Epidemiologic and Economic Analyses on Highly Pathogenic Avian Influenza H5N1 in Nigeria and Egypt

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Epidemiologic and Economic Analyses on Highly Pathogenic Avian Influenza H5N1 in Nigeria and Egypt

Epidemiologische en economische analyses op hoogpathogene aviaire influenza H5N1 in Nigeria en Egypte

(met een samenvatting in het Nederlands)

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Folorunso Oludayo Fasina

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Contents

Chapter 1

General Introduction

Background on Avian Influenza virus and the current outbreaks in Africa

Avian influenza viruses are Type A Influenza viruses that belong to the *Orthomyxoviridae* family (Acha and Szyfres, 2003). Other members of the same family include Influenza B and C types. These viruses are single-stranded, negative sense RNA viruses that allow minor changes in their surface. Thus, viral antigens can re-align, resulting in variations (virus strains) that influence pathogenicity (Cunha, 2004; Hampson and Mackenzie, 2006). While mutation and sequence divergence are possible in all three types of influenza viruses, influenza B evolves more slowly than type A but more rapidly than C type viruses (Yamashita et al., 1988).

While wild aquatic birds are the natural hosts for influenza A viruses, such viruses have also been transmitted to other species (Klenk, Matrosovich and Stech, 2008). Influenza A viruses have caused several devastating outbreaks in poultry and —after adapting to humans— also induced human pandemics (Klenk, Matrosovich and Stech, 2008). In addition, pigs, tigers, leopards, domestic cats, viverrids, stone martens, dogs, and racoon dogs have all been infected in the past by Avian Influenza viruses, particularly by H5N1 viruses (Qi et al., 2009). Previous notable pandemic and epidemic outbreaks of influenza A viruses have involved: (i) H1N1 (Spanish Flu, 1918, and Swine Flu, 2009); (ii) H2N2 (Asian Flu, 1957); (iii) H3N2 (Hong Kong Flu, 1968); (iv) H5N1 (Bird Flu, which started in 2004); (v) H7N7 (an unusual zoonotic reported, in The Netherlands and elsewhere); (vi) H1N2 (endemic/enzootic in humans, pigs and birds), as well as H9N2, H7N2, H7N3, H10N7 and the H7N9 types (Hay et al., 2001; Fouchier et al., 2004; Klenk, Matrosovich and Stech, 2008).

The less common type B viruses cause, in humans, acute viral disease of the upper respiratory tract. Characteristic symptoms include acute fever, chills, headache, myalgia, weakness, runny nose, sore throat, and cough. However, this virus has also been reported in horses, seals, and ferrets (Jakeman et al., 1994; Osterhaus et al., 2000; Ohishi et al., 2002). Compared to the type A viruses, type B viruses mutate at a slower rate and are less antigenically diverse. In addition, type B viruses do not cause pandemics (Webster et al., 1992; Zambon, 1999; Nobusawa and Sato, 2006).

Type C viruses have been reported to infect humans, dogs and pigs, causing both severe illness and local epidemics (Matsuzaki et al., 2002; Taubenberger and Morens, 2008). However, influenza C viruses are less common than the other types and usually only cause mild diseases in children (Matsuzaki et al., 2006; Katagiri, Ohizumi and Homma, 1983).

Between December 2003 and January 2004, outbreaks of highly pathogenic avian influenza (A) subtype H5N1 (HPAI H5N1) were reported in many Asian countries. The Guangdong virus (A/Goose/Guangdong/1/96 (Gs/Gd/1/96) was the precursor of the viruses that caused these outbreaks. Since the year 2000, many reassortants of the H5N1 have been reported (Sims et al., 2005; Duan et al., 2007). Between 2003 and 30 May 2015, a total of more than 7,560 outbreaks of HPAI H5N1 involving domestic and wild bird from 63 countries have been reported to the World Organisation of Animal Health (OIE, 2015).

Specifically, in Africa, the following countries have been infected: Nigeria, Egypt, Sudan, Ghana, Benin, Togo, Cote d'Ivoire, Burkina Faso, Niger, Libya, Djibouti and Cameroun (OIE, 2015). Since the outbreaks of 2006-2008 in Nigeria, the country has had a period of quiescence with regards to new infections. However, since the beginning of the year 2015 (January 2), reported cases have occurred on 401 farms and live bird markets in 76 local government areas in 18 states in Nigeria and neighbouring West African countries of Niger, Ghana, Burkina Faso and Cote d'Ivoire have been re-infected as well. A rapid molecular assessment of the new virus identified it as belonging to Clade 2.3.2.1c (Monne et al., 2015). This new virus is closely related to and clustered with the influenza viruses that circulated and is still circulating in China and Vietnam. Specifically, the haemagglutinin of the virus clusters with influenza viruses collected in China in 2013 and with an H5N1 virus (A/Alberta/01/2014) isolated from a Canadian returning from China (Monne et al., 2015). It was hypothesised that perhaps the new infection has an association with migratory bird movement but more evidence is needed to confirm this hypothesis. Because Nigerian conduct international trade (legal and illegal) with other countries in Africa and elsewhere, the alternative hypothesis may be that the source of the incursion of this recent virus into Nigeria may have been related to international trade while the spread was facilitated by informal poultry trade in Nigeria. Since the time of these outbreaks, strict quarantine, culling and compensation policies have been instituted by the Nigerian government.

Risk factors associated with outbreaks in poultry farms

Several risk factors have been investigated in association with the HPAI H5N1 outbreaks. They include: (i) animal (chicken density, domestic waterfowl density), (ii) biology- and management-related (population density, farm management protocols, adherence/non-adherence to biosecurity), (iii) geographic and environmental (proportion of land covered, agriculture, surface water, cropping intensity, elevation), (iv) socio-economic and trade-related (wild bird trade), and (v) demographic (population density and movement) factors (Martin et al., 2011; Gilbert and Pfeiffer, 2012).

Specific factors associated with HPAI H5N1 outbreaks include: (i) remnants of slaughtered chickens used to feed other chickens (Biswas et al., 2009), (ii) slaughtering of birds in live bird markets (Indriani et al., 2010), (iii) slaughterhouses located within the district of chicken houses (Tiensin et al., 2009), (iv) purchase of live poultry from another farm (Paul et al., 2011), (v) high poultry flock density (Henning et al., 2008), (vi) high native chicken density (Gilbert et al., 2006; Tiensin et al., 2009, Paul et al., 2010), (vii) free-grazing chicken and duck densities (Hogerwerf et al., 2010; Gilbert et al., 2010), (viii) cock densities (Gilbert et al., 2006; Tiensin et al., 2009, Paul et al., 2010), (ix) quail flock in district of chicken farms (Tiensin et al., 2009), (xi) high human population density (Gilbert et al., 2006, 2008; Loth et al., 2010; Yupiana et al., 2010), (xii) presence of high road density and road length (Ward et al., 2008; Rivas et al., 2010; Loth et al., 2010; Yupiana et al., 2010) amongst others. Six countries (Bangladesh, Egypt, Indonesia, Peoples Republic of China, Eastern part of India and Viet Nam) have been declared as endemically infected countries (FAO, 2011; FAO, 2013).

Prevention and Control of Influenza viruses

In an effort to control and eradicate HPAI H5N1, a number of intervention strategies have been implemented in affected countries. In addition, the FAO/OIE has jointly issued guidelines for control and eradication of avian influenza, including: (i) capacity building in human and animal health services, (ii) master coordination plans for effective surveillance, (iii) diagnosis and control of the disease, (iv) sustainable funding on the part of the government to support surveillance and diagnostic activities, (v) adherence to international standards and surveillance for international trade, (vi) joint activities in national/regional/international co-ordination and co-operation, (vii) confirmed and tested strategies for surveillance and control of avian influenza, (viii) continuous awareness of the implications of HPAI H5N1 virus for human health, (ix) setting of research priorities for Avian Influenza, and (x) careful consideration of the economics and policy issues related to avian influenza (FAO/OIE, 2005).

Biosecurity is the implementation of measures that reduce the risk of introduction into and spread of pathogens out of a farm. The fundamental principles of biosecurity include: segregation, cleaning, and disinfection (FAO, 2008). Its implementation depends on farm type, farm size, and management practices. To demonstrate biosecurity has been implemented, all bio-exclusion (keeping disease out) and bio-containment (preventing a resident infection from spreading out of the farm) aspects should be implemented (Swayne and Akey, 2005; FAO, 2008). Aimed at controlling and eradicating infection where present, different levels of biosecurity have been recommended for the different sectors of poultry in developing countries

(FAO, 2008). With regards to HPAI H5N1, disease-specific biosecurity includes: (i) cleaning and disinfecting poultry pens and surrounding areas, (ii) keeping out wild birds and scavengers, and (iii) reducing environmental contaminations, as well as visitors (Cardona, 2008; Swayne and Akey, 2005; FAO, 2008). Biosecurity, including movement controls, natural and artificial boundaries, good farming practices, and compartmentalization have assisted in reducing the burden of infections in many countries (FAO, 2008; Conan et al., 2012; OIE, 2014a, 2014b).

Furthermore, good surveillance systems backed up by early diagnosis, as well as effective laboratory-based diagnosis is critical to reduce the risk of spread of infection to naïve flocks and new territories. It is known that a good surveillance approach (active and passive) is necessary to aid containment of infection. Such surveillance will be influenced by variables such as management practices, poultry species at risk, different biosecurity levels and production systems, and the frequency of contacts between domestic poultry and wild birds (OIE, 2007).

Vaccination has been identified as an important tool for the control of HPAI H5N1, because it can reduce susceptibility to and infectivity upon infection and decrease the effect of infection on disease and production. Its implementation has reduced the risk of infection in some countries (Ellis et al., 2004; Capua and Marangon, 2006; Fasina et al., 2007; Kim et al., 2010). However, some limitations of vaccination have been reported in parts of Africa (Kim et al., 2010). Moreover, a high quality surveillance programme and good biosecurity measures are essential parts of an adequate vaccination programme.

General aim and scope of the thesis

The goal of the research described in this thesis was to evaluate the situation of avian influenza A H5N1 in two of the African countries most affected by this virus. To that end, two aims were pursued: (i) to identify the factors that played important roles in the dissemination and circulation of the virus, and (ii) to explore the potentials of the virus from becoming endemic in the poultry production system in Africa.

In **Chapter 2**, the extent of H5N1outbreaks in Nigeria is described. Outbreaks were reported in 2006 and 2007. In July 2008, there was a report of a distinct outbreak (Fusaro et al., 2009). A combination of active and passive surveillance was used to collect samples from infected, at-risk and non-infected farm premises; in addition, live bird markets, infected states and non-infected states were also investigated. The samples (tracheal and cloacal swabs, parenchymatous tissues and sera) were analysed using virus

isolation, reverse-transcription polymerase chain reaction and serology as appropriate.

Chapter 3 was based on the primary data on influenza A H5N1 in human as recorded by the Egyptian government in reports to the WHO. The timeline of confirmed cases and deaths reported between 20 March 2006 and 31 August 2009 were statistically analysed using variables including date of exposure, date of onset, course of symptoms, and time from hospitalisation until death/recovery, gender, age and patterns of deaths.

While the second Chapter investigated the outbreaks through the evaluation of passive and active surveillance of HPAI H5N1 in Nigeria, **Chapter 4** was based on the investigation and identification of risk factors for HPAI H5N1 virus infection in poultry farms in Nigeria during 2006-2007 based on available data. Risk factors were analysed statistically using the conditional logistic regression models, and factors that supported infections in new premises included: receiving visitors on farm premises, purchase of live poultry/products, farm workers that live outside the premises.

Because of the fact that biosecurity remains the most important means of reducing risks of infection in poultry farms, the effects and costs associated with the implementation or neglect of biosecurity, as well as the feasibility of implementation in the household poultry in Egypt were explored (**Chapter 5**). Risks of particular importance for the household poultry were categorized into people-related risks, environmental-related risks and other birds/animal-related risks. Biosecurity measures were applicable in the household poultry and it is 8.45 times better to implement biosecurity than doing nothing against HPAI H5N1.) The financial risk analysis was robust and withstood sensitivity analysis because changes that may occur in the course of the annual household poultry project cycle can be accommodated profitably with biosecurity implementation.

To determine whether epidemics, such as HPAI H5N1, exhibit properties of network theory, wherein rapid microbial dissemination will require both susceptible hosts and the environment as known in the host-pathogen-environment triad, work was conducted to investigate the effect of road network (a geo-spatial factor) on the HPAI H5N1 epidemics in Nigeria in **Chapter 6**. Using geo-temporal data collected from epidemics in which all hosts were susceptible to HPAI H5N1 in Nigeria in 2006, 'connectivity', 'nodes' and 'contacts' were investigated and compared to a FMD outbreak. Spatial aggregation of cases (disease clusters), links among similar 'nodes' (assortativity), simultaneous activation of similar nodes (synchronicity), epidemic flows moving from highly to poorly connected nodes (directionality), and a few nodes accounting for most cases (a "20:80" pattern) were

observed. In this spatio-temporal model, synchronicity and directionality properties explained where and when an HPAI H5N1 epidemic spreads.

Finally, in **Chapter 7**, an exploratory analysis was conducted on the probability of human infection through the oral contacts with meat contaminated with the virus [avian influenza A (H5N1)]. Detailed quantitative risk assessment was conducted using predictive microbiology-process risk model. According to the outcomes of the model, up to 15,159 humans may have contracted HPAI H5N1 in Africa, resulting in approximately 1,964 deaths. These figures are much higher than those officially reported. Besides limitations of the model bias associated with official epidemiological statistics which are often prone to underreporting, lack of awareness especially in the rural communities and censoring effects may account for this discrepancy.

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Chapter 2

Serologic and Virologic Surveillance of Avian Influenza in Nigeria, 2006-2007

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Serologic and Virologic Surveillance of Avian Influenza in Nigeria, 2006-2007

Abstract

Since January 2006, H5N1 avian influenza has affected Nigeria's poultry population causing enormous loss of resources. The current circulating virus is a potential candidate for pandemic influenza which may severely affect the human and animal population worldwide especially in the resource poor countries. In this study, we report our field and laboratory surveillance efforts in Nigeria. A total of 1,821 tissue samples, 8,638 tracheal swabs, 7,976 cloacal swabs and 7,328 avian sera were analysed over a period of two years, with 312 positive results. Limited human serosurveillance involving 320 individuals was also carried out but yielded no positive result. We recovered 299 isolates of highly pathogenic avian influenza virus H5N1 mainly from the poultry diagnostic samples arising from birds kept in backyard, small scale and free range farms. This finding emphasised the role played by these farming systems in the dissemination of avian influenza in Nigeria and calls for a continued surveillance in humans since human-animal interaction is a key feature in Africa. Furthermore, there is a need for the strengthening of border controls. Since October 2007, there has been no reported and confirmed outbreak of avian influenza in Nigeria.

Introduction

In late 1996, a farm in Guangdong, China was affected by infections with highly pathogenic avian influenza (HPAI) virus H5N1 [1,2]. Since the time of these reports, several countries in Asia (n=17), Europe (n=27), the Middle East (n=7) and Africa (n=11) have reported infection or re-infection of poultry flocks and/or wild and migratory birds [3].
In parts of the continents that reported infection, with the exception of Europe, it has been documented that the virus is becoming entrenched in the poultry populations and many clades and sub-clades are emerging [4]. Several human infections (n=372) and fatalities (n=235) have similarly been confirmed [5], and most of these human infections have been linked to exposure to domestic poultry [6].

The expanding geography (infection of new locations), biology (acquiring of new biological properties) and ecology (adaptation to new host range) of H5 influenza viruses necessitated that every country should actively search for H5 avian influenza viruses within its territories. Nigeria, a country with an estimated human population of over 140 million, first reported infection in poultry in January 2006 [7], and in humans in January 2007 [5], and since that time, efforts to carry out active surveillance for the influenza viruses have been intensified by the national authority. Poultry production is a key economic activity in Nigeria. It contributes significantly to the family income, especially in peri-urban and poor rural communities [8]. The effect of growing urbanisation the rural, peri-urban and urban poultry production and on human-animal interaction has previously been reported [9]. Backyard poultry production thrives in view of the level of poverty and the economic return associated with the venture. Free-range systems of poultry production are also widespread in various parts of the country [10].

Due to H5N1 avian influenza infection in Nigeria, millions of poultry have been destroyed and one human death has occurred. A recent serological survey in humans in those administrative regions in Nigeria that were most heavily affected by HPAI H5N1, showed that despite the widespread infection in the poultry population, human infection is rare [11]. In this report, we describe our surveillance efforts in Nigeria and discuss the role of poultry and backyard flocks and their implications for humans vis-à-vis our laboratory findings.

Materials and methods

Poultry surveillance on farms and live bird markets
System 1 (October to December 2007). Based on available records, a biased random sampling procedure was adopted that included locations around previously infected farm premises and live bird markets as well as locations with suspected outbreaks and dense poultry populations. Each state of

Nigeria was visited three times at intervals of two weeks, and samples were taken at two new locations during every visit. At each location, cloacal, tracheal and serum samples were taken from 29 birds, and six moribund, clinically ill or dead birds were purchased. All samples were transported in appropriate media and the cold chain was maintained throughout the activities.

System 2 (May to July 2008). The national active surveillance covered all 36 Nigerian states and the Federal Capital Territory (FCT), irrespective of whether or not HPAI H5N1 infections had been reported from the area, but was carried out in two parts: Part A of the targeted live bird market surveillance covered only the states with infections (25 states and FCT), while part B covered the 11 states without infections.

System 3 (February, 2006 till December, 2007). While these activities were going on, additional routine diagnostic samples (mostly tissue samples) were submitted to the National Veterinary Research Institute (NVRI) or collected in the field by the NVRI staff. The active surveillance programme, which started in October 2007, is still ongoing.

National surveillance programmes and team

In response to the outbreak of H5N1 influenza in the poultry population in 2006, the Nigerian government set up an inter-ministerial committee comprising health (Federal Ministry of Health), veterinary/agricultural (Federal Ministry of Agriculture and Rural Development) and information personnel (Federal Ministry of Information) to tackle the growing problem. Several surveillance efforts were jointly carried out at various times by the national teams in collaboration with representatives from the Food and Agricultural Organisation of the United Nations (FAO), the United States Centers for Disease Control and Prevention (US CDC), the World Organisation for Animal Health (OIE), the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE) and others. Teams were regularly dispatched to suspected farms nationwide to collect samples and identify infected birds, advise on compensations and carry out cullings.

Sample collection, virus isolation and serology from avian species

Following sample collection, *post-mortem* examinations were conducted on moribund, dead or freshly killed birds, and tracheas, lungs, livers, spleens, brains, hearts, intestines as well as intestinal contents were collected in sterile containers.

Virus isolation was done in 9 to 11-day-old embryonated chicken eggs according to standard protocols [12]. The eggs were candled daily to determine viability and dead eggs were removed and kept at +4^{o}C. All eggs were opened aseptically and the allantoic fluids (ALF) were spot-tested by haemagglutination test. The chorio-allantoic membranes (CAM) of positive eggs were tested by agar-gel immunodiffusion (AGID) to detect influenza A virus group antigen.

Haemagglutination-inhibition (HI) test was conducted to determine the virus subtype. All negative ALF were further passaged in a second set of embryonated chicken eggs. Any samples negative after the second passage were declared negative. As of May 2008, no isolates of influenza A virus have been obtained from the second passage**.
Serological assays including AGID test using the H5 antigen and HI test using standardized H5, H7 and H9 panels of antigens (OIE reference laboratory for Newcastle disease virus and avian influenza, Padova) were conducted on all sera submitted to the laboratory.

Molecular analysis
Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) were carried out. A cascade-type analysis was performed starting with the gene for the viral matrix protein (M). Every positive result was subjected to an RT-PCR for the haemagglutinin gene (HA) of subtype H5 and an additional RT-PCR for the N1 gene was done for all HA-positive cases. The following oligonucleotide primers were used: M forward: 5′-AGA TGA GTC TTC TAA CCG AGG TCG-3′; M reverse: 5′-TGC AAA AAC ATC TTC AAG TCT CTG-3′; H5 forward: 5′-CCT CCA GAR TAT GCM TAY AAA ATT GTC-3′; H5 reverse: 5′-TAC CAA CCG TCT ACC ATK CCY-3′.
Conventional RT-PCR has been shown to detect titre as low as 3 EID_{50} (Fifty percent egg infectious dose). In addition, our results were confirmed by the OIE reference laboratory for avian influenza and Newcastle disease, Padova, Italy.

Human sero-epidemiological surveillance
Several locations (poultry farms, live bird markets) with suspected or confirmed HPAI H5N1 infections were visited [21 March to 3 April 2007] following the compilation of a list of affected areas by the Federal Ministry of Agriculture and Rural Development in Nigeria. Specifically, a total of 295 poultry workers (76% farm workers, 15% market workers, 5% poultry cullers and 4% veterinarians), from 83 farms and four live bird markets in Kano state, and 25 laboratory workers were included in the surveillance.
Surveillance in humans was carried out between 21 March and 3 April 2006 [11]. Human sera were collected with the informed consent of participating individuals. In addition, serum samples were collected from people potentially exposed to the HPAI H5N1 virus including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples were transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera were prepared in the Human Virology Laboratory, Abuja, split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera were tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay.

Details of the tests have been reported comprehensively in another paper [11]*.

Results

Poultry sero-surveillance

In the period between 2006 and 2007, farms located in 25 Nigerian states and the FCT reported poultry infections with HPAI H5N1 virus. The geographical distribution of the positive cases is shown in Figure 1.

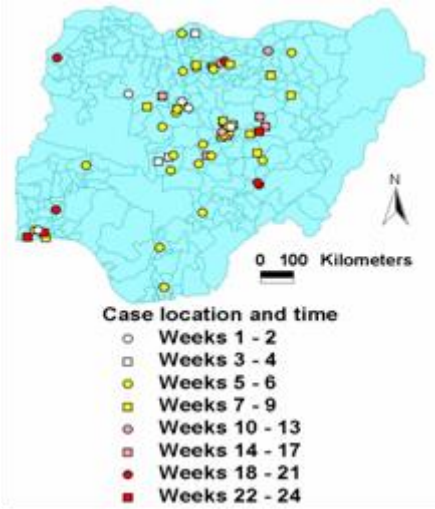

Figure 1. Temporal and geographical distribution of HPAI H5N1 poultry cases, Nigeria January-June 2006 (n=113)
Different colours indicate temporal variation in infection of farm premises. The surveillance in birds was carried out in the whole country with particular attention paid to the infected locations and live bird markets around them. Further 186 cases occurred between June and December, 2007, bringing it to a total of 299 cases, but the overall geographical distribution did not change, with additional infections happening only in already affected locations.

Details of the results are shown in the table below. During the two-year study period from January 2006 to December 2007, a total of 1,205 suspect routine diagnostic samples, 8,638 cloacal swabs, 7,976 tracheal swabs, 7,328 sera and 616 carcasses were received either from the field staff or directly from the farmers.

The samples submitted as part of routine surveillance (system 3) yielded 300 positive results (Table 1).

Table 1. Avian diagnostic samples tested in Nigeria between 2006 and 2007

	Suspected total nu	Positive sample
Diagnostic samples (tissues/swabs) tested in 2006	619	145
Diagnostic samples (tissues/swabs) tested in 2007	586	154 + 1*
Total	1,205	299 + 1*

Note: 52 isolates have been fully sequenced and are published [13]. A large majority (98%) of the isolates originated from farms. 1* represents a sample from Benin republic diagnosed in Nigeria.

Table 2 shows the results of the national surveillance using stratified sampling procedure covering farms and live bird markets in the 36 Nigeria states and FCT (system 1). To date, all of these 10,961 samples have been negative. For the targeted live bird market surveillance (system 2), results are available for part A covering only the 25 infected states and the FCT (Table 3). A total of 13,597 samples were analysed, of which 12 were found to be positive. The targeted live bird market surveillance for the 11 states without report of avian influenza infection (part B) is on-going. They have since been declared negative.

Table 2. National active surveillance covering the 36 states and the Federal Capital Territory, Nigeria, October 2007 to July 2008 (n=10,961 samples)

Samples Collected	Number analysed	Number positive
Tracheal swabs	4,253	0
Cloacal swabs	3,608	0
Sera	3,100	0

Table 3. Targeted live bird market surveillance covering 25 states and the Federal Capital Territory, Nigeria, October-November 2007 (n=13,597 samples)

Samples Collected	Number analysed	Number positive
Tracheal swabs	4,385	3
Cloacal swabs	4,368	0
Sera	4,228	6
Carcasses and moribund	616	3

In the period from January 2006 to December 2007, 299 isolates of HPAI H5N1 have been obtained and characterised. The haemagglutinin genes of 52 isolates have been sequenced and deposited in the GenBank and EMBL databases [13]. All of the positive isolates that were characterised belonged to clade 2.2. Efforts to genetically characterise more of the remaining isolates are currently underway.

