in the state, not only reduces the observed number of exposed and infectious farms, but it also slows the epidemic curve, delaying the phase of exponential growth.

In Figure 4.3 we see that the number of exposed and infected farms is projected to be less than two during the five days after contamination of the index farm, when detection of this first case is predicted to occur [25]. But the number of exposed farms starts increasing exponentially thereafter. Table 4.2 shows the total number of compromised farms (sum of farms exposed, infectious and reported) during days 5–15 after infection of the index farm. During this period, many farms would be exposed but not yet infectious, and would be difficult to trace for isolation. The results show that for all scenarios in which biosecurity measures are in place, the dynamics of spread is similar up to day five. As the number of exposed farms starts growing exponentially, differences among scenarios become apparent. Virus spread will be slower in areas of lower density and when there is a higher adoption of biosecurity measures.

Table 4.2: Expected number of farms exposed to HPAIv (sum of farms exposed, infectious and reported) from days 5–15, in the low and high density models, for varying scenarios of biosecurity adoption. Numbers represent the average after 100 iterations

		Low density model											High density model										
	Day	5	6	7	8	9	10	11	12	13	14	15	5	6	7	8	9	10	11	12	13	14	15
No Biosecurity	Exposed	2	2	3	4	5	7	10	12	15	19	23	1	2	3	4	5	7	10	13	17	24	31
	Total	4	5	7	9	11	15	20	26	33	42	53	3	4	6	8	10	14	19	25	34	46	61
Typical biosecurity	Exposed	1	1	1	1	2	2	3	3	4	4	5	1	1	1	2	2	3	3	4	5	6	8
	Total	2	3	3	4	5	6	7	8	10	12	14	2	3	3	4	5	7	8	10	12	15	19
Increased awareness	Exposed	1	1	1	1	2	2	2	3	3	4	5	1	1	1	2	2	2	3	4	5	6	7
	Total	2	2	3	4	4	5	6	7	9	10	13	2	3	3	4	5	6	8	9	11	14	17
Vehicle biosecurity	Exposed	1	1	1	1	1	2	2	2	2	3	3	1	1	1	1	1	2	2	2	3	3	4
	Total	2	2	3	3	4	4	5	6	7	8	9	2	2	3	3	4	5	6	7	8	9	11
Use of PPE	Exposed	1	1	1	1	1	2	2	2	3	4	4	1	1	1	2	2	2	3	3	4	5	6
	Total	2	2	3	3	4	5	5	6	8	9	11	2	2	3	4	5	6	7	8	10	12	15
Controlled Access	Exposed	1	1	1	1	2	2	2	3	4	4	5	1	1	2	2	2	2	3	4	5	5	7
	Total	2	3	3	4	5	5	7	8	10	11	14	2	3	3	4	5	6	8	10	11	14	17
Visitors biosecurity	Exposed	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1	1	1	2	2	3	3	3
	Total	2	2	2	3	3	4	4	5	6	6	7	2	2	3	3	4	4	5	6	7	8	10
Complete biosecurity	Exposed	0	1	1	1	1	1	1	2	2	2	2	0	1	1	1	1	1	1	2	2	3	3
	Total	2	2	2	3	3	4	4	5	6	7	8	2	2	2	3	3	4	4	5	6	7	8

The increase in the number of exposed farms coincides with an increase in the daily rate of disease transmission, as shown in Figure 4.4. The impact of increased compliance to biosecurity in reducing this rate is also shown.

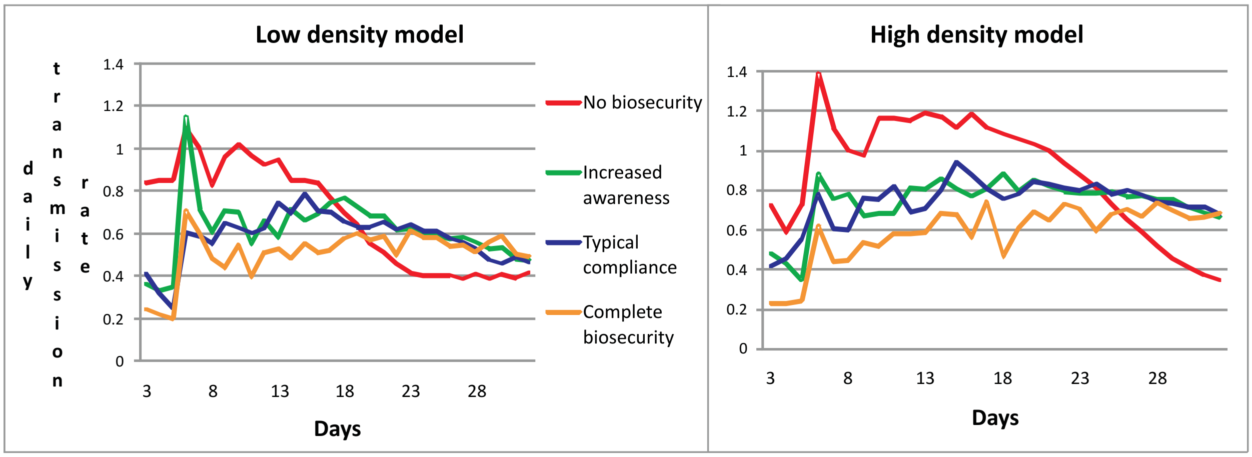

Figure 4.4: Daily transmission rate calculated as the number of farms exposed per day, divided by the number of infectious farms on the same day, for the low and high density models. Curves represent the average of 100 iterations.

Despite the effect of higher biosecurity adoption in reducing the daily transmission rate, once the epidemic reaches a phase of exponential growth (after day five) the effective reproduction rate (R_o) over the course of the infectious period of each farm is likely to be higher than one, since the infectious period is at least three days and the daily transmission rate is always > 0.4 in this period. This was the case even for the scenario with the highest level of biosecurity compliance.

The numbers in Table 4.2 and Figure 4.4 are the mean of 100 iterations of the model. The adoption of biosecurity measures was also shown to reduce the maximum number of farms exposed on any given day. Figure 4.5 shows the minimum and maximum number of compromised farms during days 5–15 under the typical level of biosecurity and when no biosecurity measures have been implemented. The number of compromised farms was defined as the sum of the exposed, infected, and reported farms.

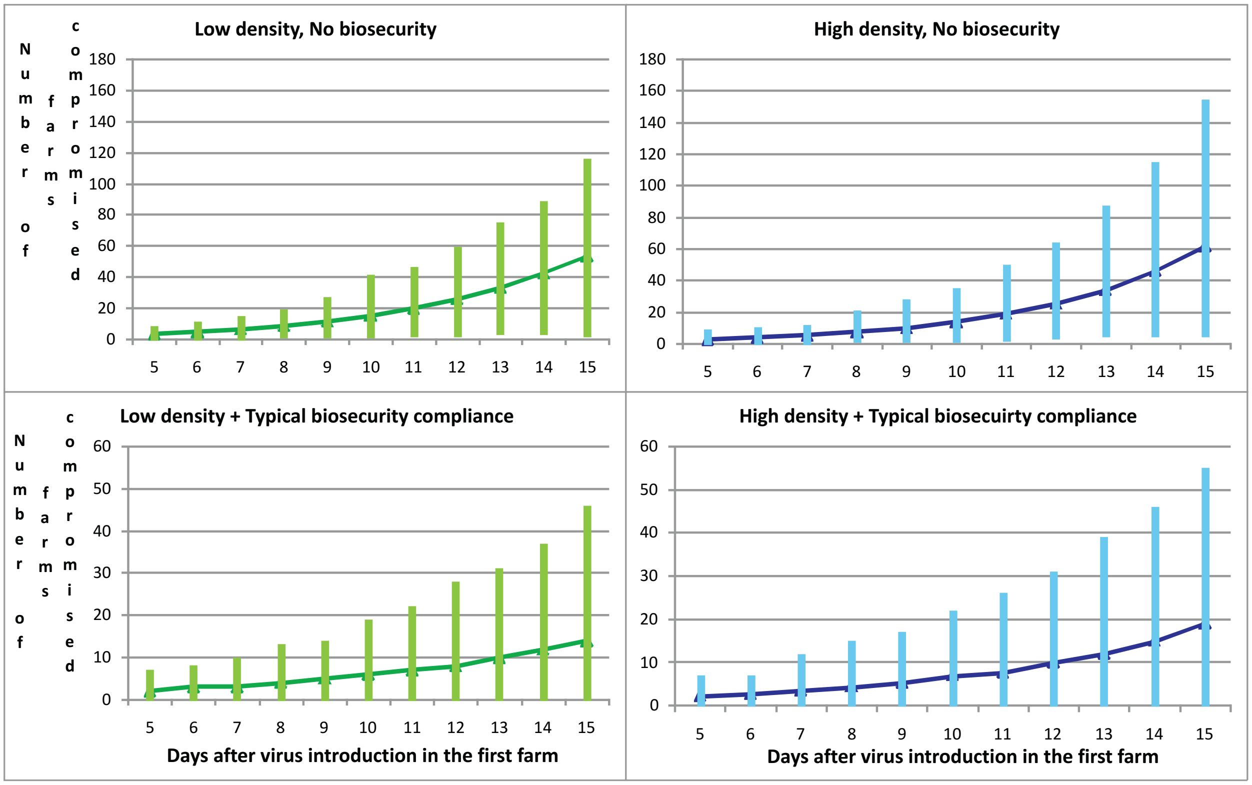

Figure 4.5: Minimum, average and maximum for the total number of farms compromised by the HPAI virus during days 5–15 (sum of the exposed, infected, and reported farms for each day)

4.4 Discussion

Several outbreaks in industrialized countries have been reported and studied to investigate details of the transmission dynamics at the farm level [30, 88, 91, 13, 47, 50, 17], but extrapolation from such events to other regions is problematic due to regional differences in the poultry industries [84] and the characteristics of the viruses. Detailed consideration of the specific features of the population at risk and potential transmission routes within this population are necessary in order to provide policymakers and animal disease control agencies with the necessary information to design prevention and control strategies [84].

Building on the work of Dorea *et al.* (Chapter 3), which captured specific characteristics of the contact network and biosecurity compliance by growers in the commercial poultry system in Georgia, the intention of this study was to simulate the dynamics of HPAI transmission in this system. To assess the potential for silent spread (virus transmission before detection of disease) under different scenarios of biosecurity adoption, we modeled the transmission dynamics in two different geographic regions of the state. Dorea *et al.* (Chapter 3) showed that the North of the state has a higher frequency of contact between farms and a greater number of farms visited on a typical day, and consequently has a higher potential for disease transmission. Spatial information was incorporated to determine how the different densities in the two areas might impact transmission.

The model of Dorea *et al.* (in peer review) [25] predicted that bird mortality would reach a threshold for detection on the fifth day after introduction of HPAIv on the farm. Implementation of control measures the same day as the detection was considered a best case scenario, but the impact of delayed detection, reporting, or implementation of control measures is also of interest. Most broiler growers in Georgia (84%) [101] indicated that they would notify their company about flock illness on the same day of detection, but some would wait up to 48hs. Consequently, even though detection may occur on the fifth day, farm isolation to contain spread may not occur until six to seven days after virus introduction. Busani *et al.* (2008) [17] reported an average of seven days between introduction of the virus on a farm and detection by growers in the H7N1 outbreak in Italy in 1999–2000, and Sharkey *et al.* (2008) [84] found an average time to first detection of 9.8 days when simulating spread in the British poultry industry.

Therefore, infected farms were never removed from the model in the current study, and even though farms that passed the expected day for grower detection were identified, these farms were still considered to be infectious. Consequently, the results for any day represent the expected spread if control measures had not been previously implemented.

The predicted spread in a network completely free of any biosecurity measures led to simulated outbreaks that infected three times more farms than would be expected for the typical levels of biosecurity measure adoption in the state, and 5–8 times more farms than the best case scenario of complete compliance with all biosecurity measures. In such a situation, the number of exposed farms was expected to reach 1/3 of all farms in less than 30 days in both density models (a couple of days earlier in high density area). However, it is not realistic to expect that detection would be delayed this long, and as control measures are put in place and infected farms are removed it is reasonable to expect that transmission would be reduced.

If reporting and implementation of control measures was achieved as early as detection by the grower is predicted to occur — on the fifth day — the results do not vary markedly among scenarios, not even when low and high density areas are compared. In fact, the expected number of exposed and infected farms is two for all scenarios considered except for the complete absence of biosecurity measures. After day five, however, the numbers of exposed farms increase quickly, making timely notification crucial.

After day five, the differences between biosecurity compliance scenarios and between the two density models become apparent. The number of infected farms is always greater for the high density model, except under the scenario of complete biosecurity adoption. If very high compliance with biosecurity measures is not in place in the high density areas, control measures put in place after disease detection may not be sufficient to halt the epidemic, as stated by Boender *et al.* (2007) [11]. In the scenario of complete biosecurity adoption, however, the number of exposed farms in the high density model is not different from the model for the low density region.

Because notification and implementation of control measures can be delayed, and the successful tracing of all exposed farms may take a long time, high compliance to biosecurity measures is the most effective way to prevent an unexpected pathogen introduction from becoming an epidemic. If control measures cannot be efficiently put in place until day 15, for instance, increased biosecurity towards vehicles alone could reduce the expected number of exposed farms by 36% in the low density region and by 42% in the high density region, when compared to the typical level of biosecurity compliance in the state. Complete compliance with biosecurity measures, also in comparison to the typical frequency of biosecurity adoption, could reduce the expected number of exposed farms by 43% in the low density region and by 58% in the high density region. Therefore, if a high level of biosecurity adoption is in place, more time is afforded to gain control of the epidemic, even if the introduction is detected late. Having a high level of biosecurity gives stakeholders more time to trace and isolate exposed farms, as the exponential increase in the number of exposed farms is considerably delayed.