Tables 1-3 reveal a certain pattern in that H5N1 influenza virus isolates were obtained mainly from routinely submitted diagnostic samples and live bird markets. Following infection of farms, farmers promptly report outbreaks to the NVRI or other appropriate government agencies since this will ensure payment of compensation. It is also very likely that viruses that escape detection at the farm level will get to the live bird market and can be detected there. These two locations (farms and live bird markets) are important in the epidemiology of avian influenza viruses in Africa. However, we are aware that the level of education may affect reporting in certain circumstances and our systems may have inadvertently missed some outbreaks situations.
Figure 2 gives an overview on HPAI H5N1 outbreaks in the years 2006 and 2007 as determined by the routine diagnostic poultry surveillance.

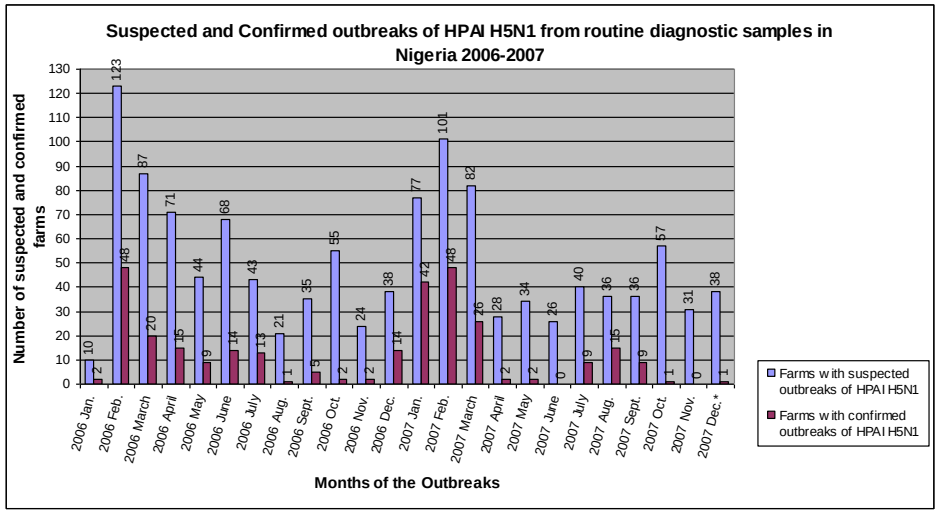

Figure 2. Suspected and confirmed outbreaks of HPAI H5N1 from routine diagnostic samples of avian species in Nigeria, 2006-2007 (n=1,205 reports and 299 confirmations)

2007 Dec: The only isolate in that month originated from Benin Republic.*

The overall rate of confirmed outbreaks was 24.8%. The peaks of infection around January and February in both years may be linked to poultry movement which is usually on the increase around festive periods (December/January). The peaks in the June/July 2006 and July/August 2007 period similarly represent the times when seasonal guineafowl eggs are available. The same period is accompanied by sale of commercial poultry due to a surplus in the egg market caused by the cheaper guineafowl eggs.

Human sero-surveillance
As previously reported, none of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test despite the degree of possible exposure to H5N1 influenza virus[11]*.

Discussion

Before the first occurrence of avian influenza in Nigeria, active surveillance on wild fowl and migrating birds was conducted between September and November 2005 (results not shown) at the Nguru-Hadejia wetlands covering an area of about 4,125 km^2. Similar surveillance was done in the same period in the high risk agroecological/farming areas and live poultry markets, but failed to detect H5 or H7 avian influenza virus.

However, after the first avian influenza outbreak in Nigeria in January 2006, surveillance efforts in the period between January, 2006 and December, 2007 yielded a total of 299 Nigerian isolates of HPAI H5N1. Mutations at antigenic sites were identified in the haemagglutinin genes of the viruses, the significance of which need to be confirmed by further analyses. The implications of these mutations for human and animal health is yet unknown [13]. Although the H5N1 virus has not yet adapted to effectively infect human, there remains a potential pandemic threat in view of continuous infections on farms in the West African sub-region. Furthermore, there is a need to carry out routine surveillance for other influenza viruses in human and animals as a recent report using animal models indicated that the H9N2 influenza virus showed increasing pandemic potential [14].

We are aware that our surveillance systems are subject to certain limitations. Firstly, the systems were limited and not all locations within each state were considered. They also have some bias which may prevent the surveillance of birds in difficult terrains. However, we made every effort to give priority to locations that serve as point of aggregations of poultry products from many locations.
We may have underdetected some cases in view of the availability of more robust and sensitive analytic system like real time reverse transcriptase polymerase chain reaction (R-RTPCR), we are currently making effort to put in place such analytic system. It was also difficult to get paired serum samples in most location since farmers were at liberty to dispose their birds without giving consideration to the on-going surveillance systems.
Despite these limitations, we are of the opinion that this nationwide effort is critical and important since Sub Saharan Africa faces many challenges of controlling and eradicating H5N1 in poultry and implementing a good surveillance system for H5N1 in humans.
The human sero-epidemiological survey reported in this study did not detect any human H5N1 infections in Nigeria. This result is similar to the data

recorded in previous studies in Cambodia (0/351) and Guangdong, China (1/110) [15,16]. This probably confirms that the virus has yet adapted to effectively infect humans.
Although the human serosurveillance was negative, human H5N1 infections in Nigeria cannot be excluded. It is common practice in the northern part of the country, for reasons of culture, religion and poverty, to bury a deceased person within 24 hours of death, sometimes without ascertaining the cause of death through post-mortem and detailed laboratory examinations. The only human case in Nigeria, which was officially reported by the World Health Organization on 3 February 2007, was diagnosed following a thorough investigation of a fever complicated by respiratory distress which finally led to death. It is important to ensure in the future that at least diagnostic specimens are collected before burial for proper retrospective analysis. Since it is beyond the mandate of NVRI to do a nation-wide serosurveillance in humans, the Nigerian Federal Ministry of Health, human medical practitioners, virologists and immunologists are encouraged to carry out a similar study in humans in Nigeria and parts of the West African sub-region.

Globalisation can affect animal and human health and change the disease ecology especially in those countries that presently claim to be free from HPAI infection in humans and animals [17], and risk assessment studies have shown that the European Union and parts of North America are at high risk of infection with animal diseases,in particular those originating from Africa [18-21]. These countries will need to strengthen their borders with respect to animal disease controls.
To date, the majority of the HPAI H5N1 cases in Europe has been introduced through wild birds. The source of contamination as well as the movement pattern of these wild and migratory birds needs to be studied more critically in order to exclude cross-continent infection of a potentially pandemic influenza virus.

Since October 2007, there has been no confirmed outbreak in Nigeria despite the on-going intensive surveillance. This situation has helped to stabilise the Nigerian poultry industry and has had a positive psychological effect on consumers. However, the continued absence of HPAI H5N1 will depend on sustained surveillance of poultry farms and live-bird markets, changed agricultural practices and a heightened biosecurity system entrenched in the farming system in Nigeria. Cross-continent collaborative research is encouraged and a network of funding systems, especially from the rich countries, to support research and diagnosis in developing economies like Nigeria will be greatly valued.
** Note added in proof: Since the time of submission of this report, the FAO laboratory has recently (June and July, 2008) isolated and molecularly characterised new HPAI virus isolates obtained from live bird markets and from outbreaks in farms in a total of four Nigerian states. While the viruses

from two states, Kano and Katsina, (isolated from farms) belonged to the old clade (2.2) circulating in Nigeria, the isolates from two other states, Gombe and Kebbi, belonged to a new sublineage of clade 2.2, EMA3, that is novel to the African continent. This sublineage was previously circulating in Europe (Italy), Asia (Afghanistan) and the Middle East (Iran) in 2006.

** Erratum: The following amendments were made to correct the fact that supporting data on sero-surveillance in humans had mistakenly not clearly been labelled as cited from a previous publication: The sentence "Limited human sero-surveillance involving 320 individuals was also carried out but yielded no positive results" was removed from the abstract. The paragraph "Surveillance in humans was carried out between 21 March and 3 April 2006 [11]. Human sera were collected with the informed consent of participating individuals. In addition, serum samples were collected from people potentially exposed to the HPAI H5N1 virus including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples were transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera were prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera were tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. Details of the tests have been reported comprehensively in another paper [11]." was changed to "In addition, surveillance in humans had been carried out by Ortiz et al. between 21 March and 3 April 2006 [11]. In that study, human sera had been collected with the informed consent of participating individuals. In addition, serum samples had been collected from people potentially exposed to the HPAI H5N1 virus, including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples had been transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera had been prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera had been tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. For details see Ortiz et al. [11]. " The sentence "None of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test …" was changed to "As previously reported, none of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test … [11]." The sentence "The human sero-epidemiological survey reported in this study did not detect any human H5N1 infections in Nigeria." was changed to*
"The human sero-epidemiological survey reported by JR Ortiz et al. did not detect any human H5N1 infections in Nigeria [11]."

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Chapter 3

Avian influenza A (H5N1) in humans: lessons from Egypt

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Rapid Communication

Avian influenza A (H5N1) in humans: lessons from Egypt

Abstract

Highly pathogenic avian influenza A (H5N1) has ravaged the Egyptian poultry population. Ninety human cases, including 27 fatalities have been recorded by 30 December, 2009. However, epidemiological information on the infection in humans in Egypt is scarce. We analysed the first three years of highly pathogenic avian influenza A (H5N1) in Egypt between 20 March 2006 and 31 August 2009) and found that more cases occurred in females than males, especially in 2006 and 2007. Women in the age group 20-39 years had the greatest tendency to be infected. It took an average of one day and 18 hours to seek medical assistance in patients who recovered and of six days in fatal cases. Children sought treatment much earlier than adults. On average, a patient died 11 days after the onset of symptoms. Exposure to infected poultry remained the most important risk factor.

Introduction

On 17 February 2006, highly pathogenic avian influenza A (H5N1) was first reported in the poultry population in Egypt [1]. Since that time, the infection had affected at least 21 governorates forcing over 1.5 million individuals to loose their source of livelihood [1]. Overall, 370 backyard poultry flocks, 850 farms, and four zoos have been affected, and more than 36 million birds (mainly chickens) have died or have been culled in Egypt at an enormous cost to the country [1]. Currently, the virus is endemic in the Egyptian poultry population.

The first human case of avian influenza A (H5N1) in Egypt occurred on 17 March 2006 [2], and to date (30 December 2009), the statistics of human infection and fatalities continue to rise. Specifically, 90 human cases (ca. one fifth of the total global count), including 27 fatalities (ca. one eleventh of the global count) have been recorded in Egypt as of 30 December 2009 [2]. These numbers rank Egypt third in the list of recorded human cases and fatalities in the world, after Indonesia and Vietnam, and remain by far the highest in Africa. The World Health Organization (WHO) had previously stated that "countries around the world had improved their defenses against bird flu, but the situation remained critical in Egypt and Indonesia where the risk of the H5N1 virus mutating into a major human threat remains high" [3].

Worrisome with the situation in Egypt is the frequency with which women and young people are being infected and the very current trend of rising infections in children: in 2009 alone, 79% of all infected individuals were under 10 years-old. Between January and December 2009, 17 of the 34 recorded cases involved children between 12 and 30 months-old. Similarly, at the time of this report, human cases of 2009 pandemic influenza A(H1N1) had also been confirmed in the Egyptian population, which raises the possibility of co-infection and the emergence of reassortant viruses.

While the situation in Egypt remains critical, empirical evaluation of its peculiarities seem to be lacking, except for a very recent report by Dudley [4]. Assessment of the scientific literature and epidemiological data returned little or no concrete evidence from Egypt. However, the country has provided adequate records to international organisations like the WHO and the World Organization for Animal Health (OIE) and these reports have improved significantly since the first submission in terms of spatial and temporal data, and clinical records of affected persons.

In this study, we analysed the records on avian influenza A (H5N1) in Egypt between 20 March 2006 and 31 August 2009 and explain the epidemiological significance of our findings.

Materials and Method

The Egyptian government reports to the WHO, available on the WHO website [2], were the primary source of data for these analyses. We considered all laboratory-confirmed human cases of avian influenza A (H5N1) reported to the WHO from Egypt between 20 March 2006 and 31 August 2009. All positive samples reported and used in these analyses had earlier been confirmed by microneutralisation assay on serum or by PCR on respiratory tract specimens as reported [5]. Similar confirmatory tests were done in the Egyptian national reference laboratory and at the WHO reference laboratories for diagnosis of influenza A(H5) infection, including the United States Naval Medical Research Unit 3 in Cairo, Egypt [4].

The parameters included in our analysis were: date of exposure, date of onset, course of symptoms, and time from hospitalisation until death/recovery, as listed in the WHO situation report on avian influenza [6].

In the absence of complete information, reports were based on approximate dates and times from the reports. However, in cases of ambiguity arising from the records, such data were excluded from the calculations. In total, 85 confirmed cases were reported during the study period, of which 27 were fatal. After the exclusion of ambiguous data, only 63 of the 85 reported cases and 20 of the 27 fatal cases were evaluated for symptoms and hospitalization; and 44 of the 58 cases who recovered or were stable were analysed for symptoms and recovery. Analyses were performed using StatGraphics v2.0. Distributions were compared using chi-square test, and medians were compared using Fisher's exact test.

Results

Demographic characteristics

In the period under analyses, 85 cases were evaluated, 32 of whom were male and 53 were female. Eighteen cases had been reported in 2006, 25 in 2007, eight in 2008 and 34 to date (31 August) in 2009, including a total of 27 human fatalities over the three and a half-year period.

The youngest cases were one year of age (two boys), and the oldest case was a 75 year-old woman. The median age of all confirmed cases was six years. The age of the cases (n=85) ranged from 12 months to 75 years, with a mean of 13 years and two months. The median age of all fatalities (n=27) was 25 years (range: four to 75 years) and the mean was 26 years and three months. The median age of the female cases (n=53) was 15 years, (range: 14 months to 75 years) and the mean was 16 years and 10 months, while the median age of the male cases (n=32) was four years (1range: two months to 32 years) and the mean seven years and two months (Table, Figures 1 and 2).

Table 1. Human cases and fatalities associated with avian influenza A (H5N1) in Egypt, 20 March 2006-31 August 2009 (n=85)

Year	Number of human cases and fatalities distributed according to sex						Number of human cases distributed according to age					
	Number of human cases		male		female		Age range of human cases					
	disease	fatalities	disease	fatalities	disease	fatalities	<10	10 to 19	20 to 29	30 to 39	40 to 49	>50
2006	18	10	5	1	13	9	4	5	2	6	0	1
2007	25	9	7	0	18	9	13	4	5	2	0	1
2008	8	4	4	1	4	3	3	3	1	1	0	0
2009	34	4	16	1	18	3	29	1	1	3	0	0
	85	27	32	3	53	24	49	13	9	12	0	2

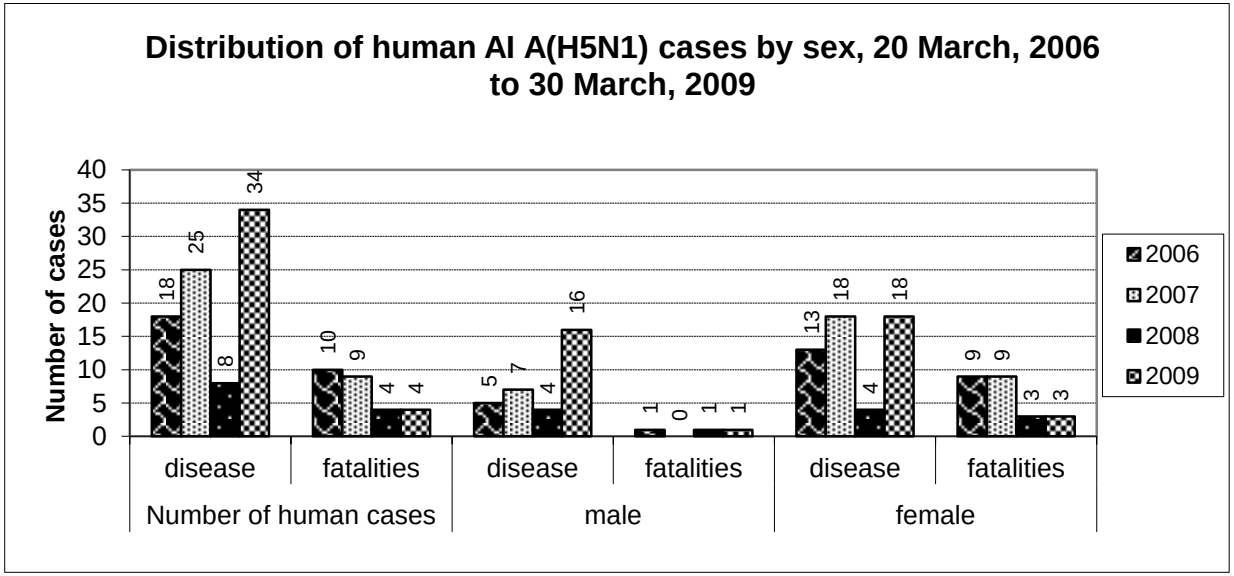

Figure 1. Distribution of human cases of avian influenza A (H5N1) by sex, Egypt, 20 March 2006–31 August 2009 (n=85)

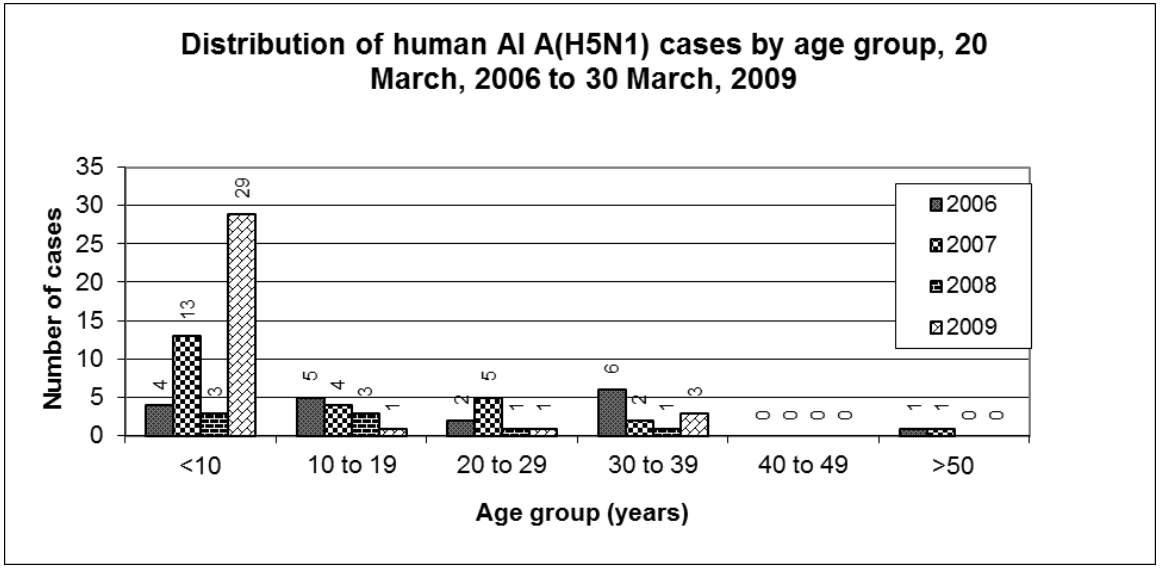

Figure 2. Distribution of human cases of avian influenza A (H5N1) by age group, Egypt, 20 March 2006–31 August 2009 (n=85)

The overall sex ratio (male:female) was 0.6 and the annual sex ratios were 0.4 (2006), 0.4 (2007), 1.0 (2008) and 0.9 (2009). By age, the sex ratios (male:female) were 1.1 (<10 years), 0.3 (10-19 years), 0.1 (20-29 years), 0.2 (30-39 years), 0 (40-49 years; no case) and 0 (>50 years; all cases female), with χ=11.87 in Pearson's chi-square test, p=0.001 in Fisher's exact test, and degree of freedom (DF)=1 (see Table).

Intervals

The number of days from onset of symptoms to hospitalisation (S-H) for all cases was calculated for 63 of the 85 cases. The median was two days (range: 12 hours to 11 days), the mean was two days and 19 hours. For the fatal cases (17 of 27 were included in the analysis), the median (S-H) was six days (range: two to 11 days) with a mean of six days. Among the recovered cases (47 of 58 were included in the analysis), the median (S-H) was one day (range: 12 hours to five days), and the mean was one day and 18 hours.

The time from onset of symptoms to death had a median of nine days (range: five to 30 days) and a mean of 11 days, while the time from hospitalisation to death had a median of four days (range: one to 25 days) and a mean of six days. The S-H in children and teenagers between the ages of 10 and 19 years (n=51) had a median of one day (range: 12 hours to eight days) and a mean of two days and 12 hours, in contrast to the adults over 20 years of age (n=12), in whom the median was four days (range: 12 hours to 11 days) and the mean was four days. Many (19) of the adults did not present with full hospital records and were not included in the analysis for hospitalisation.

Mortality

The overall case fatality rate was 32% (27/85). It was much lower in male (3/32) than in female (24/53) cases. According to age, the case fatality was two of 49 in the under 10-year-olds, eight of 13 in the 10-19-year-olds, seven of nine in the 20-29-year-olds, eight of 12 in the30-39-year-olds, and two of two in the over 50-year-olds (there were no cases among the 40-49-year-olds). In the years under review, the case fatality was 10 of 18 for 2006, nine of 25 for 2007, four of eight for 2008 and four of 34 for 2009, with χ=10.81 in Pearson's chi-square test and DF=4).

Discussion

This study is subject to some limitations. We conducted our analyses based on the limited data available for scrutiny. We suspect that cases have been missed because of the current surveillance system in

humans which targets only severe infections backed by laboratory confirmation [7]. If this is so, Egypt may have had many more cases and possibly fatalities than reported and used in this work. People trying to avoid hospitalisation, especially among the adults, may also have contributed to underreporting.

In this analysis, the female cases had a wider age window (14 months to 75 years) than the male cases (12 months to 32 years). Since exposure to poultry remains the most important risk factor for human infection in Egypt, this may reflect the fact that across all age groups, more women than men are involved in poultry-related activities. All infected individuals with the exception of three (whose exposure status was uncertain) had been exposed to infected poultry or poultry products or to slaughtered or defeathered infected birds. In children and young adults, however, infection was more prevalent among males, although it is not clear why. Although infections in children peaked in the years 2007 and 2009, the reason for which is not yet clearly understood. Strong peaks of infection usually appear to follow periods of relaxation of preventative measures [7].

It also appears that especially in the group of the 20-39-year-olds, women had a greater tendency to be infected and more women died post infection. Fifteen of 21 infected women in this age group died. These groups face the highest risk of exposure as it is mainly they who are involved in home slaughtering and defeathering of chicken and preparation of food, farm work and visits to infected farms. A recent study has analysed the age and sex bias with regards to the situation in Egypt [4], and it has been reported that farmers from other infected African countries believe that there is little or no risk of infection from culling, defeathering, home slaughtering and visit to infected premises [8,9]. In addition, failure of the government to pay compensation in Egypt for culled birds and the practice of keeping of poultry on rooftops and in close association with humans may have played a role. Although no association has yet been established between the level of exposure to avian influenza A (H5N1) and fatalities in Egypt, reports on workers in Asia showed that a high prevalence of infection in the poultry population is associated with a higher incidence of infection in humans, and that controlling such outbreaks of H5N1 influenza in the poultry flocks can stop human infection [7,10,11]. In addition, genetic characterisation of viruses from both the human and avian populations in Asia revealed that the viruses from both species were very similar [9,10].
According to our analysis, early hospitalisation following infection increased the chances of recovery. Children tend to be hospitalised earlier than adults and this may have contributed to the significantly

lower death rate in the children (only two cases in children under the age of 10 years were fatal). Similarly, although 62 of the 85 cases were under 19 year-olds, this does not represent national demography since only approximately 32% of the population are 15 years and younger [12]. In most parts of Africa, people are known to visit a hospital less frequently as they advance in age, and supposedly non life-threatening conditions such as seasonal influenza are often treated at home and therefore underreported [8].

The overall case fatality in this study was 32% (27/85). This percentage may appear small when compared with statistics from other places, for example 82% in Indonesia (115/141), 68% in Thailand (17/25), 66% in China (25/38) and 50% in Vietnam (56/111). Nevertheless, with the exceptional surge in number of cases (especially in children) arising in Egypt in 2009 and the recent reoccurrence of human cases of avian influenza A (H5N1) in China and Vietnam despite an intensive control programme in the poultry populations, the pandemic potential of this virus is still very evident. Case fatality was significantly higher in females compared with males, but whether this is related to exposure dose can not be confirmed in this analysis.

As previously suggested by Briand and Fukuda [9], public health guidelines in Egypt will need to be tailored to meet the local situation taking into consideration the agricultural practices and the people's perceptions. It will also be necessary to conduct more studies on human H5N1 influenza infection in Africa to evaluate the situation of asymptomatic carriers and unreported cases.

Finally, as evident in this analysis, exposure to infected poultry remains the only common denominator and an important risk factor for the spread of avian influenza A (H5N1) in humans in Egypt. Other workers had identified and reported the same risk factor-exposure to sick poultry-previously [10,11].

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Chapter 4

Identification of risk factors associated with highly pathogenic avian influenza H5N1 virus infection in poultry farms, in Nigeria during the epidemic of 2006-2007.

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Identification of risk factors associated with highly pathogenic avian influenza H5N1 virus infection in poultry farms, in Nigeria during the epidemic of 2006-2007.

Abstract

We conducted a matched case-control study to evaluate risk factors for infection with highly pathogenic avian influenza (HPAI) H5N1 virus in poultry farms during the epidemic of 2006-2007 in Nigeria. Epidemiologic data were collected through the use of a questionnaire from 32 case farms and 83 control farms. The frequency of investigated exposure factors was compared between case and control farms by using conditional logistic regression analysis. In the multivariate analysis, the variables for (i) receiving visitors on farm premises (odds ratio [OR] = 8.32; 95% confidence interval [CI]= 1.87, 36.97; $P < 0.01$), (ii) purchased live poultry/products (OR = 11.91; 95% CI = 3.11 - 45.59; $P < 0.01$), and (iii) farm workers live outside the premises (OR = 8.98; 95% CI = 1.97, 40.77; $P < 0.01$) were identified as risk factors for HPAI in poultry farms. Improving farm hygiene and biosecurity should help reduce the risk for influenza (H5N1) infection in poultry farms in Nigeria.

Keywords: avian influenza, H5N1, risk factors, Nigeria, poultry.

Introduction

In 1996, a geese farm was infected in Guangdong, China with the highly pathogenic H5N1 strain of avian influenza (HPAI H5N1). That particular incident was to mark the onset of a major catastrophe in the poultry industry worldwide as the virus from the farm acquired several new biological properties and became the progenitor of several other HPAI H5N1 viruses-the key amongst which is the Z-genotype (Xu et al., 1999; Guan et al., 2002; Li et al., 2004). The virus has caused the death or culling of over 300 million multiple avian species and spread to at least 50 countries, including 467 human infections and 282 human fatalities (OIE, 2010; WHO, 2010).

Several factors have been determined to be responsible for the dissemination of influenza virus in poultry. Bavinck et al., (2009)

showed that raising of multiple species in backyard poultry and living in close proximity to infected commercial farm premises increases the risk of infection. Backyard poultry have also been found to be an important source of spread and persistence of HPAI H5N1 in South East Asia (Tiensin et al., 2005). Thompson et al., (2008) also found out that large population of birds within farm premises, poor biosecurity and presence of outside birds, especially the Egyptian geese, were associated with increasing the risk of infection of ostriches with the H5 influenza virus. Layer type operation has been associated with infection in the Netherlands (Thomas et al., 2005). Finally, a study by Nishiguchi et al (2007) proved that introduction of end-of-lay chickens, sharing of farm equipment, poor biosecurity and proximity to infected farms are important risk factors for infection with HPAI H5N2 in Japan.
Nigeria was the first African country to report HPAI infection in February 2006, when the HPAI H5N1 virus was first detected in a commercial poultry farm in Kaduna, Northern Nigeria. The virus thereafter spread rapidly to other commercial and backyard poultry and infected 25 of 36 states of Nigeria including the Federal Capital Territory (National Veterinary Research Institute records). Approximately 711,000 birds of various species died and a total of 1,264,191 were culled. One human fatality was also reported and confirmed in Lagos, South-West Nigeria in early 2007.

In Africa, despite reports on the genetic/biological constituents/properties of HPAI H5N1 viruses of African origin (Ducatez et al, 2006; Ducatez et al, 2007; Njouom et al, 2008; Couacy-Hyman et al, 2008; Cattoli et al, 2009; Fasina et al, 2009), the epidemiologic aspects of HPAI H5N1 virus infection in poultry have not been investigated. Although previous studies have provided an epidemiologic framework for investigation of risk factors for HPAI H5N1 virus in poultry (Henzler et al, 2003; Thomas et al, 2005; Bouma et al, 2009; Busani et al, 2009), study findings and proposed prevention strategies may not apply to the conditions of Nigeria in view of variations in climatic conditions, poultry practices, and the socioeconomic and cultural orientations of agricultural populations in this geographic region. The aims of this study were to investigate and identify risk factors for HPAI H5N1 virus infection in poultry farms in Nigeria during 2006-2007.

Materials and Methods

Study location and time period

Field and laboratory investigations were conducted in Nigeria from February 2006 to October 2007. During this time-period, a total of 1,205 poultry farms with suspected disease outbreaks were investigated, and 299 virus isolates of HPAI H5N1 were recovered from 26 of 36 states in Nigeria (Fasina et al, 2009). This epidemiologic investigation was conducted within a subset of the 26 states where one or more poultry farms reported HPAI H5N1 virus infections in 2006-2007. Seven states were randomly selected so that at least two states from each of the affected regions would be investigated. The final selection included: 2 states from the North (Kano, Kaduna); 3 states from the Central region (Bauchi, Plateau, Federal Capital Territory); and 2 states from the South (Ogun, Lagos; see Figure 1). This design was meant not to assess the farms representative of all Nigerian poultry farms, but high-risk farms (those located within infected states). Within the selected states, case and control farms were selected as indicated below.

Selection of cases

Case farms were randomly selected from the list of infected farms so that five farms from each of the selected states would be sampled (n = 35). Of those, questionnaires were completed and retrieved from thirty-two farms (n=32). All the 32 farms met the double criteria of a case farm (clinical signs- sudden heightened mortality, dyspnoea, coughing, wheezing, decreased food and water intake, decreased or cessation in egg production; and positive virological tests conducted at the National Veterinary Research Institute, Vom; Joannis et al., 2008).

Selection of controls

Control farms were randomly selected from a group of farms located at the same states that reported infections in 2006-2007 (sample frame: 2,111), from which serum samples had been collected at the same time the epidemic occurred (to monitor other diseases), which produced seronegative test results for HPAI H5N1 virus. Control farms were selected to generate an excess of at least 2.5:1 ratio between control and case farms. A total of 83 control farms (a 2.59:1 ratio of control/case) returned questionnaires.

The control farms were matched for flock size (<500 or >500; P-value = 0.015, two-tailed at 95% confidence interval, McNemar test value = 5.898).

Epidemiologic questionnaire

A structured questionnaire was designed, validated, and applied during farm visits for data collection of epidemiologic interest that took place during the months of July-October 2007, when farm managers were interviewed. Data on the following items were collected from each farm: location, primary occupation of the farm owner, education level of key decision makers (owner or manager), method of product disposal, number of layers, raising of other birds/animals, farm workers visit neighbouring farms, farmer and workers receive visitors within the farm premises, whether live poultry were purchases from the market during the epidemic in other farms, if there were infected farms within 1.0 km, presence of footbath, presence of gate/fence, changing room, workers are provided with own cloth/boots, shower bath, tire dip, log book, presence of preventive materials and application of procedures meant to reduce burden of diseases, disposal of farm waste, commercial/local source of feed, use of borrowed farm equipment, water source (well, lake/pool, stream, portable/chlorinated/borehole water), presence of pool/bird sanctuary, presence of water bird, rat problems, vaccinator of birds (resident vets, shared vaccinator/outside vets, para-veterinarians), whether the house is screened with netting to exclude wild birds, whether the workers live outside the farm premises, and the disposal method for dead bird.

Statistical analyses

Conditional logistic regression on the dichotomic items was used to model the odds of being an HPAI H5N1 case as a function of investigated risk factors. Initial screening of potential risk factors for HPAI H5N1 infection was performed by use of univariate conditional logistic regression (Hosmer and Lemeshow, 1989; Cytel Software Corporation, 2000). Variables associated ($P \leq 0.20$) with the outcome of interest (HPAI H5N1 virus infection) were considered for inclusion in a multivariate logistic regression analysis. Associations between exposure variables ($P \leq 0.20$) were examined, and when a pair of variables was associated ($P = 0.05$ by use of a χ^2 test, two-tailed), the exposure variable judged as most biologically plausible was used

as a candidate in the multivariate analysis. A forward stepwise approach was used to identify variables associated with infection by use of a 2-sided *P*-values-to-enter and *P*-values-to-remove of 0.05 and 0.10, respectively. Values for the final model were considered significant at $P \leq 0.05$. Fit of the model to the data was assessed by a visual examination of residual plots (standardized Δ-β values vs observation number and Δ-β vs fitted values). Case-control sets that had farms with extreme Δ-β values and low fitted values were excluded from the analysis to evaluate their influence on estimated ORs. In the final model, the adjusted odds ratio (OR) and 95% confidence interval (CI) were reported.

Results

This study included 32 case farms and 83 control farms in Nigeria. The median flock size of study poultry farms was 764 (first quartile = 351; third quartile = 2500). In the univariate analysis, 7 variables (kept other birds/animals, receive visitors on the farm, purchase live poultry, have infected neighborhood, untreated water source, outside workers, and inappropriate disposal of dead chickens) were identified and further analyzed for biological plausibility, magnitude of association, and statistical significance ($P \leq 0.20$, Table 1). In the multivariate analysis, the variables 'farm receives visitors within the farm premises', 'farm purchases live poultry/products', and 'farm workers live outside the premises' were associated ($P \leq 0.05$) with HPAI H5N1 virus infection (Table 2). Visual examination of residuals revealed that the delta-beta values for the three variables kept in the final model were not extreme (ie, not > 1), which supported overall goodness of fit. Analysis of residuals (set of case and control farms with the largest delta-beta value and lowest fitted value) indicated the existence of influential observations; however, removal of these observations did not change the finding of greater risk associated with 'farm receives visitors', purchases of live poultry/products', and 'farms with workers living outside the farms'.

Table 1. Variables collected in the questionnaire to test for risk factors for infection with HPAI H5N1 in backyard poultry farms, Nigeria.

Variable		**Case (infected farms)**	**Control (non-infected farms)**
Location	Urban	20	50
	Rural	12	33
Main occupation	Animal farming (poultry)	21	48
	Other occupation	11	34
Educational level	Primary/Secondary	12	26
	Post-secondary	19	54
Methods of product disposal (eggs/meat)	Egg buyers visit farm	26	67
	Take to open market	3	13
	Dispose by other means	2	3
Number of birds	0-500 laying birds	14	33
	500 and above	16	42
	0-500 broiler birds	13	24
	500 and above	10	13
Kept other birds/animals	Yes	8	5
	No	23	77
	Duck/geese	8	5
	Turkey	8	13
	Ostrich	0	1
	Quails	1	2
	Pigeon/G. fowl/Peacock	1	3
	Cattle	6	7
	Sheep	11	9
	Goat	10	14
	Pig	1	4
	Horse	0	1
	Dog	3	17
	Cat	3	1
	Rabbit	3	1
Visit other farms	Yes	15	55
	No	14	25
	Not sure	3	3
Receive visitors on the farm	Yes	9	29
	No	9	43
	Not sure	10	1
Purchase live poultry/poultry products	Yes	16	13
	No	8	62
	Not sure	-	1
Have infected neighbourhood	Yes	10	12

	No	10	51
	Not sure	12	18
Foot bath	Yes	19	52
	No	13	25
Gate/fence	Yes	17	44
	No	15	33
Changing room	Yes	8	25
	No	24	52
Farm cloth/boot	Yes	17	36
	No	15	41
Shower bath	Yes	2	5
	No	30	72
Tyre dip	Yes	1	5
	No	31	72
Log book	Yes	-	7
	No	32	70
Crate/bag control	Yes	1	-
	No	31	77
Usage of available biosecurity systems	Yes	26	69
	No	4	7
Disposal of farm waste	Burn/bury	5	14
	Sell as fertilizer	19	47
	Dispose in the refuse dump	7	13
	Spread in the farm site	11	28
	Other means	-	-
Source of feed	Self milling	13	48
	Commercial/industrial source	18	39
Borrow farm equipment	Yes	2	7
	No	30	74
Water source	Treated water	13	22
	Bore hole	7	22
	Well	15	49
	Lake/other sources	1	-
Pool/bird sanctuary in the vicinity	Yes	4	15
	No	28	65
Water bird visit	Yes	5	9
	No	27	71
Rat problem	Yes	17	52
	No	15	29
Who vaccinate your birds	Self/family	4	61
	In-house vet	9	21
	Shared vet	8	28
	Para vets	12	19
	others	-	-

Bird proof house	Yes	22	61
	No	10	15
Outside workers	Yes	22	34
	No	7	19
Disposal of dead chickens	Burn	9	6
	Bury	6	23
	Process for human/animal consumption	5	14
	Submit to professionals for post-mortem	1	1
	Dispose indiscriminately on the refuse dump/others	7	31

Table 2. Univariable analysis of risk factors associated with poultry farms diagnosed with HPAI H5N1 virus infection

Variable	Category	Cases	Controls	OR	95% CI	P
Location (urban)	0	19	46	1.00	Ref	NA
	1	12	32	0.95	0.39, 2.27	0.91
Primary occupation (poultry)	0	11	31	1.00	Ref	NA
	1	20	47	1.16	0.47, 2.84	0.73
Education (Secondary maximum)	0	19	51	1.00	Ref	NA
	1	12	27	1.25	0.51, 3.05	0.61
Egg/spent layers disposed on farm premises	0	3	11	1.00	Ref	NA
	1	28	67	1.57	0.39, 6.22	0.51
Number of layers in the farm (<500)	0	17	46	1.00	Ref	NA
	1	14	32	1.25	0.49, 3.21	0.63
Kept other birds/animals	0	23	73	1.00	Ref	NA
	1	8	5	5.91	1.53, 22.85	0.01
Visit other farms	0	14	24	1.00	Ref	NA
	1	17	54	0.60	0.27, 1.35	0.22
Receive visitors on the farm premises	0	8	47	1.00	Ref	NA
	1	23	31	3.69	1.50, 9.08	<0.01
Purchase live poultry	0	11	64	1.00	Ref	NA
	1	20	14	6.50	2.55, 16.53	<0.01
Have infected neighbourhood	0	10	49	1.00	Ref	NA
	1	21	29	3.48	1.40, 8.69	<0.01
Foot bath	0	12	24	1.00	Ref	NA
	1	19	54	0.65	0.25, 1.64	0.36
Gate/fence	0	15	34	1.00	Ref	NA
	1	16	44	0.74	0.31, 1.73	0.49

Changing room	0	23	53	1.00	Ref	NA
	1	8	25	0.66	0.23, 1.89	0.44
Farm cloth and boots	0	15	41	1.00	Ref	NA
	1	16	37	1.11	0.49, 2.50	0.78
Shower bath	0	29	73	1.00	Ref	NA
	1	2	5	1.16	0.18, 7.32	0.87
Tyre dip	0	30	73	1.00	Ref	NA
	1	1	5	0.52	0.05, 4.89	0.57
Log book	0	31	72	1.00	Ref	NA
	1	0	6	ND	ND	ND
Use of biosecurity	0	3	6	1.00	Ref	NA
	1	28	72	0.76	0.19, 3.08	0.70
Indiscriminate disposal of farm waste	0	26	69	1.00	Ref	NA
	1	5	9	1.52	0.46, 5.00	0.48
Self prepared feed	0	17	38	1.00	Ref	NA
	1	14	40	0.83	0.34, 2.05	0.70
Borrow farm equipment	0	29	71	1.00	Ref	NA
	1	2	7	0.71	0.13, 3.77	0.68
Untreated water source	0	22	69	1.00	Ref	NA
	1	9	9	3.51	1.13, 10.92	0.02
Pool/bird sanctuary	0	27	65	1.00	Ref	NA
	1	4	13	0.69	0.21, 2.25	0.54
Water birds visit farm premises	0	22	69	1.00	Ref	NA
	1	9	9	0.96	0.28, 3.25	0.95
Problems with rodent pests control	0	14	26	1.00	Ref	NA
	1	17	52	0.60	0.25, 1.46	0.26
Outside vaccinators operate in the farm	0	18	46	1.00	Ref	NA
	1	13	32	1.10	0.48, 2.52	0.80
Bird proof buildings/pens	0	8	17	1.00	Ref	NA
	1	23	61	0.71	0.26, 1.96	0.51
Workers live outside the farm premises	0	7	40	1.00	Ref	NA
	1	24	38	4.06	1.44, 11.47	<0.01
Inappropriate disposal of dead chickens	0	4	22	1.00	Ref	NA
	1	27	56	2.51	0.79, 7.98	0.11

Table 3. Multivariable analysis of risk factors associated with poultry farms diagnosed with HPAI H5N1 virus infection.

Variable	Category	Adjusted OR	95%CI	P
Receive visitors on the farm	No	1.00	Reference	NA
	Yes	8.32	1.87, 36.97	< 0.01
Purchase live poultry	No	1.00	Reference	NA
	Yes	11.91	3.11, 45.59	< 0.01
Workers live outside premises	No	1.00	Reference	NA
	Yes	8.98	1.97, 40.77	< 0.01

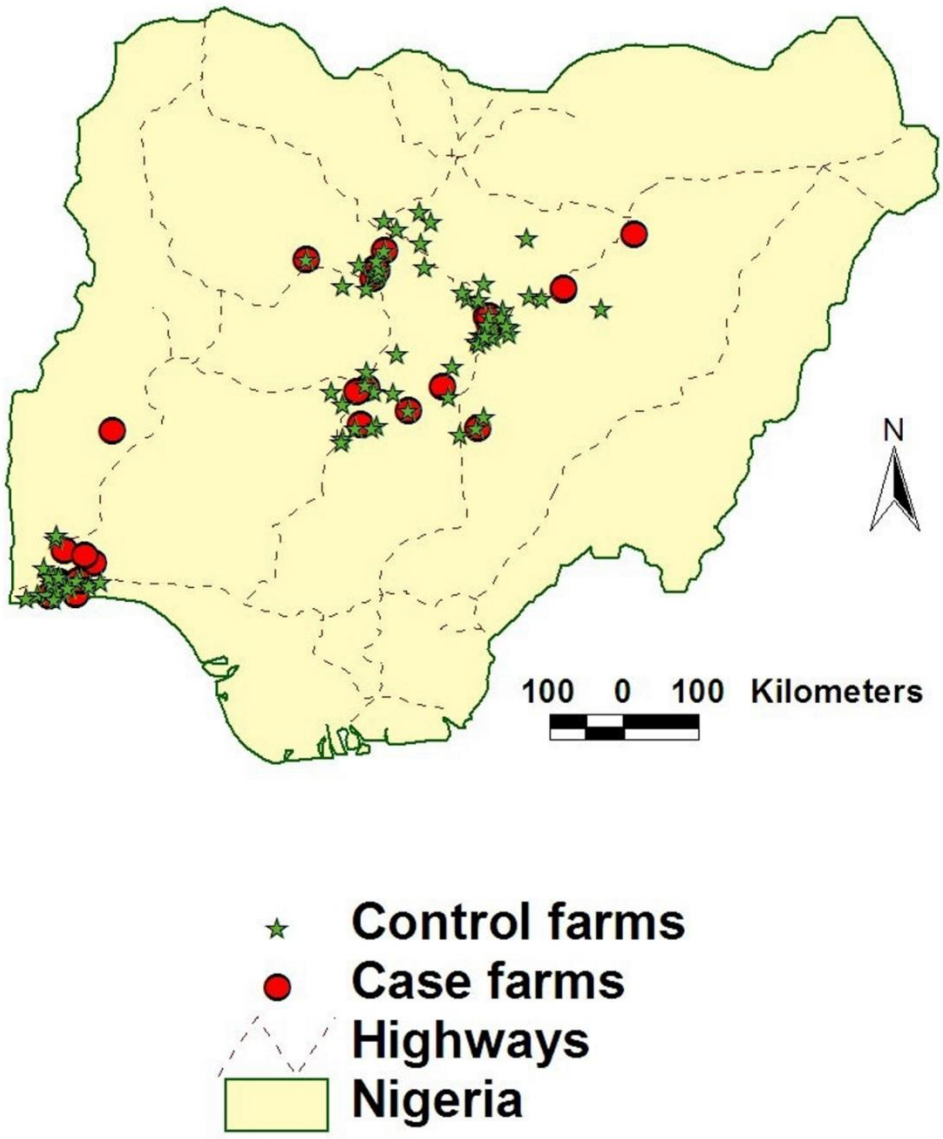

Figure. Spatial representation of infected locations. Sampled populations were taken from the infected areas using a stratified random sampling approach.

Discussion

The use of epidemiologic research methods proved useful in the identification of risk factors associated with poultry farms classified as infected with HPAI H5N1 virus during the epidemic of 2006-2007 in Nigeria. While under-reporting and /or over-reporting (due to confusion with other diseases) may have occurred during the epidemic, given the laboratory results conducted, such possibilities did not influence the case and control farms here investigated. Overall, the epidemiologic analysis revealed that low biosecurity standards contributed to the wide spread of HPAI H5N1 in poultry farms in Nigeria during the epidemic. Study results should help the national veterinary authority in Nigeria to justify the allocation of funds to formulate, implement, and evaluate enhanced education and communication activities on the importance of biosecurity to prevent future epidemics of HPAI in Nigeria.

Statistical significant associations were found among three risk factors (presence of visitors on farm premises, purchase of live poultry and poultry products by farmers, and farm workers living outside the farm premises). The odds of HPAI H5N1 virus infection in farms that received visitors was higher than that in farms where visitors were received outside the poultry premises (OR = 8.32; 95% CI = 1.87, 36.97). It is noteworthy that most of the visitors that patronize farms in Nigeria are farm gate buyers who have higher chance of visiting several farms per day. In the course of conducting their trade, they may inadvertently transmit infection from farm to farm especially from a newly infected farm where clinical signs are not yet fully displayed. Similarly, farmers may offer for sale, at a lower cost, birds suspected to be infected. A previous study has indicated that transfer of poultry and their products from one part of the country to another enhances the spread of avian influenza H5N1 (Fasina, et al., 2009).

Poultry farms that purchased live poultry and poultry products in the course of the outbreak had higher odds of being infected with HPAI H5N1 virus (OR = 11.91; 95% CI = 3.11, 45.59), compared to farms that did not. Sales of live poultry (a common practice in Nigeria and most parts of Africa) are associated with rapid dissemination of infections through slaughter, evisceration, improper disposal of

visceral, flying feathers and contaminated equipment used by farmers (Henzler et al., 2003).

Farms with farm workers that live outside the farm had higher odds of infection, compared with farms where workers live within the premises (OR = 8.98; 95% CI = 1.97, 40.77). One explanation we can offer for this association is that outside workers are less likely to observe biosecurity principles and guidelines set by the farms. Such workers raise poultry in their own homes, offered services to other farms, exchange items with workers from other farm premises, and rarely change their clothes and shoes when they report for work (Adene and Oguntade, 2007).
The established risk factors are inherently behavioural: they can be modified by human decisions. Given the fact that both the control and case farms were located in the same states, the difference observed between non-infected and infected farms in terms of risk factors ruled out the role of geography as the determining factor of infection. Instead, findings suggest that, at least partially, epidemic dissemination may be diminished, if not prevented, by simple measures that essentially, involve behaviour. This study highlight the low cost but high benefit associated with educational measures meant to prevent epidemic dissemination.

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Chapter 5

The cost-benefit of biosecurity measures on infectious diseases in the Egyptian Household Poultry

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The cost-benefit of biosecurity measures on infectious diseases in the Egyptian Household Poultry

Abstract
Increased animal intensification presents with increasing risks of animal diseases. The Egyptian household poultry is peculiar in its management style and housing and this present with particular challenges of risk of infection to both the flock and humans. Biosecurity remains one of the most important means of reducing risks of infection in the household poultry, however not much information is available to support its feasibility at the household level of production. In this study financial feasibilities of biosecurity were modeled and evaluated based on certain production parameters. Risks of particular importance to the household poultry were categorized and highly pathogenic avian influenza H5N1 was the most risky disease while people-related risk was the most important risk category. It was observed that basic biosecurity measures were applicable in the household poultry and it would be 8.45 times better to implement biosecurity than to do nothing against HPAI H5N1; 4.88 times better against Newcastle disease and 1.49 times better against coccidiosis. Sensitivity analyses proved that the household poultry project was robust and would withstand various uncertainties. An uptake pathway for basic biosecurity was suggested. The outcome of this work should support decisions to implement biosecurity at the household sector of poultry production.

Keywords: Egypt, Biosecurity, Financial, Technical, Feasibility, Household Poultry, Profitability.

Introduction

The Egyptian poultry industry experienced tremendous changes in the past three decades with the development of large scale operations with high level of intensification. These changes have been comprehensively described in available literatures and reports (CAPMAS, 2006; Hosny, 2006; Geerlings et al., 2007). The Egyptian household poultry industry remains very important and represents a major economic activity. It is intensive in nature, usually done on rooftops or in empty rooms in-house and involves close human-animal interaction. It is not comparable to the scavenging-type of management practiced by household poultry producers in many developing economies.