Moreover, the adoption of biosecurity measures, besides reducing the expected (average) number of farms exposed to the virus, also has the potential to increase the chance that no farm is exposed following introduction of HPAI to an index farm, as well as of reducing the possible number of exposed farms in a worst case scenario. This again ensures that even if the virus is introduced, in a scenario of high adoption of biosecurity measures propagation of the outbreak can either be prevented or minimized

Contact tracing, and quarantine and depopulation of infected farms are measures established by the United Stated Department of Agriculture (USDA) as part of the national response plan to prevent spread of HPAI [54]. BBecause farms in downtime can be exposed to the virus, as they share some visitors with farms in growout, possible contacts in downtime should also be traced, and subjected to a full change of litter, and cleaning and disinfection of all houses. In the current study, no assumption was made as to the number of days that the virus would survive in the litter during downtime, and infection of farms resulting from the re-introduction of birds to premises that were contaminated during downtime was not considered.

Because the adoption of biosecurity measures can delay transmission, but not prevent the exponential growth in the number of farms exposed, whenever detection occurs there is great potential for increase in the number of affected farms if the exposed farms are not all identified and isolated/depopulated. This is highlighted by the daily transmission rates, which indicated that the reproductive ratio will most likely remain above one for the entire period modeled (each farm is expected to infect at least one other farm during their infectious period, ensuring propagation of the outbreak). The daily transmission rate remained above 0.40 for any scenario modeled.Variability in the estimated transmission rates over time was caused by random effects captured when modeling farm density and spatial location, which caused the number of susceptible contacts to vary not only in time, but also depending on the location of the infected farms.

For the purposes of this study, it was assumed that every farm exposed to the virus would get infected. For that reason, the calculated daily transmission rates are higher than those observed in previous outbreaks. While the calculated daily transmission rate up to day five (the expected day for detection) averaged 0.54 farms for the low and high density models under the scenario of typical biosecurity adoption in the state, and 0.46 farms exposed per infected farm per day under the scenario of increased disease awareness, Stegeman *et al.* (2005) [88] reported average daily transmission rates of 0.47 and 0.39 for two different regions in The Netherlands for the H7N7 outbreak in 2003. Even though it is not likely that this 100% transmission efficiency would be observed in the field, it gives a reasonable approximation of the risk associated with each visitor, and allowed an exploration of the impact of biosecurity measures.

4.5 Conclusion

If HPAI virus were introduced in the commercial poultry industry in Georgia, the number of farms exposed to the HPAIv would be expected to increase exponentially after the fifth day, the same day predicted for detection of disease on the farm where the virus was first introduced. Therefore, immediate reporting following detection is critical to contain spread. After notification, fast contact tracing and isolation and depopulation of infected farms is crucial, since the ratio of disease reproduction indicates a high potential for outbreak propagation throughout the entire epidemic period modeled. If an efficient biosecurity system is in place, spread is slower and the exponential growth phase of the epidemic delayed, giving stakeholders more time to effectively trace exposed farms and eradicate the outbreak before a large number of farms are compromised.

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APPENDIX

QUESTIONNAIRE USED IN THE POULTRY GROWERS SURVEY

1785
The University of Georgia

Dear Poultry Grower:

The University of Georgia, in cooperation with Georgia poultry companies, is conducting a scientific study to identify potential vulnerabilities in the state that could result in infectious disease introduction. To be successful, we need your help.

Please take a few moments to complete the attached questionnaire about biosecurity measures in place on your farm. The form should take about 10 minutes to complete. Please answer all questions to the best of your ability. Please return the completed questionnaire to the study investigators using the pre-addressed, stamped envelope provided.

All questionnaire data is collected completely anonymously. No identifying information will be collected that will allow the study investigators or anyone else to identify you, your company, or your farm. All study results will be reported in a statistical summary form only.

If you have any questions about the purpose of the study, the questionnaire, or the disposition of study results, please contact Dr. Fernanda Dorea or Dr. Charles Hofacre at the address and phone numbers provided below.

Thank you for cooperation and support in this important research project.

Sincerely,

Fernanda Dorea,
Graduate Researcher,
Poultry Diagnostic Laboratory Research Center
University of Georgia
College Station Road
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Phone: (706) 353-2401

Dr. Charles Hofacre
Professor, Population Health Department
Poultry Diagnostic Laboratory Research Center
University of Georgia
College Station Road
Athens, GA 30602
Phone: (706) 542-1904

We need your help! The University of Georgia is conducting a study of the biosecurity protocols in place on Georgia poultry farms. The information will be used to better understand the mechanics of disease transmission among commercial poultry operations. All information is collected anonymously, and there will be no way for anyone to identify you, your company, or your farm. If you have any questions or concerns please contact Dr. Charles Hofacre at (706) 542-5653.

Please read the questions thoroughly and answer them to the best of your ability. Please note that survey questions are on front and back of both enclosed pages. Return the completed survey to study investigators in the addressed, stamped envelope provided

1. How many poultry farms do you own or operate? ________ farms

2. How many poultry houses do you have on your farms (total)? ________ houses

3. What is the total one-time bird capacity of all of your houses? ________ birds

4. Does anyone in your immediate or extended family (like brother/sister, son, etc) own a poultry farm or work for a poultry company? [ ]Yes [ ]No

Considering the **routine activities** on your farm(s), please answer the following:

5. When performing daily activities on your farm, which of the following do *you and your employees do before entering the poultry houses*: (please put an“X” in the appropriate column at right)

	Always	Usually	Sometimes	Rarely	Never
Put on disposable **plastic boots or shoe covers**?					
Step in a **footbath** containing disinfectant?					
Put on **hairnets**?					
Put on **coveralls**?					
Shower and change clothes before entering and exiting farm premises?					

If you or your employees ever wear coveralls, when are the coveralls removed? (please check all that apply)

[ ]After exiting each poultry house [ ]Prior to leaving the farm premises

[ ]At the end of the day

6.Do you or your employees clean hands with soap and water or hand sanitizer after handling birds?