It has been proved that a higher degree of intensification (increasing population density within an increasingly reduced available space) is often associated with higher burdens and risks of infection with animal pathogens (Graham et al., 2008), and it is virtually impossible to achieve zero risk in the extensive or intensive system of management. In Egypt, the poultry industry is affected by a number of poultry diseases including the zoonotic Highly Pathogenic Avian Influenza A H5N1 (HPAI H5N1).

The challenge of high levels of poultry infections, with huge economic consequences and chanced human infections, therefore necessitated major interventions from the Egyptian health and agricultural authorities including the following: control of wildlife reservoirs, stamping out, quarantine, movement control, screening, vaccination in response to outbreaks and disinfection of premises amongst others (OIE, 2011). Other interventions were widespread public health and extension messages, training and specific biosecurity projects implemented at various levels. However, to date outbreaks are still being reported, including in the household poultry (HHP).

Biosecurity is defined here as the implementation of a set of measures that reduces the risks of the introduction and spread of disease agents in animals. The three principal elements of biosecurity are segregation, cleaning and disinfection. Segregation is the creation and maintenance of physical or virtual barriers to limit the potential opportunities for infected animals and contaminated materials to enter an uninfected site. This step, properly applied, will prevent most infection. Cleaning will remove most of the contaminating virus. Properly applied, disinfection will inactivate any virus that is present on materials that have already been thoroughly cleaned. Biosecurity requires the adoption of a set of attitudes and behaviors by people to reduce risk in all activities involving domestic, captive exotic and wild birds and their products (FAO, 2008).

Biosecurity has been proposed as a good intervention strategy to reduce the continuing spread of poultry diseases in the HHP in Egypt.

While the proposal was taken as acceptable and a possible long-term solution to the recurring cases of HPAI H5N1 in Egypt, its implementation and adoption in the HHP was seen as difficult in view of its seeming technicalities, the cost

involved and the possibility of recouping invested funds within the project period. The control and eradication efforts aimed at poultry diseases in Egypt may be better achieved by involving all the stakeholders, including the HHP producers. However, to convince poultry producers to implement biosecurity measures, it will be important to show a cost-benefit analysis, emphasizing the benefits of spending more resources on biosecurity.

The aim of this study was to assess the current level of implementation of biosecurity measures in HHP and whether or not their implementation is beneficial in term of costs using a model. A financial risk analysis was done by subjecting the model to a variety of changes that may occur in the course of the annual HHP project cycle.

Materials and Method

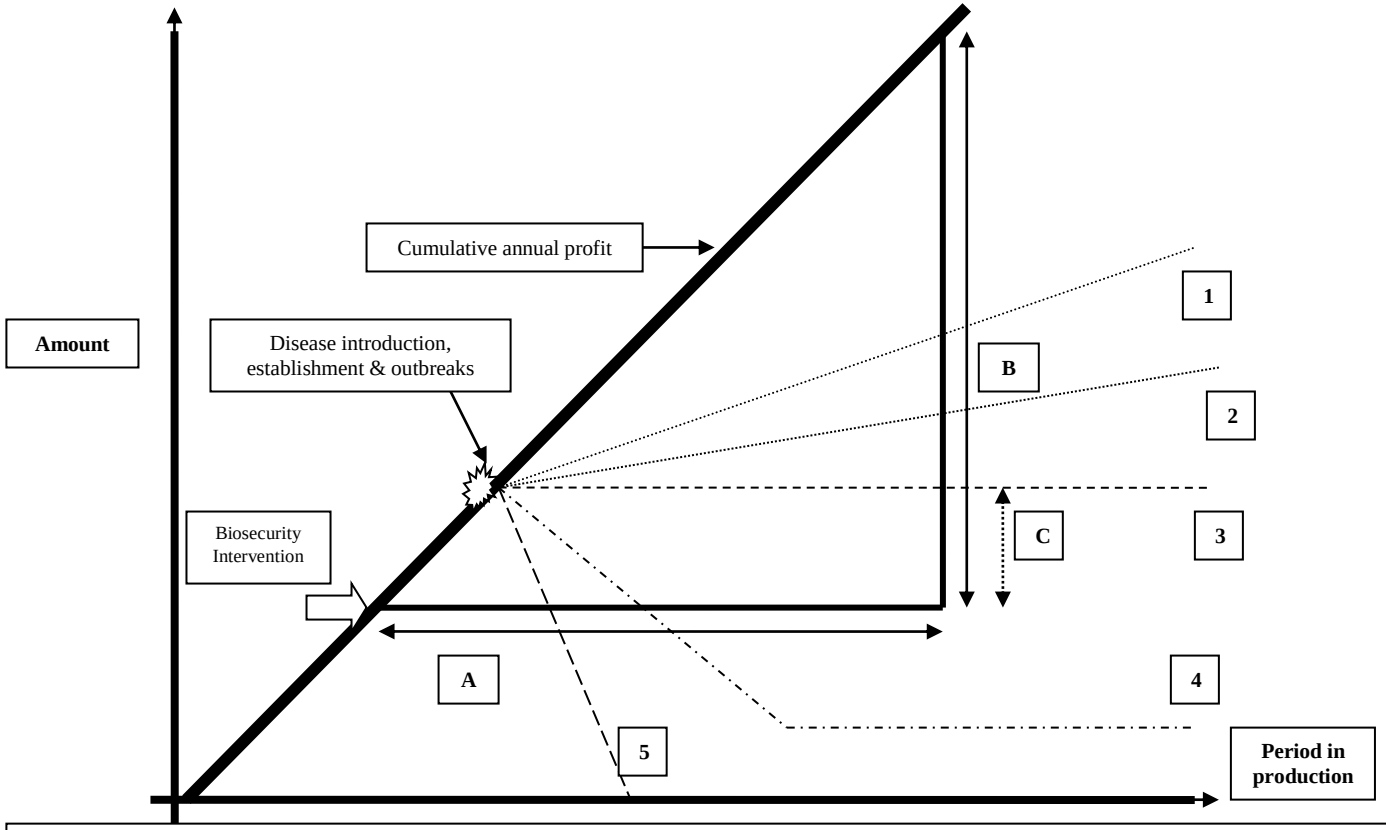
Model development

To assess the impact and cost-benefit of the implementation of biosecurity measures in household poultry, a schematic representation of cumulative annual profit (Figure 1) was developed with the following assumptions:

1. There will be a linear growth in cumulative profit for a year operation (12 months) totaling LE 2,389.67 (US$415.05) (Fasina et al., 2010; Appendix 1).
2. While some birds (laying birds and geese) will remain in the flock for the whole year, fattener birds and young birds will revolve 3-4 times (based on field observations).
3. In the theoretical situation where none of the proposed biosecurity measures are implemented, both HPAI H5N1 and Newcastle disease would occur twice a year in the flock and coccidiosis will occur with every batch within a year, based on field observations. These estimates take into account the 20% HPAI H5N1 vaccination coverage and 10% flock immunity reported for HHP by GOVS in Egypt (unpublished report), and the level of endemicity of each disease in the country.
4. Biosecurity will be introduced in the earlier part of annual farm operation.
5. Disease agents can be introduced, get established within the flock and cause outbreak at any period within the one-year operation.
6. Disease may cause minimal/graded reduction in levels of profit [1] or [2], prevent further profit but allow the farm to operate at break-even level [3], cause moderate losses [4], or cause losses above and beyond the profit made before the outbreak [5] (see Figure 1).
7. Biosecurity intervention will minimize losses [B], but some "gains" will also be made even in the absence of biosecurity intervention especially in a disease situation where 100% mortality will not be recorded [C]. [A] represents total costs spent on biosecurity for the whole year operation (Figure 1).

All calculations were done using the Egyptian Pound (LE). The exchange rate at the time of analysis was 5.7575LE = US$1.00.

Figure 1. GRAPHICAL REPRESENTATION OF THE EFFECTS OF BIOSECURITY INTERVENTION AND DISEASE SITUATIONS ON CUMULATIVE ANNUAL PROFIT IN HOUSEHOLD POULTRY PRODUCTION



Assuming that the cumulative annual profit will continue to grow linearly throughout the production period, disease may be introduce at any point and it will interfere with the linear growth in profit causing deviation and reducing maximum profit or tend to losses (1,2,3,4,5). Should Biosecurity intervention be implemented before the disease, it has a potential of preventing disease and still enable maximum profit to be achieved.
"A" represents total costs associated with the biosecurity intervention; "B" represents total benefits arising from the new intervention (discounting for benefits associated with probability of no disease "C"); "C" is benefit associated with probability of no disease without intervention. Following the point of disease introduction/outbreak, the effect on profitability may be 1, 2, 3, 4 or 5. While 1 may represent a mild/managed disease that continually reduce profitability e.g coccidiosis or endoparasitosis, 5 may represent disease like highly pathogenic avian influenza (leading to zero profit). The Benefit costs of biosecurity will be [B-C]/A

Assessment of actual production parameters

To collect production and other quantitative parameters, a field survey was conducted in Qalyubia, Gharbia and Menoufia governorates based on a pretested and validated questionnaire. Governorates are equivalent to States or Provinces; it is a second level of government administration which is below the national or federal government system and above the local government or provincial administration.

The choice of the governorates was based on their relative importance in poultry production in Egypt in general and in household poultry production in particular. Details of the selection criteria have been described elsewhere (Fasina et al., 2010).

A total of 191 household interviews were conducted in the selected governorates but three with inconsistent answers were excluded and 188 interviews were used in the analysis. Fifteen (15) villages were sampled in all. To ensure the reliability of data collected from the farmers, physical observations/counting of the flocks and photographic documentations were done. These were correlated with the interview responses. Where minimal disparities were noticed, observed data were used. It should be noted that household producers are sometimes unwilling to disclose certain information for fear of possible taxation/surcharge or governmental intervention in culling of the flock should HPAI H5N1 supervene.

Though pigeons are also widely kept in the households, pigeon production was not included in this study as they usually do not mix with poultry.

Based on the numbers of households in these governorates (2,808,982) and an assumption that approximately 95% of the rural households have poultry (2,668,533) (Geerlings et al., 2007), a minimum of 146 households was needed. The calculation of sample size was done using the formula:

Sample size $\boldsymbol{n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]}$

Where N = Population size (for finite population correction factor or fpc); p = Hypothesized % frequency of outcome factor in the population =95%±5; d = Confidence limits as % of 100 (absolute +/- %) = 5%; and the Design effect (for cluster surveys-DEFF) =1
(http://www.openepi.com/OE2.3/SampleSize/SSPropor.htm).

Experts' opinions survey: The following information was required for the analysis:

- Risk of introduction of major poultry diseases to the household flock without biosecurity.
- Associated mortality with each of the selected diseases.
- Associated income losses with each of the selected diseases.
- People-related risks for household poultry (people-related risks are risks of infection in the poultry flocks that are to a large extent directly related to human activities, e.g poultry manager taking care of other farms).
- Environment/flock-related risks for the household poultry (environment/flock-related risks are risks of infection that are associated with the flocks directly or their micro-environment, e.g multi-age or multi-species birds managed together).
- Other birds and other animal-related risks for the household poultry [these risks are those that are associated with other bird species (wild, free flying or scavenging) and other animals].

A list of national poultry experts was obtained from the national consultant. Ten experts were selected based on the following criteria: 2 each of poultry consultants, government veterinarians, private veterinarians, academia and poultry farm leaders, spread across governorates and districts. The opinions of all the 10 selected experts were sought using Delphi survey tools.

Delphi opinion surveys have been used in previous studies to evaluate the risks of poultry diseases but also for other studies where hard data were not available (Verhagen et al., 1998; Vaillancourt, 2002; Ferri et al., 2006). In this instance, the questionnaire was mailed electronically to each of the selected experts. 100% (10/10) returned the completed documents. All receipts were harmonized and a mean score arising from each question was statistically evaluated, calculated and tabulated against each expert score. This was mailed back to each expert to signify the degree of agreement or disagreement. Phone calls were employed to get a good return rate at the second instance. Ninety percent (90% or 9/10) approved the final questionnaire comparing the initial response of each expert to the mean experts' score individually. The last expert was not available

for the approval of the questionnaire. There was a 93% agreement score for all evaluated responses. Field validation of the scores was obtained through personal interviews with rural and district veterinarians in selected locations where farmers' interviews (to collect production parameters) were held. Similarly, the answers were compared with those available in the literature (Table 2b). There was a high degree of agreement between the experts' opinions, field surveys and the literature.

Statistics

All the descriptive statistics (means, medians, modes, quartiles, standard deviations and percentages) were evaluated at 95% confidence levels using Graphpad Quickcalcs® (statistical calculator, http://www.graphpad.com/quickcalcs/index.cfm). Means, medians, modes, quartiles and percentages were used for the production parameters used and disease risks. Biosecurity cost and other costs were evaluated using means and standard deviations. Analysis of variance (ANOVA) was used to compare means of category risks using Openepi® (http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm).

Benefit-cost assessment and sensitivity analysis

Based on the annual profitability of a household poultry project with a mixed flock size of 73, and the outcomes of experts' opinion survey, an assessment of the effect of biosecurity in a household poultry project was undertaken using partial budgeting (Chase, 2010) to evaluate the benefits and costs of the project. The choice of a flock size of 73 and mean annual profitability was based on previous assessment of mean flock size of household poultry in Egypt. Details have been reported elsewhere (Fasina et al., 2010; Box 1; Appendix 1 & 2-*Available on request and online at the journal site*).

Partial budgeting is a planning and decision-making framework used to compare the costs and benefits of alternatives faced by a farm business. It focuses mainly on changes in incomes and expenses that would result from the implementation of a specific alternative (PSU, 2002). Benefit-costs analysis has previously been used for evaluating the effect of biosecurity (Gifford et al, 1987) and other interventions in poultry (Sen et al., 1998, Fasina et al., 2007). In the present study, we used the tool to evaluate benefit-cost of biosecurity on highly pathogenic avian influenza (HPAI H5N1) but also Newcastle disease and coccidiosis. These three diseases were selected as representative disease situations based on the following factors:

- Rapidly fatal disease that will cause major production losses, lead to stamping-out of the remaining flock and closure of facilities with consequent downtime costs-**HPAI H5N1**.
- Important poultry disease that will cause economic loss (not in the magnitude of Highly pathogenic avian influenza), but is currently endemic in the Egyptian poultry populations-**Newcastle disease**.
- An endemic disease that will not cause major mortality but will reduce profitability continuously throughout the production cycle if not checked-**Coccidiosis**.

Biosecurity costs were estimated for the "**observed biosecurity-**the current level of application of biosecurity practices observed in the flocks surveyed", (Figure 2 and Table 1) and for the desirable level of biosecurity "**desirable biosecurity-**mimimum acceptable standards expected to be implemented to ensure effective protection for the flock", using the prevailing prices.

A list of basic biosecurity measures applicable to the household poultry production was drawn based on available documents (Nespeca et al., 1997; Pagani and Kilany, 2007; FAO, 2008 and Negro-Calduch, 2010) and evaluated on each farm (Table 1). The adherence to the complete list as outlined in Table 1 was taken as "desirable biosecurity" while partial application of these measures as observed on each farm was termed as "observed biosecurity". The annual financial involvement in all observed biosecurity per each household was calculated based on available information and a mean annual cost of observed biosecurity was derived from this evaluation (Figure 2). To enable the estimation of the real cost of desirable level of biosecurity per annum, the quantitative data were evaluated in financial terms and these evaluation included measures not currently adopted by the farmers. On measures whose cost was not available from the household producers, a market appraisal was undertaken to cost such items and these were added to the overall annual cost of biosecurity.

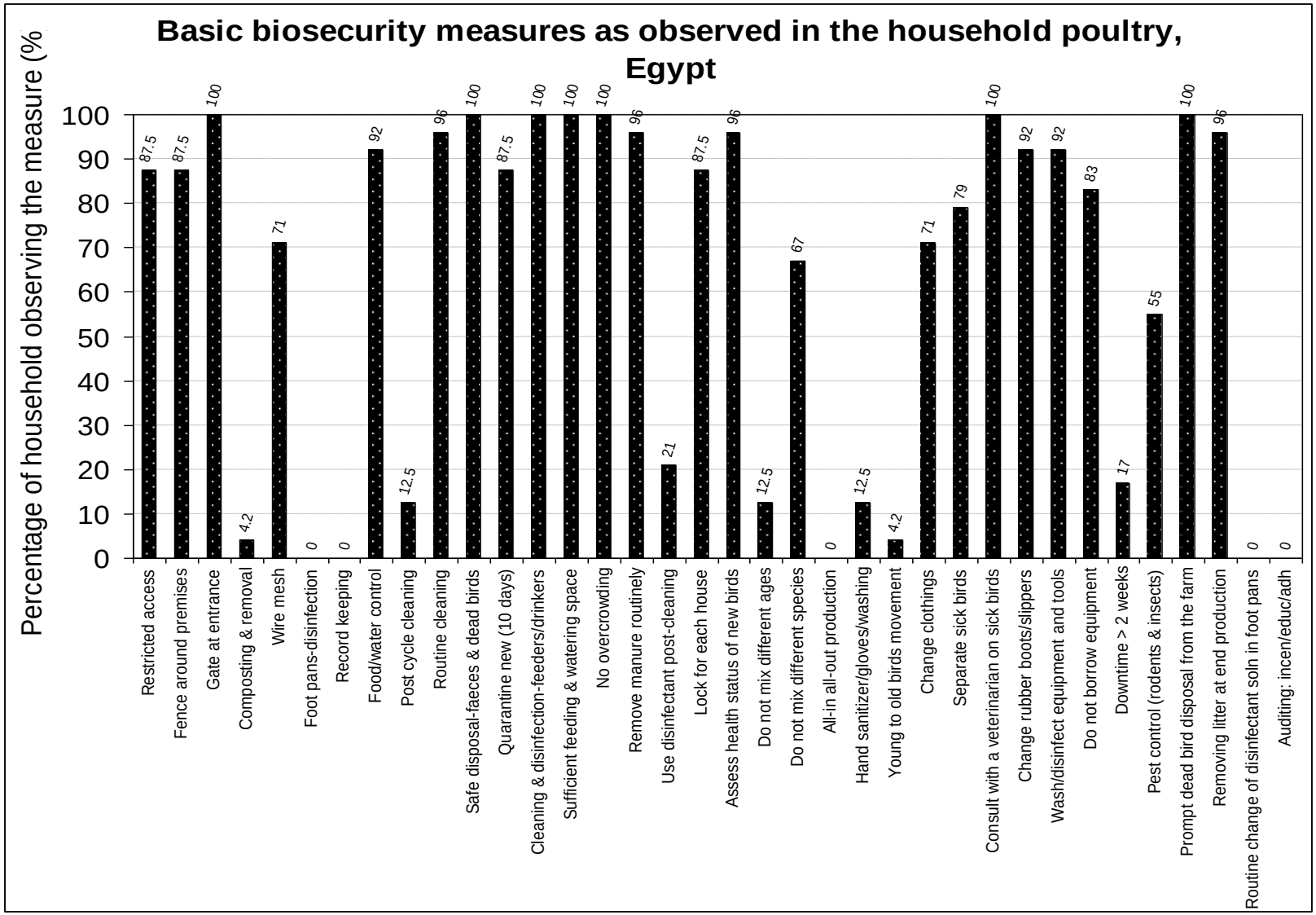


Figure 2. Basic Biosecurity measures as observed in the Egyptian household poultry.

Table 1. Description of basic biosecurity as observed amongst the Household poultry producers in Qalyubia, Menoufia and Gharbia Governorates, Egypt.

s/no.	**Biosecurity**	**Description**
1	Restricted access	Most poultry exist on rooftops or within fenced yards. Permission of the household is needed to access birds. This is taken as restricted access.
2	Fence around premises	Household fence sufficed for poultry fence
3	Gate at entrance	Household gate sufficed for poultry gate
4	Composting litter before removal	No producer compost.
5	Wire mesh window	These are provided but inadequate because it is torn in certain cases without immediate replacement.
6	Foot pans for disinfection before the house	Small wide-based household basin can be adapted to suit this purpose.
7	Record keeping	Provision of a simple exercise book to track visitors and activities in the poultry
8	Food and water control	Exclusion of feed and water from direct access by wild birds and other animals.
9	Terminal (Post cycle) cleaning	Thorough end-of-cycle cleaning.
10	Routine (regular) cleaning	Sweeping with broom and packing-off of debris.
11	Safe disposal of faeces and dead birds (is animal and insect proof)	Faeces and dead birds are removed in tightly sealed bags, delivered to refuse collector who take them away far from the premises
12	Quarantine new purchased birds for at least 10 days	Have a small section of the farm or a cage for new birds before further mixing with the flock.
13	Regular cleaning and disinfection of feeders and drinkers	Washing is done mainly by the use of water. Only in 17% cases was soap used in addition to water.
14	Sufficient feeding and watering space available for all birds	Freedom of each bird to access water and feed easily.
15	Sufficient space for each bird (No overcrowding)	Freedom to move without restrictions within the poultry and perch if necessary
16	Remove manure and litter routinely.	Routine is dependent on flock size and perception of the owner. No scheduled cleaning exists.
17	Usage of Disinfectant after cleaning	Usage of standardized disinfectants like phenols, quaternary ammonium etc
18	Lock for each house	Padlock or firmly secured entrance/door
19	Assess Health status of birds coming in	Assessment is only based on observation of brightness and conformation to normal birds. No empirically based assessment is done.
20	Do not mix different ages	Raised young birds separate from older birds. However, grouping was not based on sharply divided age but based on groups of 0-9 weeks and > 9weeks.

21	Do not mix different species	Have separate pens for rearing ducks and chickens. However, physical barriers between these two are not wide enough in several cases and may still permit spread of inter-species infection.
22	All-in all-out production	Clearly defined end of production cycle.
23	Hand sanitizer, gloves and washing	Only 4.2% use gloves in addition to hand sanitization. Usage of gloves is not regular also.
24	Going from young to older birds	The house is arranged so that young birds are visited first.
25	Change clothing when going in/out	Comprise mainly of old clothes. Though this may exist for the farmer, it is non-existent for the visitors.
26	Separate sick birds	Have a form of physical barrier for sick bird's isolation.
27	Consult with a veterinarian in case of sick birds	Usage of cheap or non-cost district veterinarian services
28	Change rubber boots/slippers	Have at least one pair of rubber slippers. Minimum of 2 pairs is desirable to permit twin changes or accommodate visitors.
29	Wash/disinfect equipment and tools	Most washing were done with water and none reported regular/routine washing using disinfectants
30	Do not borrow equipment from neighbors	Represents only poultry-related simple farm equipment.
31	Downtime $\geq$ 2 weeks	Observed minimum of 2 weeks downtime post one cycle. Most of the farmer observed forced biosecurity following outbreaks but not as part of the routine management system.
32	Pest control (rodents & insects)	Rodent poisons but also cats are used for pest rodent control. Rarely no effort is made to control insect pests
33	Prompt dead bird disposal from the farm	Removal of carcass as soon as sighted and packaging as part of the items for disposal to exclude further contacts.
34	Removing litter after each flock	End-of-cycle litter removal for use on farms.
35	Change solution in foot pans regularly	Application of disinfectants as foot dip and regular usage and maintenance of the prepared solution.
36	Auditing: incentives, education, adherence (encourage assistants to adhere to biosecurity)	Take care to observe that assistants (children, or other household that works in the poultry) including visitors observe the simple biosecurity rules set for the poultry.

*Compliance rate only indicated the percentage of farmers observed in the field who displayed some level of adoption of the stated biosecurity measure.
It is good to assume that measures that have a score of 70% and above can be easily adopted or intensified/improved, those with scores between 30 and 69% may need some effort to get it across to farmers and see it adopted, while those with scores below 30% will really need intensive education to get them entrenched in the poultry operation at the household level. Actually, some of them may not be adaptable to the current poultry practice in the household production sector.

Sensitivity analysis

The model was subjected to a selected list among the variety of factors that have the potential of affecting the profitability of the household poultry including 10% up to 80% increase in feed and grain prices sustained over a 1 year period (an unlikely event), 100% increase in biosecurity costs per annum, 50% increase in costs of other inputs per annum, 10% increase in the egg price, 10% decrease in the egg price and 10% increase in the price of day-old-ducks. These sensitivity assessments were done using the costs for the observed level of biosecurity and repeated using the cost for the desirable level of biosecurity for the household poultry.

Other factors that can affect the profitability of the project and the implementation of a biosecurity system include:

1. Human-related factors: Illness or death of the husband or wife, other individuals and organizations that contribute to the project.
2. Operation-related factors: Disruption to input-output supply balance in the poultry industry, loss of access to essential assets especially from the man, failure in distribution network of day-old birds, feed and other things.
3. Procedure-related factors: Failure of accountability especially in the commercially oriented household poultry, lack of complete internal systems and control including record keeping.
4. Project failure-related factors: Cost over-runs, extended periods of projects e.g delayed start of lay, delayed maturity, insufficient outputs and other products.
5. Nature-related factors: Weather change, natural disasters, accident and disease.
6. Political-related factors: change in governmental policies, change in tax systems, public opinion about household poultry and foreign influence.