[ ]Always [ ]Usually [ ]Sometimes [ ]Rarely [ ]Never

7. How frequently are dead birds removed from poultry houses?

[ ]More than twice a day [ ]Twice a day [ ]Once a day

[ ]Every other day [ ]Once or twice a week [ ]Less than once a week

8. Once removed from the poultry house, how are dead birds disposed?

[ ]Composting on the farm [ ]Composting off the farm

[ ]Dead pit/Burial on the farm [ ]Burial off the farm

[ ]Other method. Please indicate which method:________

If dead birds are disposed on the farm, the disposal site is located (please check all that apply):

[ ]Within 10ft of a poultry house [ ]More than 10ft, but <20ft from a poultry house

[ ]More than 20ft from a poultry house

[ ]Within 10ft of a road or driveway [ ]More than 10ft, but <20ft from a road or driveway

[ ]More than 20ft from a road or driveway

9. A complete change of the litter in each poultry house is performed after:

[ ]Every flock [ ]Every 2nd flock [ ]Every 3rd flock

[ ]Every 4th flock [ ]Every 5th flock [ ]Every 6th flock

[ ]After more than 6 flocks

10. Are all the houses in your farm (or each of your farms) cleaned out at one time?

[ ]Yes [ ]No

If all the houses are not cleaned out at the same time, how many are cleaned at one time?___

11. Do you use contracted services for cleaning/decaking?

[ ]Always [ ]Usually [ ]Sometimes [ ]Rarely [ ]Never

12. Do you ever share with other poultry growers equipment for cleaning, decaking or litter spreading?

[ ]Always [ ]Usually [ ]Sometimes [ ]Rarely [ ]Never

If you ever share equipment with other growers, how many growers, besides you, use the same equipment?

[ ]1 [ ]2 [ ]3 [ ]4 [ ]5 [ ]If more than five, how many growers?___

13. How is the equipment for cleaning/decaking cleaned after use? (Please check all that apply)

[ ]Disassembled, pieces cleaned separately [ ]Disinfectant used to clean all washable surfaces

[ ]Visible debris removed with water [ ]Power washer used on all washable surfaces

[ ]Not cleaned

Considering **visitors and servicepersons** that come to your farm(s), please answer the following:

14. For *vehicles* entering the farm: (please put an "X" in the appropriate column at right)

	Always	Usually	Sometimes	Rarely	Never
Do vehicles need to park before entering farm?					
Before *entering* farm, are vehicles required to wash tires and wheel wells?					
Before *leaving* farm, are vehicles required to wash tires and wheel wells?					

If vehicles ever need to park before entering farm, how close is the parking area to poultry houses?

[ ]Within 10ft [ ]Over 10ft. but within 30ft.

[ ]30-50ft. [ ]Over 50ft.

15. Which of these procedures are required for all *visitors entering a poultry house*: (please put an "X" in the appropriate column at right)

	Always	Usually	Sometimes	Rarely	Never
Put on disposable **plastic boots or shoe covers**?					
Step in a **footbath** containing disinfectant?					
Put on **hairnets**?					
Put on **coveralls**?					
Shower and change clothes before entering and exiting farm premises?					

If the visitors/service persons ever wear coveralls, when are they removed? (please check all that apply)

[ ]After exiting each poultry house [ ]Prior to leaving the farm premises

[ ]At the end of the day

Do visitors clean hands with soap and water or sanitizer after handling birds?

[ ]Always [ ]Usually [ ]Sometimes [ ]Rarely [ ]Never

16. Which of these visitors are permitted to enter a house during a flock grow out either routinely or occasionally? (check all that apply)

☐ Shaving suppliers
☐ Feed truck drivers
☐ Chick bus drivers
☐ Meter readers
☐ Repairmen
☐ Vaccination crews
☐ Decaking service personnel
☐ LP Gas delivery personnel
☐ Egg truck drivers
☐ Veterinarians
☐ Company service persons, managers
☐ Live haul service personnel
☐ Clean-out service personnel
☐ Pit inspectors
☐ Non service-related visitors (like neighbors, helpers that are not regular employees, friends)
☐ Agricultural extension agents
☐ Any regular visitor not already listed:______________

Appendix

Stochastic Model of the Potential Spread of Highly Pathogenic Avian Influenza (HPAI) from an Infected Commercial Broiler Operation in Georgia, U.S. - Article in peer review

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Keywords: broiler, poultry, stochastic model, highly pathogenic avian influenza (HPAI)

Abbreviations:

SEIR - Susceptible, Exposed, Infectious, Recovered - compartments and title of an epidemic disease model.

HPAI - Highly Pathogenic Avian Influenza

AI - Avian Influenza

U.S. - United States of America

FSIS - Food Safety and Inspection Service

FDA - U.S. Food and Drug Administration

SUMMARY

The potential spread of highly pathogenic avian influenza (HPAI) among commercial broiler farms in Georgia, U.S. was mathematically modeled. The dynamics of spread within the first infected flock were estimated using an SEIR deterministic model, and predicted that grower detection of flock infection is most likely 5 days after virus introduction. Off-farm spread of virus was estimated stochastically for this period, predicting a mean range of exposed farms from 0-5 depending upon the density of farms in the area. Modeled off-farm spread was most frequently associated with feed trucks (highest daily probability and number of farm visits) and company personnel or hired help (highest level of bird contact).

INTRODUCTION

The highly pathogenic avian influenza virus, H5N1, has become an important global health issue. The Asian H5N1 strain has caused numerous outbreaks in domestic poultry and wild bird populations (28) and hundreds of human deaths (27). The ongoing transmission of this virus presents a threat to the poultry industry worldwide (26) and increases the probability of HPAI outbreaks among poultry flocks in industrialized countries (22). Poultry comprises the largest segment of Georgia's agriculture and agribusiness. In the face of the HPAI threat to U.S. poultry, the industry and agriculture agencies need quantitative estimates of the likely size, duration and geographical distribution of an HPAI outbreak. This is especially true in states such as Georgia where there is no previous outbreak data to inform local prevention and control strategies.

Transmission of avian influenza (AI) viruses between poultry farms is mainly due to inter-farm spread of virus-contaminated media on people, equipment and vehicles prior to the detection of disease and isolation of infected farms (13, 20). However, specific information about the types and frequencies of horizontal contact between farms is limited; and the patterns of contact likely vary significantly between companies, poultry production types, and geographic areas (5). Consequently, several studies have used stochastic mathematical models of AI transmission to estimate the dynamics of virus spread under field conditions to better understand and prevent outbreaks. Many authors have used epidemic data from outbreaks to estimate transmission parameters and develop disease spread models (1, 2, 12, 13, 15, 19). Most of these studies model transmission between farms as a single step event (e.g. transmission may be based upon a single probabilistic function of inter-farm distance) and use epidemic data fit to probability distributions of farm infection to explain the observed epidemic patterns. As a result, these models do not assess the potential impacts of specific human movements between farms on the dynamics of AI transmission between commercial flocks. Sharkey *et al.* in 2008, reported the results of a quantitative assessment of the role of human movement in the potential spread of H5N1 HPAI virus using information on the contact structure of the industry in Great Britain. The estimated size of the outbreak was very dependent on the sector of the poultry industry in which it was introduced and the characteristics of the production system. Therefore, extrapolation of these results to commercial poultry systems in other countries is very limited.