Results

Experts' opinion survey

The outcome of the experts' opinion survey indicated that the risk of introduction of coccidiosis for the household poultry was 64% while those of Newcastle disease, HPAI H5N1, fowl pox, fowl cholera and endoparasitosis were 75%, 95%, 27%, 46% and 30% respectively (Table 2a). Associated mortality was highest in HPAI H5N1 with a mean score of 76.67% and lowest in endoparasitosis with a mean score of 2.40% (Table 2a). Similarly, HPAI H5N1 received the highest score of 83.33% in associated income losses as against the minimum score of 6.94% recorded for endoparasitosis (Table 2a).

People-related risks (risks of infection in the poultry flocks that are to large extent directly related to human activities) remained by far the most important risk category to the household poultry production with a score of 88.42%, while environment/flock-related risks had a score of 72.81% and other birds/other animal-related risks scored 68.00%. Although, an intra-category mean difference exists within each category, there was a statistically significant difference among the three risk categories with a *p*-value of 0.001 (Table 3).

Table 2a. Risks, associated mortalities and losses of selected poultry diseases in farms without biosecurity based on experts' opinions, Egypt

	Mean Experts' scores (range) at 95% confidence level		
Disease	**Percentage risk of introduction* ±Standard deviation (Range)**	**Percentage associated mortality ±Standard deviation (Range)**	**Percentage associated income loss ±Standard deviation (Range)**
Coccidiosis	**64±35.9** (37-91)	**11.83±10.8** (3-40)	**21.67±15.9** (0-50)
Newcastle disease	**75±21.8** (55-95)	**30.56±16.4** (5-60)	**35.55±8.3** (20-50)
HPAI H5N1	**95±10.8** (83-100)	**76.67±19.6** (20-100)	**83.33±18.3** (40-100)
Fowl pox	**27±14.1** (16-38)	**11.60±8.0** (1-25)	**16.39±11.2** (5-40)
Fowl Cholera	**46±23.5** (29-64)	**19.50±12.7** (1-40)	**24.44±11.1** (10-40)
Endoparasitosis	**30±19.6** (9-58)	**2.40±1.9** (0-5)	**6.94±3.4** (0-10)

*Zero risk is virtually impossible even in a farm with maximum biosecurity. Associated mortality is taken as percentage of total flock and associated income loss is taken as percentage of total income from poultry.

Table 2b. Risks, associated mortalities and losses of selected poultry diseases based on surveyed literatures

Diseases	**Percentage risk of introduction (Range)**	**Percentage associated mortality**	**Percentage associated income loss**	**Source**
Coccidiosis	NA	14.5%	11.86% of total income	Dana et al., 2000; Kinung'hi et al., 2004
Newcastle disease	≥70 to ≤ 100%	≤80%	30% of total income	Sen et al., 1998; Bell et al., 1995; Dana et al., 2000;
HPAI H5N1	≥70 to ≤ 100%	100%	80-100% total income and downtime costs	Fasina et al., 2007; Steensels et al., 2007
Fowl pox	NA	0-50% (5.1%)	NA	Dana et al., 2000; McMullin P., 2004; Sonaiya and Swan, 2004
Fowl Cholera	NA	25-35% (18%)	US$0.015/Kg meat	Choudhury et al., 1985; Morris and Fletcher,1988
Endoparasitosis	≥90 to ≤ 100%	1.0-78% (2.5%) Varied	≤30% of total income	Khan et al., 1999; Permin et al., 2002; Sonaiya and Swan, 2004

Table 3. Risk scoring and categorization based on experts' opinions, Egypt.

Risk Category	Risks	Percentage Mean Experts' score ± Standard deviation (range) at 95% confidence level	Percentage Category score ± Standard deviation (range) at 95% confidence level
People related	Poultry caretaker or manager own other birds	**98±6.4** (93.4 -100)	88.4±6.0[a] (80.86 – 95.94)
	Poultry owner visit live bird markets	**86±21.2** (70.8 -100)	
	Family member of poultry owner work in other farms/poultry	**82±14.8** (71.4 – 92.6)	
	Poultry owner visit other poultry	**86±16.4** (74.2- 97.8)	
	Poultry owner own other birds	**90±10.6** (82.4 – 97.6)	
Environment/flock related	Multi-aged birds kept together	**72±21.4** (56.6 – 87.4)	72.8±13.6 (63.06 – 82.54)
	Multi-sourced birds kept together	**60±21.0** (45.0 – 75.0)	
	Multi-breed birds kept together	**52±25.2** (34.0 – 70.0)	
	High density of household poultry in the area	**72±19.4** (58.2 – 85.8)	
	High density of small scale or commercial farms in the area	**66±25** (48.0 – 84.0)	
	Presence of another household producers within 250 metres	**60±13.4** (50.4 -69.6)	
	Dead bird disposal in water canal	**88±10.4** (80.6 – 95.4)	
	Workers (vaccinators, gas supplier, farm gate buyers etc)	**94±9.6** (87.0 - 100)	
	Lack of biosecurity awareness by poultry owner	**80±16.4** (68.4 – 91.6)	
	Sick and dead birds are not separated promptly	**84±15.8** (72.8 – 95.2)	
Other birds and other animal related	Rats and mice are present in the farm	**64±20.6** (49.2 – 78.8)	68.0±12.6 (47.88 – 88.12)
	Wild birds have access to poultry	**80±23.0** (63.4 – 96.6)	
	Old and new batches of birds are mixed	**86±31.0** (53.4 – 98.2)	
	Cats and dogs have access to poultry	**52±21.4** (36.6 – 67.4)	

[a]Using the analysis of variance to test for differences among the three category scores, significant difference was observed (*p*-value = 0.001) (http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm)

Note: People related risks are risks of infection in the poultry flocks that are to large extent directly related to human activities. Environment/flock related risks are those associated with the poultry flock or their microenvironment (pen, density, multi species, multiage, closeness of pens etc). Other birds/other animal related risks are those associated with outside the bird immediate environment (wild bird, scavenging animals, rodents etc)

Benefit-cost analysis

From the combination of partial budgeting and benefit-cost analysis, the implementation of all biosecurity measures (desirable level) will generate an increase in net annual income of 4386.16LE (US$761.82) for risk of HPAI H5N1 infection alone. Without biosecurity, a total of zero to 1451.59LE (US$252.13) in net annual income may be saved depending on the severity of the infection (Table 4). The benefit-cost ratio of implementing biosecurity is 8.45. For Newcastle disease, the increase in net income is 2534.26LE (US$440.17) with a benefit-cost ratio of 4.88. In the case of coccidiosis, the increase in net income is 772.10LE (US$134.10) with a benefit-cost ratio of 1.49. Details are available in Tables 4, 5 and 6. Note that it has not been possible to do a benefit-cost analysis comparing current production practices (observed biosecurity) with a situation where desirable biosecurity will be implemented. This is because a situation of partial biosecurity is equivalent to a no-biosecurity since a breach in the complete protocol will still expose the birds to high risk of infection. Moreover, benefit-cost analysis was assessed for each disease separately. The real benefit-cost ratio for the producer will be higher than that as all farms are exposed to all diseases.

Table 4. Overall Incomes and Costs from biosecurity implementation against HPAI H5N1

Increase in net incomes		**Decrease in net incomes**	
Total profit from birds due to biosecurity intervention	1891.66LE (US$ 328.55)	Cost of biosecurity implementation per annum	519.00LE (US$90.14)
Downtime cost from disease (HPAI)	46.67LE (US$8.11) x 2		
Disease management and eradication costs at farm level*	71.00LE (US$12.33)		
Wasted feeds/other supplies (1 month supplies)	339.29LE (US$58.93)x 2		
Sub total	**2734.58LE (US$474.96)**	**Sub total**	**519.00LE (US$90.14)**
Decrease costs		**Increase costs**	
Losses associated with the disease	3622.17LE (US$629.12)	Assumed profit from birds without intervention	498.00LE (US$86.50)
		Value of total costs saved without intervention of biosecurity	953.59LE (US$165.63)
Sub total	**3622.17LE (US$629.12)**	**Sub total**	**1451.59LE (US$252.13)**
Overall increase in net income	**6356.75LE (US$1104.08)**	**Overall decrease in net income**	**1970.59LE (US$342.26)**
Change in net income due to biosecurity intervention			**4386.16LE (US$761.82)**
BENEFITS OF BIOSECURITY AGAINST HPAI H5N1			
Total net benefits of implementing biosecurity			4386.16LE (US$761.82)
Total costs of implementing biosecurity			519.00LE (US$90.14)
Benefit/cost of biosecurity			**8.45**

All calculations were done in Egyptian pounds (LE) with US dollars equivalent in parenthesis. It is about eight and a half times better (845%) to implement biosecurity that not to do so against avian influenza H5N1.

Benefit cost = Net benefit divided by cost of biosecurity.

*Note the government will also bear the larger disease management costs.

Using a realistic value for biosecurity to cover all of the items listed for biosecurity, an estimate of 519LE/annum was achieved. Assuming that biosecurity is targeted at the risk of HPAI H5N1 and the Risk of introduction of HPAI H5N1 is 95% (Experts' opinions) and if the farm is infected twice a year (50:50 chance of infection of the 4 cycle). If it is taken that biosecurity will be effective in preventing infection (since zero risk is virtually impossible, and absence of the infectious organism may also be responsible for a no-disease state), and that 83.33% income losses will be achieved in HPAI H5N1 infection (Experts' opinion), then:

Risk of introduction is 95%.

Economic losses due to HPAI H5N1 = 95% of 83.33% = **79.16%**

Total profit saved = 79.16/100 x 2389.67LE (total profit) = **1891.66LE (US$ 328.55)** (see calculation on production parameters)

Downtime cost = 100% of 100/3 (housing cost/4mnths) + 20/3 (equipment-feeders and drinkers/4mnth) + 20/3 (cages/4mnth) = **46.67LE (US$8.11)** (see calculation on production parameters)

Wasted feed (one month supply) = 4071.44/12 = **339.29LE (US$58.93)**

Management and eradication costs = **71LE (US$12.33)** (post culling cleaning, farm-level disinfection and washing)

Cost of disease = 66.67% of investment= 79.16/100 x 4575.76LE (investment per annum) = **3622.17LE (US$629.12)**

Assumed profit from bird saved without intervention= [(100-79.16) % x 2389.67] = **498.00LE (US$86.50)**

Value of total cost saved without intervention= [(100-79.16) % x 4575.76] = **953.59LE (US$165.63)**

Cost of biosecurity = **519LE (US$90.14)**

The factor of 79.16% will not come into the calculation for "assumed profit from bird saved and value of total cost saved" because without biosecurity intervention and with infection, the whole birds will be culled and no extra bird will remain. ***The exchange rate at the time of analysis was 5.7575LE = US$1.00.***

Table 5. Overall Incomes and Costs from biosecurity implementation against Newcastle disease

Increase in net incomes		**Decrease in net incomes**	
Total profit from birds due to biosecurity intervention	637.32LE (US$110.69)	Cost of biosecurity implementation per annum	519.00LE (US$90.14)
Downtime cost from disease (NDV)	11.67LE (US$2.03)x 2		
Disease management and eradication costs at farm level*	71.00LE (US$12.33) + 52LE (US$9.03)(13LE/qtr)		
Wasted feeds/other supplies (1 month supplies)	254.47LE (US$44.20) x 2		
Sub total	**1292.60LE (US$224.51)**	**Sub total**	**519.00LE (US$90.14)**
Decrease costs		**Increase costs**	
Losses associated with the disease	3050.66LE (US$529.86)	Assumed profit from birds without intervention	442.59LE (US$76.87)
		Value of total costs saved without intervention of biosecurity	847.41LE (US$147.18)
Sub total	**3050.66LE (US$529.86)**	**Sub total**	**1290.00LE (US$224.05)**
Overall increase in net income	**4343.26LE (US$754.37)**	**Overall decrease in net income**	**1809.00LE (US$314.20)**
Change in net income due to biosecurity intervention	**2534.26LE (US$440.17)**		
BENEFITS OF BIOSECURITY AGAINST NEWCASTLE DISEASE			
Total net benefits of implementing biosecurity	2534.26LE (US$440.17)		
Total costs of implementing biosecurity	519.00LE (US$90.14)		
Benefit/cost of biosecurity	**4.88**		

All calculations were done in Egyptian pounds (LE) with US dollars equivalent in parenthesis. It is approximately five times better (488%) to implement biosecurity against Newcastle disease alone.

*Product saved and value of bird saved without intervention were calculated as [(100-30.56) x 26.67% x 2389.67LE or 4575.76LE] where 30.56 is percentage mortality due to Newcastle, 26.67% is 75% of 35.55% (economic losses), 2389.67LE is 100% profit per annum and 4575.76LE is value of total costs per annum. Since Newcastle will not kill all the birds and vaccination is routinely administered from the source of day-old-birds, we will assume that it will affect every other (50:50 chance) cycle (approximately 4 cycles per annum). Therefore some parameters that will be affected quarterly were multiplied by 2.

The exchange rate at the time of analysis was 5.7575LE = US$1.00.

Table 6. Overall Incomes and Costs from biosecurity implementation against Coccidiosis

Increase in net incomes		**Decrease in net incomes**	
Total profit from birds due to biosecurity intervention/annum	331.42LE (US$57.56)	Cost of biosecurity implementation per annum	519.00LE (US$90.14)
Downtime cost from disease (Cocci)/quarter	11.67LE (US$2.03) x 4		
Disease management and eradication costs at farm level (including vet services)	71.00LE (US$12.33) + 52LE (US$9.03)(13LE/qtr)		
Wasted feeds/other supplies (0.75 month supplies)/quarter	254.47LE (US$44.20) x 4		
Sub total	**1518.98LE (US$263.83)**	**Sub total**	**519.00LE (US$90.14)**
Decrease costs		**Increase costs**	
Losses associated with the disease/annum	634.66LE (US$110.23)	Assumed profit from birds without intervention	295.92LE* (US$51.40)
		Value of total costs saved without intervention of biosecurity	566.62LE* (US$98.41)
Sub total	**634.66LE (US$110.23)**	**Sub total**	**862.54LE (US$149.81)**
Overall increase in net income	**2153.64LE (US$374.06)**	**Overall decrease in net income**	**1381.54LE (US$239.95)**
Change in net income due to biosecurity intervention	**772.10LE (US$134.10)**		
BENEFITS OF BIOSECURITY AGAINST COCCIDIOSIS			
Total net benefits of implementing biosecurity	772.10LE (US$134.10)		
Total costs of implementing biosecurity	519.00LE (US$90.14)		
Benefit/cost of biosecurity	**1.49**		

All calculations were done in Egyptian pounds (LE) with US dollars equivalent in parenthesis.

*Product saved and value of bird saved without intervention were calculated as [(100-10.72) x 13.87% x 2389.67LE or 4575.76LE] where 10.72 is percentage mortality due to coccidiosis, 13.87% is 64% of 21.67% (economic losses), 2389.67LE is 100% profit per annum and 4575.76LE is value of total costs per annum. Since coccidiosis will not kill all the birds, we will assume that it will affect every cycle (approximately 4 cycles per annum). Therefore some parameters that will be affected quarterly were multiplied by 4. The factor of 13.87% comes into the calculation for "assumed profit from bird saved and value of total cost saved" because the intervention is expected to save extra costs from infected birds that did not die and can still produce.

It is approximately one and a half times better (149%) to implement biosecurity against coccidiosis alone.

The exchange rate at the time of analysis was 5.7575LE = US$1.00.

Sensitivity analysis

Subjecting the model to a variety of input and output changes revealed that the household poultry project is robust and will withstand a wide variation in prices of inputs and outputs. For the case of observed biosecurity, a 10% sustained all-year round increase in prices of feed and grains will decrease the profit margin by 17.80% while sustained feed and grain price increases of 55% will reduce profit by 97.78%. However, any increase in the price of feed and grains beyond the margin of 56% will tend to losses for the project (Table 7).

A 100% increase in costs of observed biosecurity will only reduce the profit margin by 4.46% while a 50% increase in prices of other inputs will reduce profit by 11.20%. A ten percent (10%) increase in price of eggs and day-old-ducks will reflect positively on the project by increasing the profit 5.32% and 7.39% respectively while a 10% decrease in egg price will also reduce profit by 5.32% (Table 7, Appendix 2).

Using the same parameters for the desirable level of biosecurity, a 10% sustained all-year round increase in prices of feed and grains will decrease the profit margin by 21.76% while a sustained feed and grain price increase of 40% will reduce profit by 87.05%. In this case, any sustained increase in the price of feed and grains beyond the margin of 46% will tend to losses for the project (Table 8).

A 100% increase in costs of desirable biosecurity will reduce the profit margin by 27.74% while a 50% increase in prices of other inputs will reduce profit by 13.69%. A ten percent (10%) increase in the price of eggs and day-old-ducks will reflect positively on the project by increasing the profit 6.50% and 9.04% respectively while a 10% decrease in egg pricewill also reduce profit by 6.50% (Table 8).

Overall, a huge increase in the price of feed and grains (over 40%) that is sustained over a period of time may force the closure of the poultry business as it has the most significant single effect on the project in the event of the implementation of biosecurity.

Discussion

The in-house poultry production and its intensity in Egyptian households present with some specific challenges for animal disease control as had been reported in other places (Cristalli and Capua, 2007). The current management practices provide for highly connected poultry networks within villages, districts and governorates. Within such networks, on-farm (in-house) and community-mediated biosecurity are vital components of disease prevention and control (Nespeca et al., 1997; Hogerwerf, 2010; Dorea et al., 2010). These preventative measures are especially more important in view of the likelihood of human infections should zoonotic poultry disease outbreak occur within the household (Fiebig et al., 2009). In this study, we modeled a real field situation of biosecurity implementation in the household poultry and assessed its cost-effectiveness. Implementation of all biosecurity measures will generate a higher net annual income and there is a 8.45 benefit-cost ratio for implementation of desirable biosecurity, for highly pathogenic avian influenza H5N1 alone; the ratio is 5 and 1.5 for Newcastle disease and Coccidiosis respectively.

Although the application of some biosecurity measures is part of current husbandry practices within household poultry production in Egypt (Figure 2; Appendix 2), the non-implementation of other measures will limit the impact of those implemented. The differing levels of adoption by different household poultry producers have implications for the overall breach in biosecurity with the consequent risks of infection in the household. Low frequencies of adoption of a comprehensive biosecurity package have been confirmed in previous studies to contribute significantly to disease outbreaks on farms (Bos et al., 2007; East, 2007; Kung et al., 2007; McQuiston et al., 2005; Thomas et al., 2005; Dorea et al., 2010; Fasina et al., 2011The adoption of certain measures however indicated that given the right atmosphere, the household producers were willing to improve their management and disease control practices.

In our submission, certain items of biosecurity which are practical elsewhere, for example composting are incompatible with the Egyptian household poultry. Wilkinson had earlier identified the risks associated with such measures at farm level (Wilkinson, 2007). It will be necessary to

critically assess, identify, implement and monitor practicable biosecurity measures (others with the exception of composting, see Table 1) within the Egyptian HHP and in other places where such policies are being implemented.

While the authorities may want to implement important control programmes such as prohibition/punishment including the confiscation of poultry, bans on rural poultry and vaccination alone as means of mitigation, these measures will have a negative effect on control and eradication of poultry diseases, especially at the household level. It should be understood that control and eradication policies should not be made to the exclusion of the socio-cultural value systems of the society; as such policies are bound to fail from inception (Scoones and Forster, 2010).

Since household poultry production is the way of the people, the reorganization of the Egyptian poultry industry, including the entrenchment of key aspects of biosecurity at all sectors of the industry, will yield positive results if introduced in a way that enables smooth adoption and gradual replacement or improvement on the current management and hygiene practices observed in these household flocks (FAO, 2011).

For the household poultry in particular, it will be essential to create a "biosecurity uptake pathway" that will reinforce those measures that currently have wide adoptions amongst household producers (Figure 2) and gradually introduce those whose current levels of compliance are lower. In the present circumstances, these can begin with the implementation of gates for the household; safe disposal of dead birds and faeces in polythene bags to exclude the carrion eaters, stray animals, insects and rodents; cleaning but also disinfection of drinkers and feeders, and farmers consultations with village-based veterinarians;. Subsequently, other measures can then be introduced (Figure 2). In particular, the district and village veterinarians should be used to continuously impress the messages of biosecurity to the HHP producers since they have the confidence of rural farmers, they are regularly consulted and their opinions are well respected by the latter.

Though we agreed that the evaluation of risks, associated mortalities and income losses in the household poultry based on expert opinion may be skewed, subjective and based purely on the current endemicity and waves of outbreaks in poultry in Egypt, the analytic tool (Delphi opinion survey) used in this study is time-tested and had been cited at least 2000 times in agricultural, clinical, epidemiological and social science-related research (Verhagen et al., 1998; Linstone and Turrof, 2002; Ferri et al., 2006; Halvorson and Hueston, 2006).

Furthermore, in this study the collated data from the survey were assessed against field evaluation and surveyed literatures and was also statistically analyzed, and comparable results were obtained. HPAI H5N1, but also Newcastle disease and coccidiosis, are diseases identified as posing the greatest risks to the HHP production system. While major income losses will result from an HPAI H5N1 outbreak, graded reduction in profit was associated with Newcastle disease and coccidiosis in this assessment (Table 2a). Vaccination is cited as being responsible for a reduced associated mortality in the case of Newcastle disease (Personal communication with field experts). However, biosecurity can reduce the risk of outbreak of these diseases by huge margins of up to 95% in certain cases and generate higher incomes (Table 4-6).

Since the people-related risks were identified as significantly related to infections in the HHP (p-value = 0.001), effort should be directed to immediately implement those measures that will control people's access to HHP in Egypt (Vaillancourt, 2002). Only essential persons in the HHPP system should be allowed access to the birds and these individuals must observe implemented measures. Kung and colleagues had previously advocated for similar measures in Hong Kong (Kung et al., 2007). The adoption of such measures in the Egyptian household is currently fair to good as observed in the field (≤100%) but will need to be enhanced and reinforced (Table 3 & Figure 2).

The individual and cumulative benefits-cost of biosecurity implementation is huge in the HHP in view of its effects on the different diseases assessed and this justifies the need to carry out these measures in the Egyptian poultry industry (Table 4-6). While the measures will protect the HHP, it will also greatly reduce the risks of human infection. Though cost associated with the loss of human lives arising from poultry zoonotic infections like HPAI H5N1 is difficult to estimate in this study, we believe such cost will further strengthen the reason for the implementation of biosecurity at the HHP production sector. This is aside from the outbreak-associated costs that will be borne by the government and other sectors including direct production costs, poultry traders, feed mills and

breeder losses, culling and control costs, export-import bans, reduced consumption, reduced trade and economic activities, cost of vaccination, medication, hiring, clean-up and compensation.

The HHP production is robust and will withstand very severe price variation (Table 7). In our assessment, even a sustained annual increase in the price of major items like feed and grains up to a total of 56% per annum will only reduce profit marginally. It is highly unlikely that the price of grain and poultry feed will remain at that level for a whole year without government intervention since grain remains a key item in Egyptian meals. Adopting desirable/improved biosecurity measures as outlined in Table 1 will not gravely affect the profitability of the business (Table 8). It will therefore be essential to up the level of basic biosecurity in the Egyptian HHP and farmers should be willing to adopt the various measures.

The current study has identified in financial terms that the implementation of basic biosecurity measures will contribute to the overall objective of household poultry production in Egypt. However, field trials (in the form of a controlled study) may be essential to validate the effectiveness of the model presented herein in the field (Nespeca et al., 1997).

Finally, to the best of our knowledge, this work represents the first attempt that used a combination of epidemiological techniques to explore the possibility of adapting biosecurity measures to the household poultry production system.

Table 7. Sensitivity analyses of the household poultry project with implementation of observed/current level of biosecurity

Percentage change (Item)	Price variation (LE)	Current price (LE)	Other inputs (LE)	Sub total (LE)	Biosecurity cost (LE)	New sub total (LE)	Standard annual profit (LE)	New annual profit (LE)	Change in profit per annum (%)
Standard	**0**	4071.44	504.32	4575.76	102	4677.76	2287.67	NA	
10% increase (feed+grains)	**407.1**	4071.44	504.32	4982.86	102	5084.86	2287.67	1880.57	407.10 (17.80%)
20% increase (feed+grains)	**814.2**	4071.44	504.32	5389.96	102	5491.96	2287.67	1473.47	814.20 (35.60%)
30% increase (feed+grains)	**1221.3**	4071.44	504.32	5797.06	102	5899.06	2287.67	1066.37	1221.30 (53.39%)
40% increase (feed+grains)	**1628.4**	4071.44	504.32	6204.16	102	6306.16	2287.67	659.27	1628.40 (71.18%)
50% increase (feed+grains)	**2035.5**	4071.44	504.32	6611.26	102	6713.26	2287.67	252.17	2035.50 (88.98%)
55% increase (feed+grains)	**2239.05**	4071.44	504.32	6814.81	102	6916.81	2287.67	48.62	2239.05 (97.78%)
56% increase (feed+grains)	**2279.76**	4071.44	504.32	6855.52	102	6957.52	2287.67	7.91	2279.76 (99.65%)
57% increase (feed+grains)	**2320.47**	4071.44	504.32	6896.23	102	6998.23	2287.67	-32.8	2320.47 (101.43%)*
60% increase (feed+grains)	**2442.6**	4071.44	504.32	7018.36	102	7120.36	2287.67	-154.93	2442.60 (106.77%)*
61% increase (feed+grains)	**2483.31**	4071.44	504.32	7059.07	102	7161.07	2287.67	-195.64	2483.31 (108.55%)*
62% increase (feed+grains)	**2524.02**	4071.44	504.32	7099.78	102	7201.78	2287.67	-236.35	2524.02 (110.33%)*
63% increase (feed+grains)	**2564.73**	4071.44	504.32	7140.49	102	7242.49	2287.67	-277.06	2564.73 (112.11%)*
70% increase (feed+grains)	**2849.7**	4071.44	504.32	7425.46	102	7527.46	2287.67	-562.03	2849.70 (124.57%)*
80% increase (feed+grains)	**3256.8**	4071.44	504.32	7832.56	102	7934.56	2287.67	-969.13	3256.80 (142.63%)*
100% increase (biosecurity)	102	4071.44	504.32	4677.76	**102+102**	4881.76	2287.67	2083.67	102 (4.46%)
50% increase (other inputs)	252.16	4071.44	**504.32+ 252.16**	4827.92	102	4929.92	2287.67	2035.51	256.16 (11.20%)
10% increase (egg price)	**121.68**	4071.44	504.32	4575.76	102	4556.08	2287.67	**2409.35**	121.68 (5.32%)
10% decrease (egg price)	**121.68**	4071.44	504.32	4697.44	102	4799.44	2287.67	**2165.99**	121.68 (5.32%)
10% increase (DOD price)	**169.06**	4071.44	504.32	4575.76	102	4508.7	2287.67	**2456.73**	169.06 (7.39%)

All asterisks are negative values indicating losses. Bold values are the major changes introduced into the model to calculate the new annual profit margin.

The exchange rate at the time of analysis was 5.7575LE = US$1.00.

Table 8. Sensitivity analyses of the household poultry project with implementation of improved/desirable level of biosecurity

Percentage change (Item)	Price variation (LE)	Current price (LE)	Other inputs (LE)	Sub total (LE)	Biosecurity cost (LE)	New sub total (LE)	Standard annual profit (LE)	New annual profit (LE)	Change (%)
Standard	**0**	4071.44	504.32	4575.76	519	4677.76	1870.67	1870.67	
10% increase (feed+grains)	**407.1**	4071.44	504.32	4982.86	519	5501.86	1870.67	1463.57	407.10 (21.76%)
20% increase (feed+grains)	**814.2**	4071.44	504.32	5389.96	519	5908.96	1870.67	1056.47	814.20 (43.52%)
30% increase (feed+grains)	**1221.3**	4071.44	504.32	5797.06	519	6316.06	1870.67	649.37	1221.30 (65.29%)
40% increase (feed+grains)	**1628.4**	4071.44	504.32	6204.16	519	6723.16	1870.67	242.27	1628.40 (87.05%)
50% increase (feed+grains)	**2035.5**	4071.44	504.32	6611.26	519	7130.26	1870.67	**-164.83**	2035.50 (108.81%)*
55% increase (feed+grains)	**2239.05**	4071.44	504.32	6814.81	519	7333.81	1870.67	-368.38	2239.05 (119.69%)*
56% increase (feed+grains)	**2279.76**	4071.44	504.32	6855.52	519	7374.52	1870.67	-409.09	2279.76 (121.87%)*
57% increase (feed+grains)	**2320.47**	4071.44	504.32	6896.23	519	7415.23	1870.67	-449.8	2320.47 (124.04%)*
60% increase (feed+grains)	**2442.6**	4071.44	504.32	7018.36	519	7537.36	1870.67	-571.93	2442.60 (130.57%)*
61% increase (feed+grains)	**2483.31**	4071.44	504.32	7059.07	519	7578.07	1870.67	-612.64	2483.31 (132.75%)*
62% increase (feed+grains)	**2524.02**	4071.44	504.32	7099.78	519	7618.78	1870.67	-653.35	2524.02 (134.93%)*
63% increase (feed+grains)	**2564.73**	4071.44	504.32	7140.49	519	7659.49	1870.67	-694.06	2564.73 (137.10%)*
70% increase (feed+grains)	**2849.7**	4071.44	504.32	7425.46	519	7944.46	1870.67	-979.03	2849.70 (152.34%)*
80% increase (feed+grains)	**3256.8**	4071.44	504.32	7832.56	519	8351.56	1870.67	-1386.13	3256.80 (174.10%)*
100% increase (biosecurity)	519	4071.44	504.32	4677.76	**519+519**	4881.76	1870.67	1453.67	519 (27.74%)
50% increase (other inputs)	252.16	4071.44	**504.32+252.16**	4827.92	519	5346.92	1870.67	1618.51	256.16 (13.69%)
10% increase (egg price)	**121.68**	4071.44	504.32	4575.76	519	4973.08	1870.67	**1992.35**	121.68 (6.50%)
10% decrease (egg price)	**121.68**	4071.44	504.32	4697.44	519	5216.44	1870.67	**1748.99**	121.68 (6.50%)
10% increase (DOD price)	**169.06**	4071.44	504.32	4575.76	519	4925.7	1870.67	**2039.73**	169.06 (9.04%)

All asterisks are negative values indicating losses. Bold values are the major changes introduced into the model to calculate the new annual profit margin.

The exchange rate at the time of analysis was 5.7575LE = US$1.00.

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Box 1. Summary of calculations, costs and formulas used in the analysis.

1. Total profit due to Biosecurity intervention ($_{T}$PB) = % Risk of introduction ($_{D}$R) X % Economic loss due to disease ($_{D}$E) X Expected total Profit ($_{E}$P)

2. Downtime cost = 1/3 of cost of renting the pen per annum + 1/3 cost of equipment per annum + 1/3 cost of cages per annum (based on downtime of four month should HPAI H5N1 occur) X 2 (twice chance of occurrence)

3. Wasted feed cost = total annual cost of feed / 12 (assuming 1 month stock is kept)

4. Management and eradication costs = (based on field survey for cost of cleaning materials, disinfectant and detergent)

5. Cost of disease = % Risk of introduction ($_{D}$R) X % Economic loss due to disease ($_{D}$E) X Expected total Investment or costs

6. Profit saved without intervention = Total profit – [% Risk of introduction ($_{D}$R) X % Economic loss due to disease ($_{D}$E) X Expected total Profit ($_{E}$P)]

7. Total cost saved without intervention = Total investment costs – [% Risk of introduction ($_{D}$R) X % Economic loss due to disease ($_{D}$E) X Expected total Investment or costs]

8. Cost of Biosecurity = (based on summation of field and farmers' surveys, See Fasina et al., 2010)

Information on the basic costing used in this analysis was obtained from Table 2a and Fasina et al., 2010: - Mean number of birds = 73; Maximum turnover/year = 4; Total feed cost per annum = 4071.44 LE (US$707.15); Overall total expenses including feed = 4575.76 LE (US$794.75); Observed biosecurity cost = 102 LE (US$17.72); Desirable biosecurity cost = 519 LE (US$90.14); Total output/annum = 6965.43 LE (US$1209.80); Total annual profit = 2389.67 LE (US$415.05).

Chapter 6

Connecting network properties of rapidly disseminating epizoonotics

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Connecting network properties of rapidly disseminating epizoonotics

Abstract

Background: To determine whether epidemics exhibit, at low geo-temporal scale, properties so far only globally described, constructs designed to adapt Network Theory concepts into low-scale indicators were evaluated. Suspecting that rapid microbial dissemination requires not only susceptible hosts but also a pre-existing, connecting network, the road structure was investigated.

Methods: Using geo-temporal data collected from epidemics in which all hosts were susceptible (Foot-and-mouth disease, Uruguay, 2001, which infected mammals; and Avian Influenza H5N1, Nigeria, 2006, which infected birds), two models were compared: 1) 'connectivity', which integrated biological and physical concepts (the agent's transmission cycle, road topology) into indicators designed to measure networks ('nodes' or infected sites with short- and/or long-range links), and 2) 'contacts', which focused on infected individuals but did not assess connectivity.

Results: The connectivity model showed five network properties: 1) spatial aggregation of cases (disease clusters), 2) links among similar 'nodes' (assortativity), 3) simultaneous activation of similar nodes (synchronicity), 4) epidemic flows moving from highly to poorly connected nodes (directionality), and 5) a few nodes accounting for most cases (a "20:80" pattern). In both epidemics, 1) not all primary cases were connected but at least one primary case was connected, 2) highly connected, small areas (nodes) accounted for most cases, 3) several classes of nodes were distinguished, and 4) the contact model –which assumed all primary cases were identical– captured half the number of cases identified by the connectivity model. When assessed together, the synchronicity and directionality properties explained where and when an epidemic spreads.

Conclusions: Retrospective analyses of low-scale bio-geo-temporal indicators confirmed global network properties, detecting several types of cases, clusters, nodes, and networks. Such new information may be applied in theory and cost-benefit based practices. Prospective studies that consider low-scale, pre-epidemic predictors, such as a connecting network, are recommended.

Introduction

The first recorded effort of a successful intervention aimed at controlling an epidemic was that of John Snow –the British physician who, in 1854, discovered and prevented the dissemination mechanism of cholera epidemics [1]. Snow integrated what, today, could be described as medicine, statistics, geography, civil engineering, and cost-benefit analysi

s: he mapped London's water network and, with simple graphs, quantified the number of cholera cases associated with specific households (http://en.wikipedia.org/wiki/File:Snow-cholera-map.jpg). That led him to geographically identify the water pump suspected to be contaminated. By removing the handle of the pump –leaving it non-operational–, he stopped the epidemic.

He did not intervene on people. He did not intervene on the pathogen. He intervened on a physical structure that connected susceptible hosts with the microbe –the water distribution network– and did so before infections could occur. Snow acted on connectivity –a concept related to, but independent from both the infectious agent and the susceptible host.

His example provides a reference against which views on how epidemics spread can be analyzed. Hoping that a review of fields involved in epidemiology may identify unmet research needs, the contents of mathematical epidemiology and medical geography are summarized.

Mathematical epidemiology focuses on hosts. It asks who is in contact with whom.

[2, 3]. This field began in 1908, when Sir Ronald Ross, after discovering that mosquitoes transmit malaria, defined the 'critical mosquito density' (later known as the basic reproductive number, or R_0 [4]. The R_0 is the ratio of secondary cases generated per primary case which, if >1, indicates that the epidemic will disseminate; and, if ≤1, predicts that the epidemic will soon die out [5]. This approach has been applied both in endemic and in epidemic diseases [6, 7]. While, in some cases, this quantity or R_0–related quantities are directly estimated from epidemic data [8, 9], R_0 is usually indirectly estimated, utilizing a process whose validity depends on several assumptions [10]. One such assumption is that individuals are homogeneously mixed: the R_0 concept may be valid when hosts are in close contact with one another. Yet, R_0-based models, which do not consider low-scale geographical data, have overestimated some epidemics [11-18].

Other mathematical approaches have focused on *social structure* [2]. They consider sub-populations suspected to be the target of the epidemic, which could be under-estimated if only the total population was measured [19]. Variations of this approach assess groups, e.g., family members, co-workers, and schoolmates [2]. These models do not consider geographical data.

A third group of mathematical models has explored *networks*. They do not assume that the population is homogeneously mixed. Instead, they consider the spatial location of each individual (a 'node' or vertex, which may be represented by a circle or point), and the contact between individuals ('link' or

'edge', which may be expressed as a line that connects two nodes, [20-24]). While network models are usually labeled 'spatial', typically, they lack geographic data [25]: the term 'spatial' refers to multi-dimensional relationships among variables.

Social network analysis (SNA) is one exception to the previous statement. This approach may include geographically explicit data, as well as temporal data. It determines the location of individuals ('nodes') and the time and duration of contacts [26]. SNA has demonstrated that temporal structures may influence epidemics in several ways [27]. SNA has been reported to: 1) risk missing data on connections [27], and 2) be sensitive to dynamic changes [28].

Medical geography addresses some of the limitations described above. This approach is based on disease maps, today generated with geographical information systems. Such maps may reveal geographical data patterns likely to be missed when only tabular data are considered [29]. Geographical models are indicated when geographical heterogeneity is documented: when disease clusters (geographical aggregations of cases at higher levels than expected) are observed, homogeneous mixing-based models are not valid [15]. Coupled with spatial statistical analysis, disease maps have attempted not to explain general problems but to be applicable [30]. Potential limitations of this approach include: 1) dependence on a relatively large sample size (rarely available in the early phase of exotic epidemics), and 2) dependence on static processes (a rare event in emerging epidemics, in which, the centroid of disease clusters, may rapidly change).

To control epidemics, functional (network theory-based), geographically explicit models that measure both dynamics and connectivity are needed [31, 33-36]. Calls to study both global and local dynamics –which occur at high and low scales, respectively– have been expressed [15, 36]. Yet, the simple combination of the previous models will not generate what is needed because they focus on contacts (people or animals) and, at the earliest epidemic phase, the number of infected individuals is very low. While air-borne epidemics have been investigated [12, 37], they are atypical because their connecting structure is mobile, and –in air travel-mediated epidemics– reduced to the few yards that separate passengers sharing the same aircraft.

Therefore, a model that measures epidemic connectivity is needed. Connectivity relates to, but differs from distance [38-40], for instance, two pairs of points, separated by the same Euclidean distance, will differ in connectivity if one pair is separated by a mountain or lake but the other pair is not. Connectivity can modify or be modified by distance, time, and/or neighbors: different geographical sites may behave as nodes at different times; e. g., a factory may act as a node on week days, losing that condition on weekends, when a park may become a node. It has been proposed that, because the network's architecture influences the global microbial invasion and/or mobility, connectivity needs to be measured and, because connections change over time, geo-temporal data should be assessed [41, 42]. These

propositions have been documented: road or river networks can promote or delay disease spread [32, 43-49].

While several authors have called for methods that integrate network analysis with geographical data [27, 32, 50], the lack of low-scale geo-referenced data has been mentioned as an impediment [51, 52]. A second reason to be considered is that nature does not offer bio-geo-temporal equivalents of 'nodes' and 'edges': they should be created and validated. To build such constructs, the model to be created should: 1) utilize low-scale geo-temporal data; 2) consider both short- and long-range connections as well as geo-temporal dynamics –i.e., the geo-temporal progression of the epidemic should be clearly determined–; 3) evaluate reproducibility; and 4) facilitate comparisons against alternatives, which may include cost-benefit metrics [33, 53, 54].

In addition, the model should distinguish contact-related from connectivity-related networks, as John Snow did. While both networks are associated, they are not synonymous: while (mobile) people or animals use (non-mobile) connecting networks –such as road, water, railroad networks; as well as food networks (e.g., markets) and energy networks (e.g., gas stations)–, such networks are built before they are used by humans and animals. Hence, the properties of connecting networks can be investigated even without data on humans or animals.

However, the physical connecting network, per se, is not the concept of interest: measuring roads or railroads, alone, will not provide information usable to control epidemics. The network of interest is dynamic and much larger: it involves a *bio-geo-temporal connecting interaction*.

Accordingly, two models were evaluated: 1) one focusing on connectivity (in addition to contacts), and 2) one focusing on contacts, in which connectivity was not explicitly measured, but neighbors were considered. Both approaches were tested utilizing geo-temporal datasets of emerging or exotic infectious diseases that affect vertebrates. The validity of the connectivity model was evaluated by asking whether network properties were revealed (such as disease clustering, assortatitivy, synchronicity, directionality, and/or a Pareto distribution [12, 20, 55, 56]). The reproducibility was determined by comparing two epidemics that differed in infective agent, host species, geography, and time, but shared the fact that all hosts were susceptible prior to the epidemic. The cost-benefit impact was estimated by comparing, across models, the total number of cases, observed at the end of the study period. By determining the number of cases these models captured, we expected that the role of connectivity could be determined. It was postulated that, if network properties were detected in two epidemics, it could then be inferred that such properties are independent of infective agent, infected host species, and spatial location. We hypothesized that, to rapidly disseminate, invading microbes require not only susceptible hosts but also a pre-existing connecting structure (e.g., a river network). While many networks may exist, we focused on the one reported to be used most of the time: the commuting (road)

network [53]. Here we asked: 1) whether actual epidemics display network properties, and 2) if so, whether a connecting network –that of roads– can influence disease spread.

Materials and Methods

Bio-geo-temporal data (primary variables)

Foot-and-mouth (FMD) and avian influenza (AI) epidemics, which affected bovines and chickens, respectively, have been described elsewhere [43, 45]. Geographical variables included: 1) point (epidemic cases), 2) line (roads), and 3) surface (demographic density) data.

An *epidemic case* was defined as any farm where, based on laboratory tests, at least one animal was diagnosed as infected. The analyzed datasets differed: while the FMD dataset included data on the location and size of infected and non-infected farms, the AI dataset did not include data on non-infected farms. While the AI dataset included temporal data on daily basis for all observations, the FMD dataset had aggregate temporal data between epidemic days 7 and 60.

Description of constructs (secondary variables)

The *epidemic node* was defined as the smallest circle that included: 1) >50% of all *cases* reported per viral transmission cycle (TC), except TC I [45], and 2) a highway intersection. The reason why the smallest possible circle was measured is due to the finite dimensions of the Earth: the number of nodes is inversely related to their size (if the radius of the node were as large as that of this planet, there would be only one node and no links). The reason why data reported in TC I were not considered was that no disease dispersal has yet occurred at that time, i.e., in order to disseminate over space, a pathogen needs a time period equal to, or longer than one TC. We considered the TC of the FMD virus to be 3 days and that of the AI virus to be 2 days [43, 45]. Assuming that *epidemic nodes* were circular, their critical radius was determined by counting, at each TC, the number of cases located inside and outside circles of various radii [45].[While *epidemic nodes* always included *cases*, *cases* could also be found outside such *nodes*.

Highway intersection areas were circles of radius equal to that of *epidemic nodes*, centered on intersections. They shared all aspects of *epidemic nodes*, except *epidemic cases*.

Road segments (lines) were components of the road network. When located within *epidemic nodes*, they were assumed to estimate short-range node degrees.

An *infective link* was any segment of an Euclidean graph that connected pairs of *epidemic cases*. Depending on the location of such cases and/or the relative location of *epidemic nodes, infective links* estimated long-range connectivity. When *cases* were outside *epidemic nodes* and there was no *epidemic node* between *cases*, *infective links* did not involve *epidemic nodes*. However, when either *epidemic cases* were located within *epidemic nodes* or such nodes were

located between pairs of *cases*, *infective links* crossed *epidemic nodes*: in such situations, the number of *infective links* crossing a node's surface estimated node degrees [22].

Node rank was the number of *infective link(s)* that intersected each *epidemic node*, where 'rank 1' identified the node crossed by the largest number of *infective links* and 'rank n' was the node crossed by the smallest number of such links. It was assumed that all *infective links* were available from day 1 onward [57]. Therefore, node degrees were assessed with indicators that estimated short- and long-range connectivity: *road segments* and *infective links*, respectively.

The distance between road intersections was generated with an additional graph. It connected all highway intersections, regardless of the presence or absence of epidemic cases.

Neighbors or *contacts* were later cases found within circles of radius equal to that of the *epidemic node*, centered on the location of earlier cases. They estimated the contact model.

The difference between the two models was connectivity: not measured in the contact model, measured in the connectivity model. While the contact model focused on a post-epidemic variable (neighbors), the connectivity model assessed a pre-epidemic variable (roads). While the contact model evaluated circles centered on infected sites; the connectivity model investigated circles centered on the road network. While roads could be captured by the contact model, such inclusion was not intentional: the contact model, per se, did not measure connectivity. While the connectivity model measured contacts, the contact model did not consider how infected and susceptible individuals could be linked outside the original cluster: the contact model inherently assumed that the invading agent could jump from one place to another without using a geographically continuous, observable path. While disease spread may be mediated by wind, air travel, or migratory birds [58], such patterns were not substantiated in these epidemics [43, 45].

Borrowing metrics from civil engineering, connectivity was described by length, continuity, and/or proximity [59]. *Proximity* was defined as the Euclidean distance between pairs of road intersections. *Length* referred to that of road segments. *Continuity* described the degree of fragmentation, if any, the road segments found in epidemic nodes could reveal. By superimposing the layers described above, additional digital and graphic data were created.

Software

Connectivity estimates (e.g., infective links) were calculated with either a proprietary algorithm, *ArcView GIS 3.3*, *ArcGIS Desktop 9.0*, and/or *ArcGis 9.3* (ESRI, Redlands, CA,

USA). Geographical data and spatial statistical tests were processed with *ArcGis 9.3*. The GIS command *buffer* was utilized to create circles of various radii which were then used to *select by location* the infected farms located inside and outside such circles. The GIS commands *intersect, clip,* and/or

merge were used to group variables of various shapes (e.g., points and polygons).
Other statistical tests were performed with *Minitab 15* (Minitab Inc., State College, PA, USA).

Results

Non-random patterns and determination of Epidemic Nodes

Clustering: Both epidemics displayed clustering. Although disease clusters are typically found only in early phases [60], they were detected over the whole epidemic (60 days in FMD, 24 weeks in AI, Figures 1a, b). In addition to global and local case spatial auto-correlation [61] ($P<0.01$, Moran's Index and Getis-Ord G, not shown), clustering was observed along roads, as expressed in Figures 1 c and d.

Validation of epidemic nodes: In the FMD epidemic, the smallest circle that included >
50% of the cases, from the second transmission cycle (TC) onward, had a 7.5-km radius, while, in the AI epidemic, 31-km radius circles were the smallest that, at all times, included > 50% of the cases (Figures 1e and f). Those circles, which included roads, estimated *epidemic nodes* (Table 1 in Text S1). *Epidemic nodes* included 57.5 % (65/113, in AI) and 70 % (402/572, in FMD) of all epidemic *cases*. These circles revealed epidemic dynamics: within 3 days (between TC I and TC II), the FMD epicenter (the centroid defined by all *epidemic nodes*) moved 40 km in a SW direction, while the centroid of the AI epidemic differed 700 km between the first and the second TC (Figures 1a and b in Text S1). Epidemic nodes helped detect network properties.