The consolidated business structure and geographic concentration of the USA poultry industry presents some unique challenges for outbreak control and emergency management (6). Consequently, it is important to build quantitative risk models based upon local or regional information about the poultry industry. The aim of this study was to estimate the potential number of susceptible farms that may be exposed to a single HPAI-infected farm in Georgia, U.S. by the time of disease detection. The relative impacts of virus virulence, farm density, and time to grower detection of bird illness on the estimated number of exposed farms were stochastically modeled using data obtained from two Georgia counties (25).

Materials and Methods

Two models were used to estimate the potential horizontal spread of disease from an infected broiler poultry farm. The first was a deterministic SEIR model of virus spread between individual birds on an infected farm to determine the likely time interval between initial farm infection and grower detection and reporting of bird illness. The second was a stochastic model of potential off-farm spread of virus associated with human movement from the infected farm to susceptible farms prior to outbreak control measures being initiated.

Deterministic SEIR model of HPAI on index farm

The dynamics of virus spread within a broiler flock following introduction of an HPAI virus was estimated using an SEIR epidemic model of infectious disease spread (10). In this model, individual birds transition through each of the following disease states during the epidemic on the infected farm: S, susceptible birds; E, exposed, infected birds in the latent period of infection; I, infected, infectious birds; and finally R, recovered or dead birds. Since recovery from HPAI infection was considered to be zero and dead birds were assumed to be non-infectious, the fourth state was specified as "dead" birds. This model was designed using the following assumptions: 1) the population in a poultry house on the infected farm was constant and equal to 22,000 birds (25); 2) farm infection was initiated by the introduction of one infected bird at time= T_0; 3) all birds were susceptible to the HPAI virus; 4) detection of bird mortality by the grower is a consequence of the cumulative daily mortality of birds in the first infected house. Thus, the number of poultry houses on a farm has no impact on the day of detection; 4) all birds mixed randomly, regardless of disease status; and 5) bird mortality attributable to other diseases is negligible.

Outbreak simulations were based on a discrete time step of one day (24hrs). The model used an initial latent period of 2 days and an infectious period of 6 days (24). However, since the latent and infectious periods of AI viruses can vary greatly between strains, the estimated day of disease detection was also evaluated for latent periods varying from one to four days and for infectious periods of one to ten days. The transmission parameter, β, was initially set to 33 birds/day (24), and the daily transmission rate was also varied from 1 to 140 birds infected/infectious contact per day.

Once the model estimated the daily number of dead birds, the day of grower detection of the outbreak was estimated using the average mortality threshold reported by broiler growers in Georgia of 0.20% (reported range < 0.01 to 3.33%) (25). However, the reported grower tolerance to daily bird mortality varied, depending on the age of the flock. Surveyed broiler growers reported acceptance of a higher daily mortality in the first week of growout (mean 0.73%; range < 0.01 to 3.33%) and a slightly lower daily mortality in birds older than four weeks (mean 0.16%; range 0.01 to 0.57%). Consequently, the most likely day of grower detection of flock infection was evaluated for each of these age categories (i.e. 0–7 days, 8–28 days, and over 28 days).

Stochastic Model of off-farm spread of HPAI

The stochastic off-farm model used estimates of the daily horizontal contact rate between broiler farms associated with human activities-movement of people, vehicles, equipment-obtained from a survey of broiler poultry growers in Georgia (25). This model estimated exposure to virus-contaminated media at the farm level (i.e. a farm premise was the modeled unit of exposure). It was assumed that the amount of virus on the infected farm would not be sufficient to contaminate the boots, clothing, equipment and vehicles of visitors until the birds infected by day t=1 became infectious (day t=3). Transmission of virus by horizontal farm contact was estimated using 14 visitor activities (Table A.1) included in the broiler grower survey. Each off-farm transmission pathway was based upon the daily likelihood of each visitor contact occurring on the infected farm, an estimated probability that the farm activity was associated with contamination and off-farm transmission of infectious materials, and the daily number of farms contacted by each visitor. Off-farm spread of virus from the infected farm was assumed to occur if (i) a visitor came to the farm; (ii) the visitor contacted infected birds or potentially infective material; and (iii) the visitor went to other farms. Figure A.1 illustrates the model flow schematically. Since the grower survey (25) identified significant differences in the reported horizontal contact frequencies between broiler farms in counties with different poultry farm densities, model outputs were estimated separately for areas with a high poultry density (1.45 farms/5 sq. miles) and a low density (0.49 farms/5 sq. miles).

The daily probability of each visitor coming to the infected farm (p1) was calculated by dividing the estimated flock frequency (Table A.1) by 49, based upon a 49 day broiler growout period. For each modeled day of off-farm transmission, a Bernoulli trial determined if the visitor activity occurred on the infected farm. Contamination and off-farm transmission of infective materials associated with each visitor activity on the infected farm were also determined by the result of a Bernoulli trial using the estimated probability (p2). For different scenarios of visitor activities (for example, equipment may be owned or borrowed/contracted, some visitors may or may not enter the poultry house), a random number between 0 and 1 was generated to determine which scenario would be applied to the visitor based on the relative proportion of each response obtained from surveyed poultry growers. Each farm visitor scenario (Table A.1) was assigned a continuous uniform probability distribution of contact with infectious materials and off-farm transmission based upon incremental categories of risk as defined by an expert panel of poultry scientists, veterinarians and industry representatives: “none,” “very low,” “low,” “moderate,” “high,” and “very high.” The uniform probability distributions assigned to each risk category greater than “none” were of equal size, ranging between 0 and 1.0. The number of susceptible farms subsequently exposed by each contaminated visitor from the infected farm was estimated using a discrete uniform distribution bounded by zero and the maximum number of daily farm visits (estimated by the expert poultry panel) minus one, to exclude the infected farm. For other activities, the number of farms exposed was modeled using either a β-pert or Poisson distribution, as shown in Table A.1. The total daily number of exposed farms was calculated by summing the outcomes for all modeled activities. It was assumed that each horizontal contact exposed a unique farm, thus no susceptible farm could be exposed to the infected farm more than once during a model iteration. Monte Carlo Simulation was done using Crystal Ball® 7.3(using a fixed initial seed value for repeatability) to stochastically model all daily visitor activity outcomes during 10,000 model iterations.

RESULTS

ON-FARM SPREAD

This model used mortality thresholds to determine the most likely day of broiler grower detection of bird illness. Mortality thresholds have been traditionally used as the parameter for HPAI detection (7), and have been shown by other studies to be the most reliable indicator of AI infection in many poultry production systems (8, 16). Using a phenomenological outbreak model, Savill *et al.* (2008)(16) showed that the time to detection of both H5N1 and H7N7 strains in floor-reared birds using only clinical signs of illness is much longer compared to using mortality thresholds. In their model, significant clinical signs of illness were never detected before rising mortality. According to our model the most likely day of illness detection is the 5th day across the range of reported average mortality levels tolerated by commercial poultry growers among birds older than seven days (25). This estimated detection day of t=5 was also fairly robust across varying parameters of virus latent period (range: 1 to 4 days), infectious period (range: 1 to 10 days) and transmission rates (18 to 140 birds per infected contact) (Figure A.2). These observed low levels of variation in within-flock outbreak dynamics are similar to the findings of Savill *et al.* (2008)(16), who found that for transmission rates above 10 infected birds per infectious contact per day, the detection day is robust across a large range of other outbreak parameters. For daily transmission rates between 10 and 20 birds per day, our model estimated that detection will be delayed by one day (estimated grower detection on day 6) for virus infectious periods between 3 and 6 days. However, when daily transmission rates of less than 10 birds/day were examined (3, 21), our model estimated that detection by the growers could be delayed until day 7, and up to day 13. Since HPAI may remain infectious in the poultry environment (26) transmission rates exceeding 20 infected birds/infectious contact per day, as reported by van der Goot and others (18, 23), may be likely in broiler poultry houses containing 22,000 birds.

Off-farm spread

Most broiler growers (84%) responding to the Georgia survey (25) indicated that they would notify their company about flock illness on the same day of detection, but some of them could wait up to 48hs. Consequently, even though detection may occur as early as the 5th day, implementation of farm isolation to contain spread may occur up to 6 to 7 days or more after virus introduction. Moreover, in cases of lower daily transmission rates, the initiation of control measures on the infected farm may be delayed further-from a couple of days to over a week. Table A.2 shows the probability that no other farm has been exposed to virus-contaminated materials each day following introduction of virus on the infected farm. By Day 5 following infection (the most likely day of grower detection in our model), there is a 32–34% probability that no other farms have had a potentially infectious horizontal contact with the infected farm. However, a 48 hour delay in either grower detection or isolation of the infected farm reduces this probability to 16% or less.

Our assumption that transmission of the virus from an infected farm would not occur until the third day following introduction agrees with the reported time of 1.9–3.4 days between virus introduction and spread to other farms reported by Garske *et al.*(2007)(9) for the 2003 H7N7 outbreak in The Netherlands. However, during the H7N1 outbreak in Italy (1999/2000) and the H7N3 outbreak in British Columbia (2004) the time to off-farm spread from an infected farm was 5.0 and 8.4 on average, respectively (9). The observed time to off-farm spread of infection during an outbreak is a function of several diverse factors, including virus virulence, the number of infected birds on the infected farm, and the horizontal contact structure of the poultry industry in the area. While we assumed that off-farm spread of infectious materials was possible on day 3 following virus introduction to a farm, the model estimated nearly a 50% probability that off-farm transmission during an outbreak will not be observed until day 4. Consequently, the range of potential transmission outcomes estimated by this model—which relied on the reported horizontal contact structure of Georgia poultry farms—is feasible.

Figure A.3 shows the estimated daily cumulative number of potentially exposed farms assuming that no movement restrictions are in place on the infected farm. Since the infected farm was not considered infectious until day 3 following introduction of a single, infected bird, the estimated number of farms exposed to virus-contaminated materials begins to increase exponentially on this day, and slightly more steeply in the model of high poultry density. Although significant differences in the frequency of specific visitors to poultry farms in an area of high poultry density and low poultry density were included in this model, the cumulative number of farms exposed is similar in both modeled regions. Several studies of infection transmission between poultry farms have found that the probability of farm infection is inversely correlated with the distance between an infected farm and a susceptible one (2, 14, 15). Consequently, we hypothesized that the reported differences in the horizontal contact structure of the poultry industry in Georgia might play a role in the increased risk of disease spread between farms in close proximity. However, for most of the visitor activities included in this model, the reported daily frequencies and the range of farms visited in a day were too similar to have a significant impact on the estimated number of farms potentially exposed to virus (25). The distance between farms is likely an important factor in the probability of virus presence and viability in contaminated media as well as the risk posed by an unauthorized visitor (i.e. unauthorized visits between poultry farms may be more likely among close neighbors), and these factors were not included in this model.

Figure A.4 shows the proportion of farm exposures associated with each modeled visitor activity. Most off-farm exposures were associated with feed trucks and human visitor activities associated with in-house contact with birds, such as company service personnel and hired help. Unauthorized visitors were reportedly more likely to visit farms in the high poultry density county so this activity comprises a slightly higher proportion of the total farm exposures in this model. Although the flock frequency and risk associated with hired farm workers was the same in both the high density and low density models, this activity was associated with a higher proportion of the total risk of virus spread in the low density model. There were fewer total farm exposures in the low poultry density model and other activities, such as company servicemen and unauthorized visitors, were also less likely in this model. Service personnel traveled to fewer farms in a day and unauthorized visitors were more likely to visit the infected farm in the high density model. The dominant estimated transmission pathways in this study are consistent with published reports of avian influenza outbreaks. In the 2003 H7N7 outbreak in the Netherlands, increased risk of virus introduction was associated with the higher number of company contacts between layer finisher farms (20); and spread by personnel and equipment was implicated in the transmission of low pathogenic avian influenza viruses in Pennsylvania during 1983–1984 (4). Likewise. visitor entry in the poultry shed was responsible for a four-fold increase in the odds of farm infection in the H5N1 outbreak in Hong Kong in 2002 (11). Our findings are also similar to a phenomenological model developed by Sharkey *et al.*(2008) (17) reflecting the industry structure in Great Britain. The Sharkey *et al.*. transmission model was also robust across a large range of input parameters and company personnel were responsible for the spread in approximately 30% of the simulations.

The actual number of infected farms resulting from exposure to a single infected farm would be lower than the estimates shown here. The decreased transmission risks resulting from on-farm biosecurity and virus decay during transport were not included in this model. Since quantitative estimates of the effectiveness of specific biosecurity measures and the level of uniform application of these in the industry is unknown, the expert panel of poultry experts provided categories of risk for off-farm transmission based upon the likelihood of contamination and the assumption that biosecurity is not uniform nor completely preventive as applied in the field. Nonetheless, by using reported grower detection thresholds of disease to estimate the likely duration of unrestricted visitor movement to and from an infected farm, as well as data characterizing the horizontal visitor contact rates among broiler poultry growers in Georgia, we were able to model the likely pathways of virus spread under realistic scenarios. The observed results highlight the need for early reporting of flock illness and the importance of on-farm biosecurity focused on high risk activities.