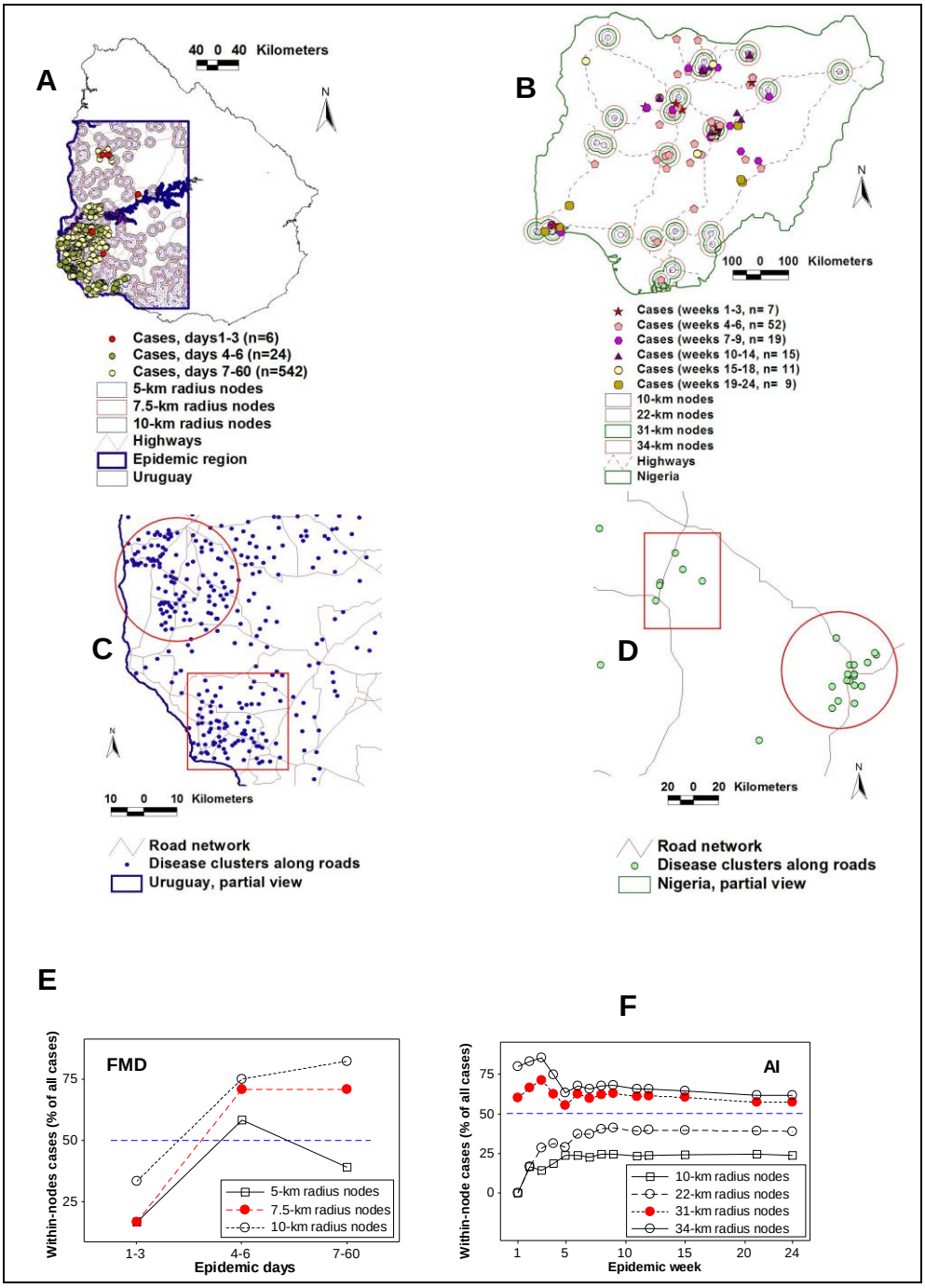

Figure 1. Detection of 'along-road' disease clusters and empirical determination of epidemic nodes. High geographical scales of the 2001 Uruguayan FMD (A) and the 2006 Nigerian AI H5N1 (B) epidemics are shown, as well as low scales (C, D). In both epidemics, low-scale data revealed that

epidemic cases not only displayed spatial auto-correlation (proximity) but also clustering along the road network (C, D).The empirical determination of epidemic nodes (the smallest circles that included one or more highway intersections[s] and epidemic cases, at any viral transmission cycle [TC] except TC I), showed that such circles, in the FMD epidemic, had a 7.5 km radius (A, E) and, in the AI epidemic, had a 31-km radius (B, F). These plots document, both visually (geographically) and numerically, that epidemics are mediated by connected nodes: in both epidemics, ≥ 57% of all cases occurred within epidemic nodes (A, B, E, F).

The FMD network properties and discriminating interactions

Differentiation of primary cases: Only one of the 6 FMD cases (16.6%) reported in TC I (days 1-3) was found within *epidemic nodes* (Figure 2a). Therefore, not all primary cases were functionally identical: only one was connected. In contrast, in TC II (after the infectious agent had enough time to disseminate), 17 of the 24 cases (71%) were reported within *epidemic nodes* (Table 1 in Text S1). Such finding indicated that disease spread depended on getting access to a disseminating (connected) network, which was observable after TC II, as shown in Figure 2b.

Differentiation of epidemic nodes and detection of Pareto's pattern: *Epidemic nodes* were differentiated by the number of cases/node: 5% (8/157) of all *epidemic nodes* reported over 60 epidemic days included 23% of all *cases* (132/572, Table 1 in Text S1). That feature displayed a Pareto's '20:80 pattern': a small percentage of nodes was associated with > 4 times more cases (23/5=4.6) than expected under the assumption of an equal number of cases per node.

Assortativity: Both the Pareto pattern and assortativity (highly connected nodes linking with similar nodes) were observed, both geographically and numerically. For instance, Figures 2b and c show that, at or after TC II, 8 *epidemic nodes* displayed similar connectivity (a high number of *road segments*), were close to one another (so much so that some of them partially overlapped), and included a much higher percentage of *epidemic cases* than average nodes.

Synchronicity: The simultaneous engagement of functionally similar nodes was observed in TC II, when 56 FMD nodes were found to be connected (Figure 1a in Text S1).

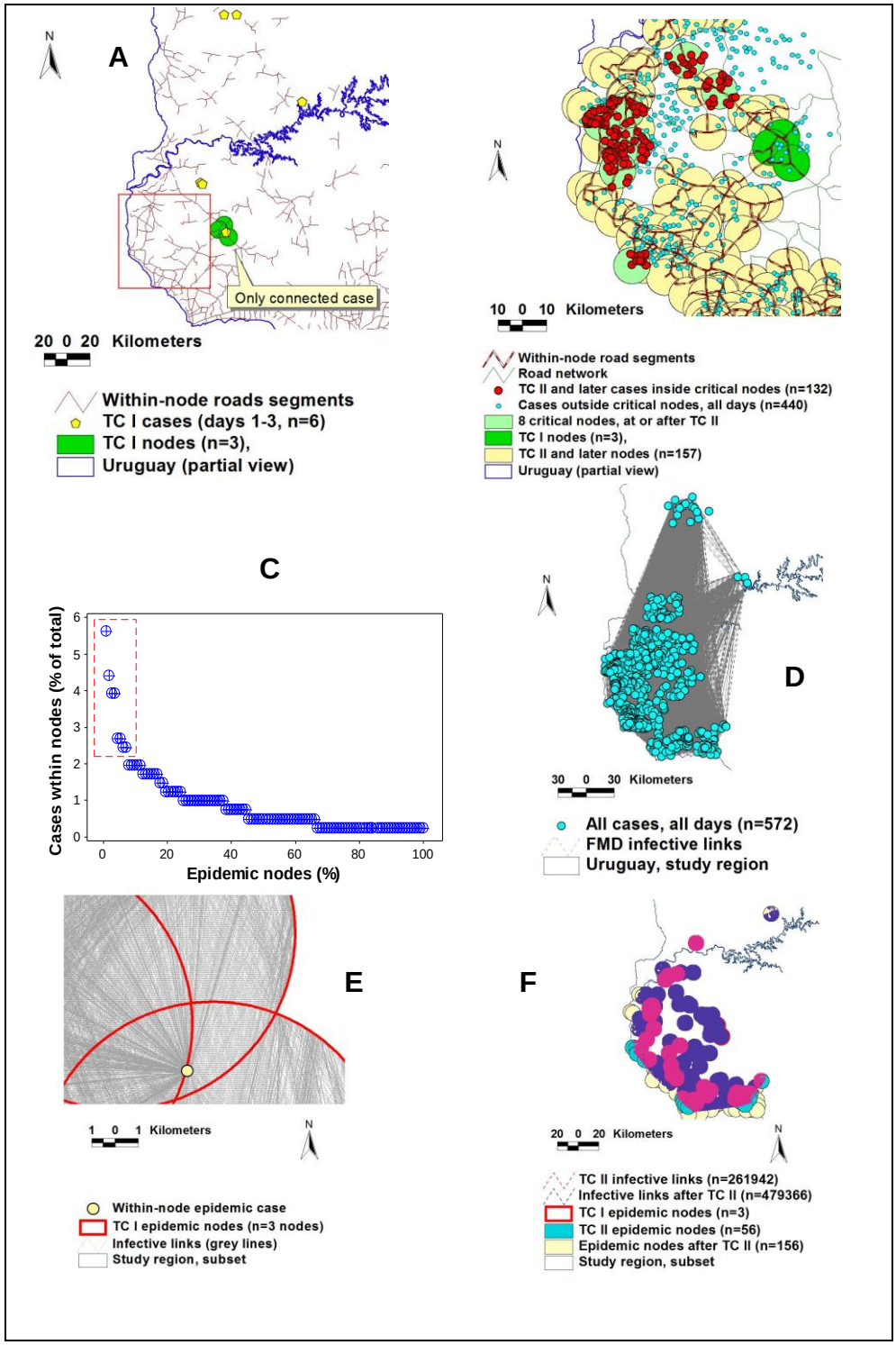
A
N
Only connected case
20 0 20 Kilometers
Within-node roads segments
TC I cases (days 1-3, n=6)
TC I nodes (n=3),
Uruguay (partial view)
N
10 0 10 Kilometers
Within-node road segments
Road network
TC II and later cases inside critical nodes (n=132)
Cases outside critical nodes, all days (n=440)
8 critical nodes, at or after TC II
TC I nodes (n=3),
TC II and later nodes (n=157)
Uruguay (partial view)
C
Cases wthin nodes (% of total)
Epidemic nodes (%)
0
1
2
3
4
5
6
0
20
40
60
80
100
N
D
30 0 30 Kilometers
All cases, all days (n=572)
FMD infective links
Uruguay, study region
E
1 0 1 Kilometers
N
Within-node epidemic case
TC I epidemic nodes (n=3 nodes)
Infective links (grey lines)
Study region, subset
F
20 0 20 Kilometers
N
TC II infective links (n=261942)
Infective links after TC II (n=479366)
TC I epidemic nodes (n=3)
TC II epidemic nodes (n=56)
Epidemic nodes after TC II (n=156)
Study region, subset

Figure 2. Differentiation of epidemic cases, detection of network properties, and estimation of long-range connectivity in the FMD epidemic. Because both epidemics involved a highly contagious and clinically observable disease (in the affected host species) and, in both cases, hosts lacked immunity because the pathogen was exotic (not reported in those environments in the previous 10 years), in both epidemics, all hosts were susceptible. Consequently, the geo-temporal progression of both epidemics was assessed unambiguously, at herd level, and, therefore, the connectivity associated with primary cases so was determined. Not all primary FMD cases –those reported in the first transmission cycle (TC)– were located within circles that included a highway intersection. Only one the first 6 primary cases was explicitly connected (**A**). In contrast, in or after TC II, most cases were connected: they were within epidemic nodes and some epidemic nodes included a much higher proportion of cases than average nodes. For instance, 8 epidemic nodes included 115 of the 402 within-node cases (**B**). Those 8 nodes were located in an area characterized by a high density of road segments (**box, A**), and were so close to one another that some of them overlapped. These 8 nodes revealed assortativity (selective connection among similar nodes) and well as Pareto's "20:80" pattern: 8 of the 157 nodes connected at or after TC II (5% of all nodes) reported 28% of all cases (115/409), i. e., these nodes included 5.6 times (28/5) more cases than average nodes (**B, C**). A graph was made, which connected every pair of *epidemic cases* with Euclidean lines –here named *infective links*– (**D**). Figure **E** shows how *epidemic cases, epidemic nodes*, and *infective links,* while different from one another, could be related: when *infective links* crossed *epidemic nodes*, the number of *infective links/epidemic node* gave an estimate on long-range connectivity. The number of *infective links/epidemic node* differed along the epidemic, indicating that at least one of the TC I node(s) possessed a higher long-range connectivity than nodes later engaged (**F**). The combined analysis of *epidemic cases* and *infective links* per *epidemic node* detected several network properties (e.g., assortativity, Pareto's pattern) and demonstrated that neither all epidemic cases nor all epidemic nodes were functionally identical.

Long-range connectivity (infective links/node): As documented in Figures 2 d-f (and also in Table 2 of Text S1), the number of *infective links* per *epidemic node* was higher before than after TC II. Therefore, several bio-geo-temporal indicators distinguished *epidemic nodes*.

Relationships between connectivity and population density: Farm density was assessed as a proxy estimate for animal density [62]. A global analysis showed that farm density was positively associated with the number of *epidemic nodes*/TC: over time, population density correlated with connectivity (Figures 3a-c). However, as Figure 3c reveals that interaction was not a simple one but mediated by a heterogenous (fragmented) bio-geographical landscape.

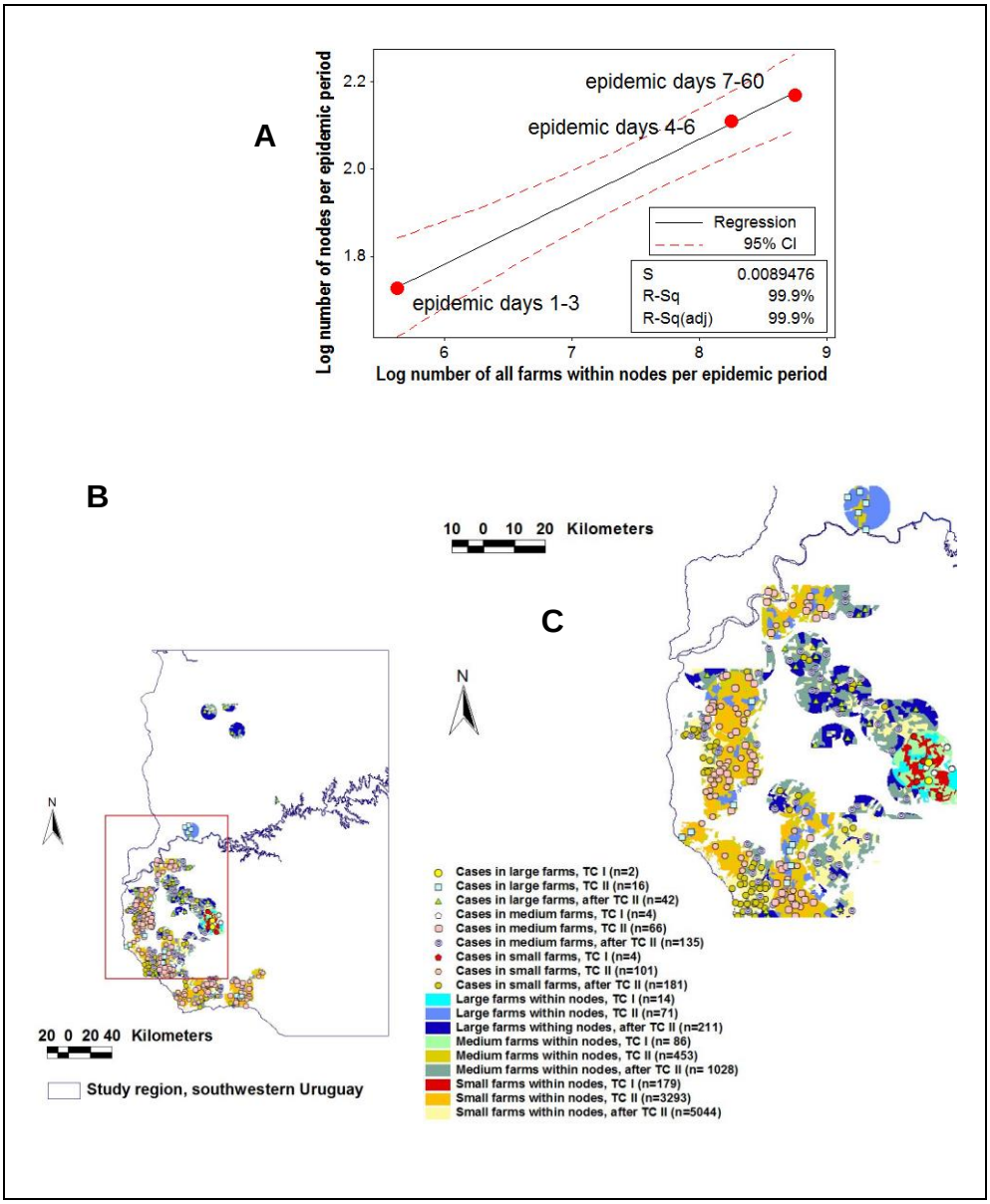

Figure 3. **Dynamics of the connectivity-population interaction and heterogeneity of the FMD epidemic landscape**. Because animal population density is known to be associated with farm density –smaller farms tend to raise more animals per sq/km–, farm density was used as a proxy variable for animal density. The association between temporal connectivity (epidemic nodes per TC) and the temporal farm density (characterized by size classes and also measured per TC) revealed a positive correlation between farm density and connectivity: over time, the greater the number of farms –which were smaller and raised more animals/sq km–, the greater the number of connected epidemic nodes found per TC (**A**). That analysis was facilitated by the bio-geo-temporal data provided by the connectivity model, which also revealed a

highly fractured (heterogenous) distribution of the population (**B, C**). The graphic data on farm size classes and cases per TC for the whole epidemic region are shown in panel **B**; a low-scale view of one subset is shown in panel **C**, which also reports, numerically, the data of the entire epidemic region. Findings document that post-epidemic data (cases, epidemic nodes) can be linked to pre-epidemic data (population, connectivity) and the numerical expression of such interaction can be generated by a simple geographical analysis. This approach integrates spatial statistical and network theory approaches with geo-referenced epidemiology.

The AI network properties and discriminating interactions

Differentiation of primary cases and epidemic nodes, and detection of Pareto pattern: Not all primary *epidemic cases* were connected. Figure 4a shows that not all primary cases were within *epidemic nodes*. *Epidemic nodes* were not functionally identical, either: four of them (44.4 % of all nodes, red pentagon, Figure 4b) included 89% (58/65) of all within-node cases, i.e., 4 nodes showed a number of cases twice higher than average. Two of those nodes accounted for 46 within-node cases (red pentagon, Figure 4b). Hence, 22% (2/9) of all nodes explained 71% (46/65) of all within-node cases, i.e., a 3.3:1 ratio –a Pareto pattern that also demonstrated not all *epidemic nodes* were similar. Epidemic node-associated clusters also met the criteria defined by Network Theory [63]: their road segments estimated node degrees (Figure 4b).

Assortativity, interactions among networks, and a second Pareto pattern: Assortativity was visually observed: the AI 3 nodes that showed the highest number of cases were linked with one another through a continuous ring of short-range road segments (red pentagon, Figure 4b). Interactions among networks were revealed: while 16 *highway intersection areas* (a network composed of circles of radius equal to that of *epidemic nodes*) were observed, only nine of them included *epidemic cases*, i.e., only 9 *road intersection areas* acted as *epidemic nodes* (Figure 4b). This network showed a Pareto pattern: 25% of all *road intersection areas* (4/16) included 80% (52/65) of all within-node *epidemic cases* (Figure 4c).

Infective links and ranking of epidemic nodes: *Epidemic nodes,* as shown in Figure 4d, were differentiated both by the number of *epidemic cases* and the number of *infective links*. *Epidemic nodes* with more *cases* tended to be crossed by more *infective links*.

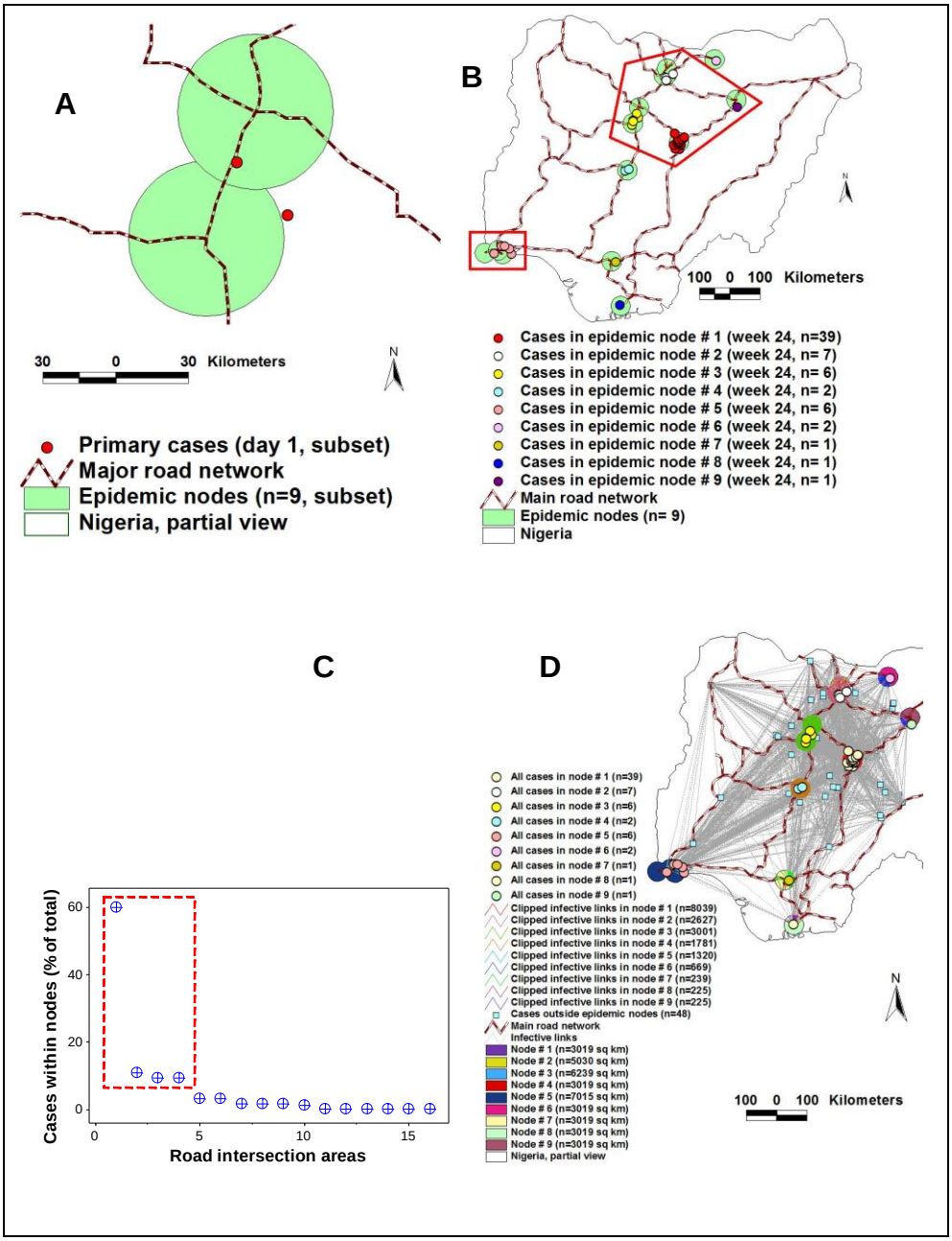

Figure 4. Differentiation of epidemic cases, detection of network properties, and estimation of long-range connectivity in the AI epidemic. Low-scale data revealed that one primary AI *case* was located close to but outside the connecting structure defined by *epidemic nodes* (**A**). In contrast, in or after TC II, most cases were found within epidemic nodes (**B**). Two *clusters* of *cases* were observed (red polygons) and some *epidemic*

nodes included a proportion of cases much higher than average nodes. For instance, 4 partially overlapping epidemic nodes included 58 of the 65 within-node cases, and two nodes (nodes # 1 and 2, red pentagon, **B**) accounted for 46 of such cases (or 71 % of all within-node cases). Therefore, both assortativity and Pareto pattern were revealed: 1) similar nodes connected to one another, and 2) 4 road intersection areas, out of 16 (or 25%), included 80% (52/65) of all within-node cases (**C**). The connection of all epidemic cases generated a graph that helped to further explore epidemic nodes: they were distinguished by the number of *infective links* that crossed *epidemic nodes* (**D**).

Synchronicity and directionality:
The number of *infective links*/*epidemic node* was used to rank nodes, e.g., *ranked epidemic node* (REN) # 1 was crossed by the highest number of *infective links*. When RENs were plotted against the number of *epidemic cases/*week, both synchronicity and directionality were observed. Figure 5a shows that nodes of similar rank were engaged at the same time and high RENs were engaged earlier than low RENs. The same figure documents that the number of *epidemic cases* grew rapidly in REN #1 and, when few or no new cases were reported, nodes of a lower rank (RENs # 2 and 3) became active, which showed the same pattern and were followed by nodes of an even lower functionality. In contrast, the last class of nodes failed to spread infections: RENs # 8 and 9 only generated one case each (Figure 5a).

Relationships between pre-epidemic and post-epidemic variables: The median distance between epidemic nodes (Figure 5b, Table 3 in Text S1) was significantly shorter in high- than in low-rank nodes (Figure 5d). Hence, the shorter the distance between road intersections, the higher the chance that such intersections could spread disease, if an epidemic occurred.