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Table A.1: Parameter estimation for fourteen potential visitor activities included in estimate of off-farm transmission of HPAI virus from an infected broiler farm

	Activity (Flock Freq.)	Prob. Cont† (min,max)	Proportion‡	Max. number of farms Visited/Day [Distribution of farms exposed]
1	Clean Out Services(0.33)			
	Owned Equipment	None	HDC* 0.50 LDC* 0.90	1
	Borrowed Equipment	Low (0.2, 0.4)	HDC 0.10 LDC 0.00	2 [β-Pert(0,0,1)]
	Contracted Services	Mod. (0.4, 0.6)	HDC 0.40 LDC 0.10	2 [β-Pert(0,0,1)]
2	Decaking, Housekeeping (0.25)			
	Owned Equipment	None	HDC 0.90 LDC 0.10	1
	Borrowed Equipment/Contracted	Low (0.2, 0.4)	HDC 0.95 LDC 0.05	2 [β-Pert(0,0,1)]
3	Off-farm Litter Disposal (0.25**)	Very High (0.8, 1.0)	1.00	2 [β-Pert(0,0,1)]
4	Live Haul (1.0)	Very High (0.8, 1.0)	1.00	HDC 4 [Uniform(0, 3)] LDC 2 [Uniform(0, 1)]
5	Shaving Suppliers (0.33)	Mod. (0.4, 0.6)	1.00	2 [Uniform(0, 1)]
6	Repairmen (0.25)			3 [Uniform(0, 2)]
	Growout/Inside	High (0.6, 0.8)	HDC 0.75 LDC 0.80	
	Growout/Outside	Mod. (0.4, 0.6)	HDC 0.15 LDC 0.10	
	Downtime	Low (0.2, 0.4)	HDC 0.10 LDC 0.10	
7	Gas Delivery			HDC 5 [Uniform(0, 4)] LDC 3 [Uniform(0, 2)]
	Winter (3.0)			
	Downtime	Low (0.2,0.4)	0.17	
	Growout	Mod. (0.4,0.6)	0.34	
	Summer (0.33 HDC, 0.55 LDC)			
	Downtime	Low (0.2, 0.4)	0.16	
	Growout	Mod. (0.4, 0.6)	0.33	
8	Feed Trucks (15.0)	Mod. (0.4, 0.6)	1.00	5 [Uniform(0, 4)]
9	Chick Bus (1.0)	Mod. (0.4, 0.6)	1.00	5 [Uniform(0, 4)]
10	Company Service Persons/ Veterinary (7.0)	Very High (0.8, 1.0)	1.00	HDC 5 [Uniform(0, 4)] LDC 4 5 [Uniform(0, 3)]
11	Utilities, Meter readers, Pit inspectors (1.0)			HDC 15 [Uniform(0, 14)] LDC 10 [Uniform(0, 9)]
	Downtime	Low (0.2, 0.4)	0.25	
	Growout	Mod. (0.4, 0.6)	0.75	
12	Non-company or Unauthorized Visitors (4.0 HDC, 2.0 LDC*)			Poisson λ=1
	Inside	Very High (0.8, 1.0)	HDC 0.25 LDC 0.50	
	Outside	Low (0.2, 0.4)	HDC 0.75 LDC 0.50	
13	Vaccination Crews (0.5)***	High (0.6, 0.8)	1.0	5 [Uniform(0, 4)]
14	Grower, Hired Help (14.0)	Mod. (0.4, 0.6)	1.0	Poisson λ=1

†Probability that contact will result in contamination: None, Low, Mod (Moderate), High, Very High.

‡Proportion is the proportional weight of each possible scenario considered for each activity.
A random number between 0 and 1 was generated to select from one of the scenarios.

* HDC=High Density County, LDC=Low Density County

**Frequency of total house clean out times the reported frequency of growers disposing litter off-farm.

**It was estimated that only half of poultry farms routinely vaccinate birds.

Table A.2: Probability that HPAI infection is not transmitted from the index farm in the days following initial flock infection (Day 0)

	Probability of a HPAI infection being restricted to the index farm										
Farm density	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day8	Day 9	Day 10
Low	100%	100%	100%	69.8%	48.5%	33.9%	23.3%	16.0%	10.9%	7.9%	5.6%
High	100%	100%	100%	66.3%	44.6%	31.6%	21.9%	15.1%	10.8	7.3%	5.3%

It was assumed that environmental contamination on the infected farm was not sufficient for off-farm spread until Day 3

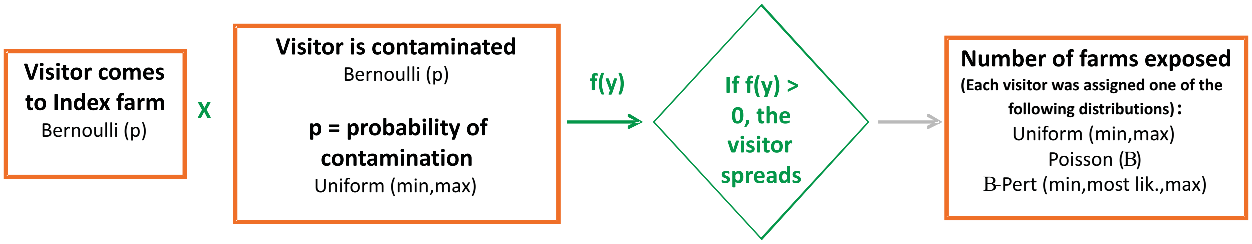

Figure A.1: Process flow of the stochastic model of the off-farm spread of highly pathogenic avian influenza

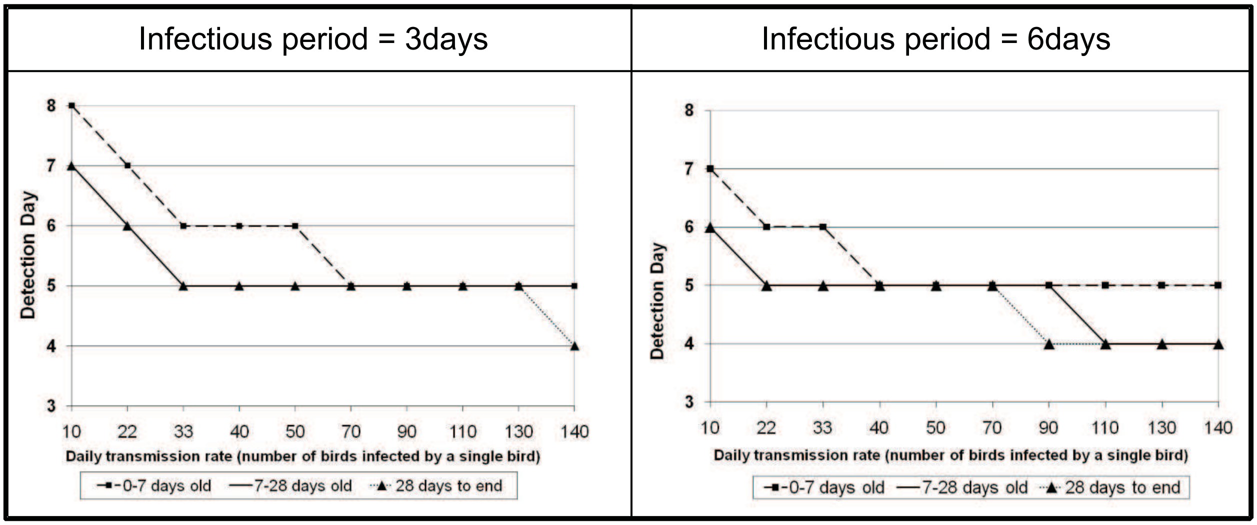

Figure A.2: The estimated number of days until grower detection of an HPAI-infected flock under varying conditions of bird age, virus transmission rates, and virus virulence (latent period of 2 days, infectious periods of 3 and 6 days).

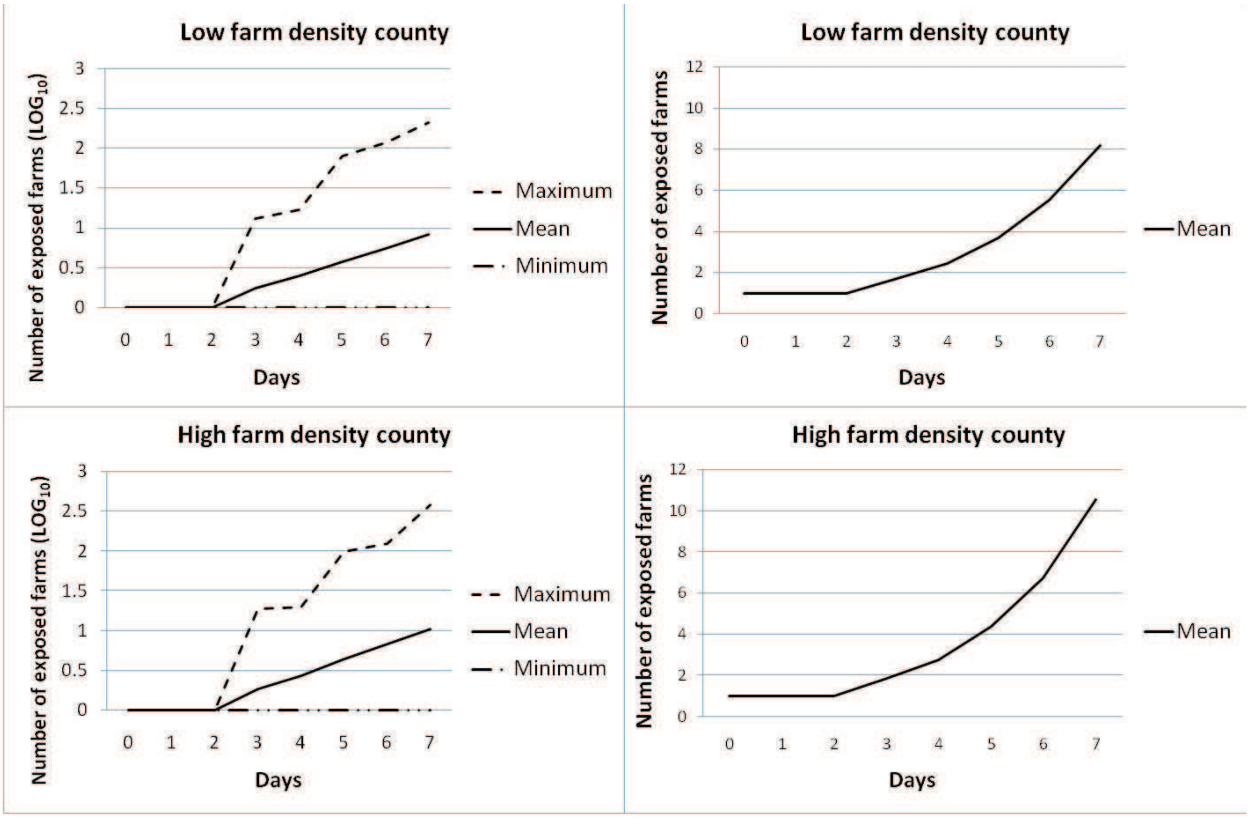

Figure A.3: . Number of farms potentially exposed to contamination from an infected farm through human movement in a 24hr interval in a high poultry farm density region (1.45 farms/5 mi^2) and one with a low poultry farm density (0.49 farms/5 mi^2). Calculations assume that off-farm spread of virus from an infected farm is not possible until Day 3

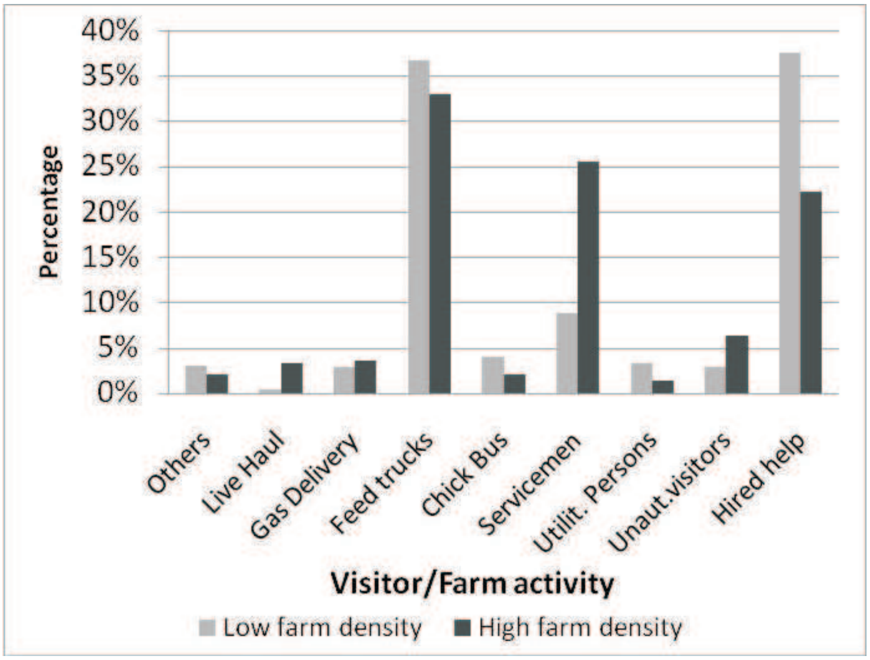

Figure A.4: Proportional contribution of each visitor to the overall spread of HPAI virus from one infected farm in one day. “Others” included all visitors with contribution equal or less than 1%, which are: clean out services, decaking services, litter disposal, shaving suppliers and vaccination crews