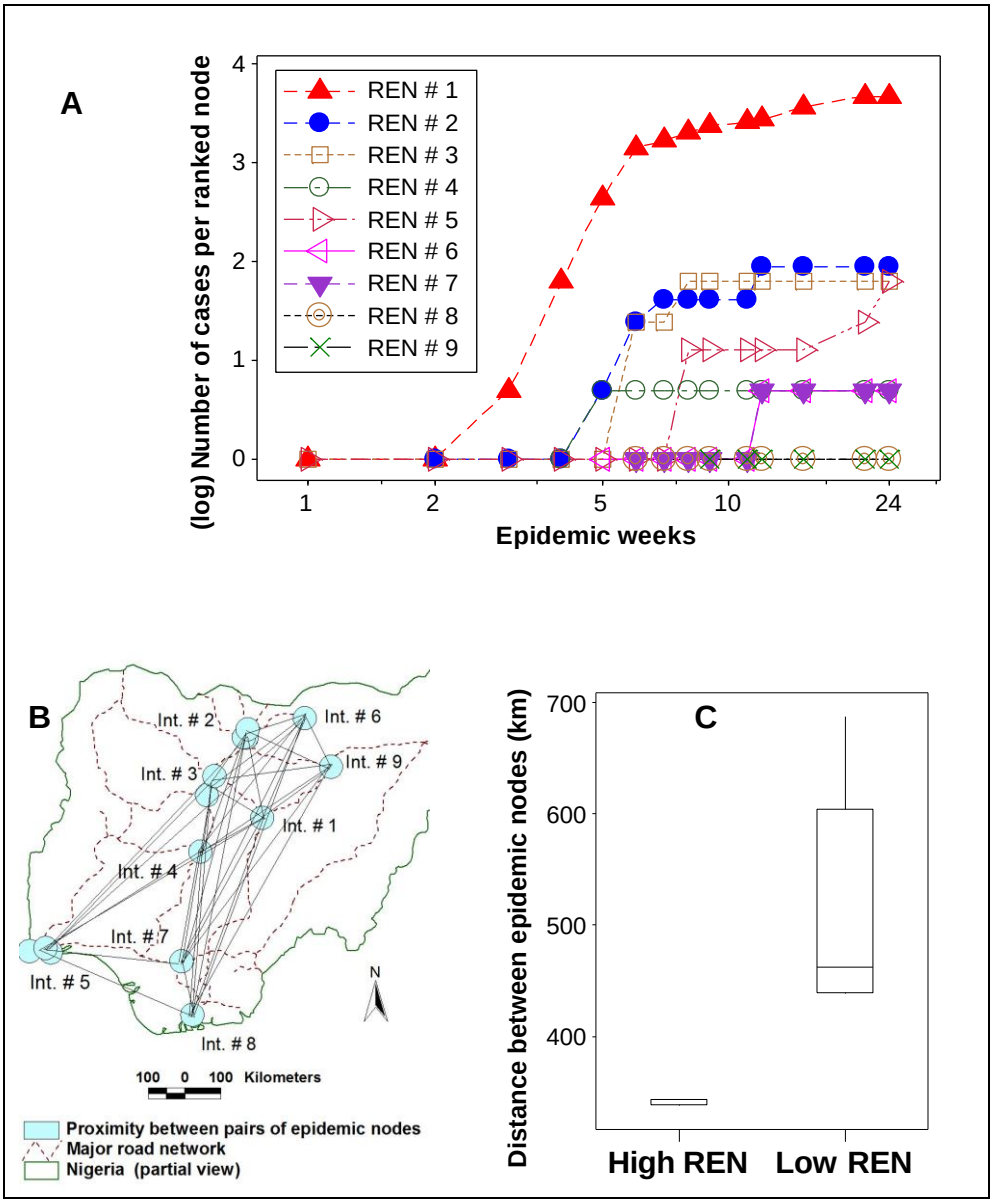

Figure 5. Synchronicity and directionality of AI epidemic flows and interactions between pre- and post-epidemic variables. A). *Epidemic nodes* were ranked according to the number of *infective links* that crossed their surface; e. g., *ranked epidemic node* (RAN) # 1 was crossed by the highest number of infective links. When RANs were plotted against the weekly (log) number of *epidemic cases* both synchronicity and directionality were revealed (**A**). Such plot distinguished at least three functional classes of epidemic nodes: 1) high-ranked epidemic nodes were engaged simultaneously, and earlier; and low RENs were engaged later; i.e., the

epidemic flow (directionality) moved from high to low RENs; 2) when high RENs achieved their plateau (no new cases were reported), lower RENs became activated, which displayed the same profile and were followed by RENs of no influence on epidemic dispersal: RENs #8 and 9 only produced one case each. Therefore, the properties associated with synchronicity and directionality lent validity to the construct here named *infective links:* the non-random and highly bio-geo-temporally structured patterns observed were the result of ranking *epidemic nodes* on the basis of *infective links.*

An additional graph, which linked the centroids of pairs of *epidemic nodes,* determined the distance between pairs of highway intersection areas that included most cases (**B**). The median distance between intersections that included cases was significantly shorter for high than for low RENs (**C**). Such finding supported the view that identification of critical hubs likely to behave as high-rank epidemic nodes could take place even before epidemics occur.

Cost-benefit comparisons between models

Performance in the FMD epidemic: After TC II, 390 cases were included within *epidemic nodes* (Figure 6a). Within the same timeframe, 181 cases were reported within neighborhoods (circles of identical radius, centered on the location of all cases reported in the first two TCs, Figure 6b). FMD *epidemic nodes* were associated with a longer connectivity –more road segments– and a more continuous structure than those of the contact model (Figures 6c, d).

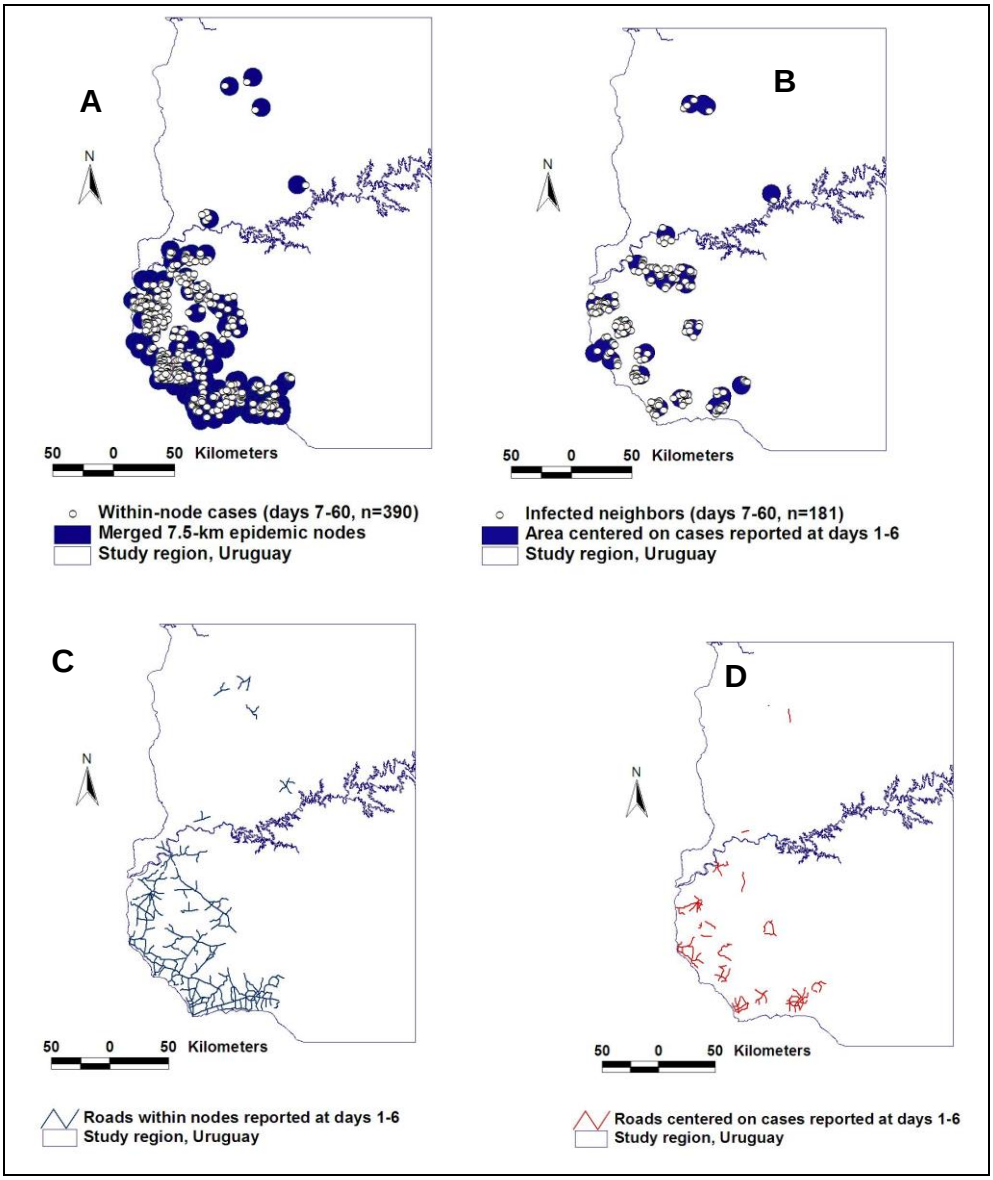

Figure 6. Comparison between connectivity and contact models—the FMD epidemic. After TC I, epidemic nodes centered on the road network (the connectivity model) showed twice as many cases as circles of equal radius that did not consider the road network (the contact model): while 360 cases were reported within epidemic nodes (**A**), 181 cases were found within the same time frame in the neighborhood of earlier cases (**B**). Longer road length and less fragmentated road segments were associated with the connectivity model (**C)** than with the contact model (**D**). Findings suggest that epidemiologic control measures are more likely to be effective when based on connectivity

than when it is assumed that all epidemic cases are epidemiological identical, so they equally influence their neighbors. Because connectivity differs, epidemic cases are likely to differ in their ability to disseminate disease. Therefore, control measures could be adjusted to the local connecting structure.

Performance in the AI epidemic: After TC II, more than twice as many *epidemic cases*
(62/30) were found within the connectivity model than within circles centered on the location of earlier cases (Figures 7a, b). Figures 7c and d document that AI *epidemic nodes* had a 3-fold longer and less fragmented road structure than circles that did not consider connectivity.

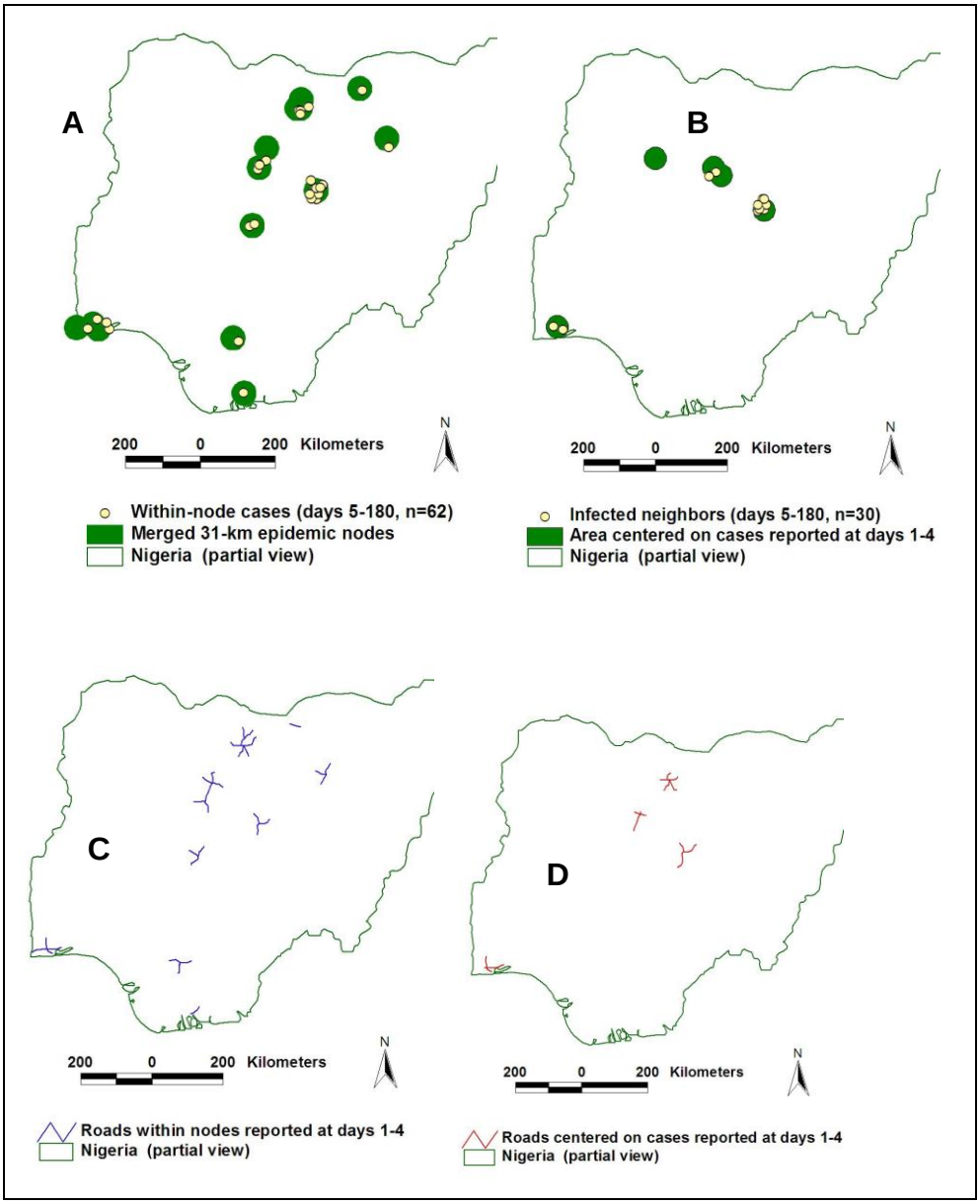

Figure 7. Comparison between connectivity and contact models—the AI epidemic. The AI epidemic displayed findings similar to those of the FMD epidemic: after TC I, the connectivity model captured twice as many cases than the contact model (**A, B**), and the connectivity structure found within the area determined by the connectivity model was three times longer and less fragmented than the road structure captured by the contact model (**C, D**). Findings documented the reproducibility of the connectivity model, and revealed, graphically, why it could capture more cases: the connecting network was less fractured (more continuous) in the connectivity than it was in the contact model.

Integration between spatial statistics, Network Theory, and bio-geo-temporal approaches: Spatial statistical (SS) and Network Theory (NT) approaches showed solutions that differed in cost-benefit metrics. While the SS version resulted in small areas (i.e., a low 'cost' per case), it did not measure directionality and, therefore, clusters could only be analyzed individually. While the NT approach revealed a non-fragmented connectivity, it covered a much larger area. The bio-geo-temporal model, as Figure 8 shows, integrated both views, producing the optimal solution.

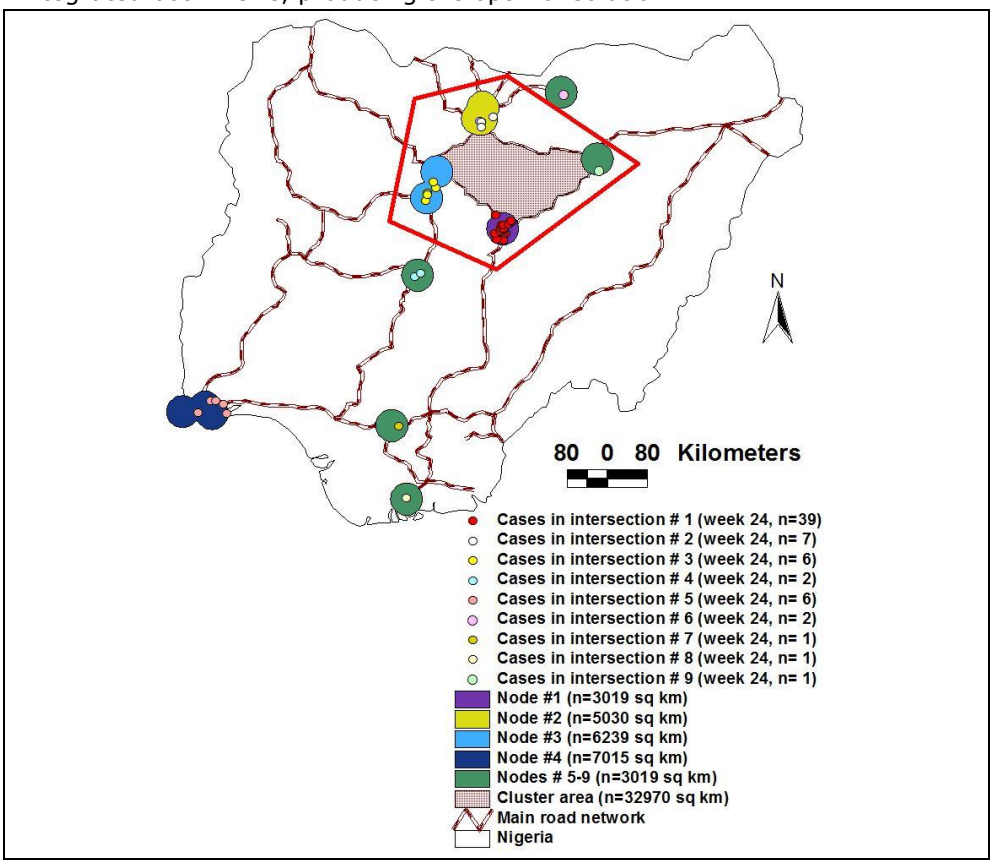

Figure 8. Three bio-geo-referenced examples of cost-benefit analyses. The AI epidemic data allowed the generation of three sets of metrics, potentially applicable in cost-benefit analyses.

I. An approach grounded in *spatial statistical (SS)* models could identify 9 disease clusters, in which *epidemic cases* would be synonymous with 'nodes', and connectivity could be estimated on the basis of within-node road segment *length* and *proximity* of each case to road segments. However, the SS approach would not possess information of directionality and, therefore, the model could not estimate the direction in which the epidemic flow is moving, i.e., the model could not measure dynamics and,

consequently, each cluster would be treated as a separate or independent event. In the SS version, one way to estimate the 'cost' of preventing a case, expressed as surface to be intervened (sq km) would be:

Cluster # 1 (3019 sq km/39 cases)= **77** sq km/case
Cluster # 2 (5030 sq km/7 cases)= **718** sq km/case
Cluster # 3 (6239 sq km/6 cases)= **1039** sq km/case
Cluster # 4 (3019 sq km/2 cases)= **1509** sq km/case
Cluster # 5 (7015 sq km/6 cases)= **1169** sq km/case
Cluster # 6 (3019 sq km/2 cases)= **1509** sq km/case
Cluster # 7 (3019 sq km/1 cases)= **3019** sq km/case
Cluster # 8 (3019 sq km/1 cases)= **3019** sq km/case
Cluster # 9 (3019 sq km/1 cases)= **3019** sq km/case

II. In a *Network Theory (NT)* version, a single cluster would be observed (the area included within the red pentagon), which would be composed of 4 nodes (regarded to be synonymous with *epidemic nodes*). In this model, connectivity among nodes could be determined by outside-node road segments. At least three calculations could be generated, e.g.: (a) if it is assumed that all within-node cases, of all nodes, will be protected if the whole area of the cluster is intervened, the 'cost'/case would be = 32970/65= **507** sq km; (b) if, instead, it is assumed that controlling just the area of epidemic nodes would prevent the number of cases found only within such 4 nodes, the 'cost' would be equal to surface of nodes #1-4 (17,307 sq km) / number of cases within nodes #1-4 (54) or **320.5** sq km/case; or (c) if it is expected that such intervention would prevent all within-node cases (including those outside the cluster), then the 'cost' would be: the surface of nodes #1-4 (17,307 sq km) / all cases (65)=**266.3** sq lm/case.

III. In the *bio-geo-temporal* version, taking advantage of both the smaller solution (in terms of territorial coverage) offered by the SS alternative and the application of NT properties (especially, directionality, which estimates *temporal mobility*), 9 clusters would be considered, which would include nodes (synonymous with *epidemic nodes*), and long-range connectivity (determined by *infective links*, not shown in Figure 8), which would distinguish node ranks (a predictor of directionality, as shown in Fig. 5a). Because, in this version, directionality or temporal mobility would be considered, control measures could focus on the node with the highest rank (node #1, identified in Figure 5a, which has a surface equal to 3190 sq km). Hence, an early intervention on such node could prevent all within-node cases and the 'cost' would be 3190 /65= **49** sq km/case.

Discussion

Both epidemics revealed highly organized data structures [64]. In spite of differences in microbial agent, host species, time, and geographical location, five inter-related network properties were observed, which seemed to be highly conserved: disease clustering, assortative mixing, synchronicity, directionality, and Pareto's data pattern. Disease dispersal was better explained by the connectivity-based model: after TC II, this model captured twice as many cases as, and displayed a less fragmented and longer length of road segments than the contact model. In addition, this model distinguished functional classes of primary nodes, nodes, and networks.

The enhanced discrimination achieved by the connectivity model was attributable to the use of two indicators: *epidemic nodes* and *infective links*. With these constructs, what previously seemed to lack 'order', became interpretable and revealed a major property of biological systems: *emergence* ('order' or a high-level function [65]). *Emergence* was observed when the weekly number of *epidemic cases* was plotted vs. the rank of *epidemic nodes*. The plot shown in Figure 5c documented that the time, the number, and the place of case occurrence were not random events but the result of *epidemic nodes* differentiated by *infective links*.

The indicators evaluated only partially related to definitions utilized in spatial statistics (SS) and Network Theory (NT). There were differences between or among: 1) 'spatial' and 'geographic', 2) 'mobility' and 'connectivity', 3) 'nodes' (as defined in NT) and *epidemic nodes*, 4) 'links' (node degrees, as defined in NT) and *infective links*, 5) 'clusters' (as defined in SS and NT) and *disease clusters*, and 6) classes of *epidemic nodes*, *primary cases*, and *networks*.

For instance, the data indicated that several networks may overlay and interact with one another [66]. While related, they were not identical: the *epidemic node* network did not include all road intersections, and the *road intersection area* network did not include all cases.

The connectivity model revealed that not all primary cases were connected. That finding may explain why the basic reproductive number (R_0) has overestimatd some epidemics, in the past [16]: the inclusion of non-connected cases should overestimate the number of primary cases.

Because, in emerging infections (when all hosts are susceptible), secondary cases can only be generated by some of the primary case(s), tertiary cases can only be produced by primary or secondary cases and so on [67], it follows that epidemic cases are neither independent nor functionally identical: connected *primary cases* are much more influential than any later case. Interventions based on identical control zones, centered on the location of all earlier cases

–i.e., the contact model, which assumes that all earlier cases have an identical probability of disseminating the infection to its neighbors– are likely to be sub-optimal in preventing disease spread, as shown here. Instead, based on their connectivity, primary cases could be distinguished.

The highly connected *disease clusters* found within *epidemic nodes* differed from both spatial statistical (SS) and Network Theory (NT) definitions: in SS, a *cluster* denotes a spatial aggregation of *epidemic cases* (point data, in this study), which may or may not be located within *epidemic nodes*; while, in NT, a cluster refers to groups of *epidemic nodes* (surface data, in this study) connected by *road segments*. In other words, the NT version of a cluster, which is based on the clustering coefficient [63], is geographically larger than the SS version. These different definitions were visually distinguished in Figure 4b, where either a *single cluster* composed of 4 epidemic nodes (the NT version, red pentagon), or *6 clusters*, each with ≥ two cases within an epidemic node (the SS version), can be seen. Figure 2b displayed a similar example.

While compatible with SS and NT approaches, the bio-geo-temporal approach was more discriminant. For instance, if two disease clusters displayed identical SS (e.g., Moran's I) indices or identical NT cluster coefficients ([61]), the bio-geo-temporal approach could distinguish them in terms of *continuity*, *long-range connectivity*, *proximity*, and/or *transmission cycle*.

Because some disease clusters showed outbound flows, earlier views on disease clusters, which assume disease clusters are only recipients of infective flows [68], could be revised.

Together, findings revealed that cases, nodes, clusters, and/or networks can be distinguished.

Distinguishing the functional role of epidemic nodes is crucial to identify not only where, but also when an optimal intervention should be applied. Defining the 'critical response time' (time available to implement an intervention and achieve the results that intervention promotes [9]) is meaningful only if associated with information on where control measures can be applied.

Such geo-temporal information was provided in this study because *epidemic nodes* were distinguished. Enhanced discrimination was possible because connectivity was not regarded to be synonymous with mobility. While the non-geographical literature assumes that mobility (the movement of people or animals, i.e., 'contacts') is equal to connectivity, that literature does not assess the structure that facilitates mobility. While 'contacts' are mobile, the connecting structure (e.g., a river network) is not. This distinction has practical effects: because in early epidemic phases the number of infected 'contacts' (mobile individuals) is close to zero, the 'contact' version of connectivity (mobility) cannot be applied to this type of infections. However, because there is no shortage of data on the connecting network (e. g., a road network), better and geographically contextualized calculations can be done if the focus of the analysis –and that of interventions– is the non-mobile connecting network, as John Snow did.

The demonstration of network properties in emerging or exotic epidemics, in which the number of observations is marginal –when information is most needed– means that, instead of approaches that focus on the host (based on counts of cases), it is possible to emphasize not the number of cases but

whether the epidemic is facilitated by a measurable connecting network. If that is shown, the practical question becomes where and when the network can be disrupted.

That could be achieved if indicators were designed to facilitate cost-benefit analysis. To that end, the *epidemic node* and *disease cluster* constructs differed from the Network Theory (NT) counterparts. While nodes, in NT, are defined as dimensionless points [69], *epidemic nodes* were defined as surfaces (the smallest circle containing most cases). Such adjustment of NT concepts to bio-geographical realities resulted in both the lowest cost (interventions applied to the smallest circle) and the highest benefit (where most epidemic cases can be prevented).

Retrospective data indicated: (i) a positive correlation between population density and connectivity, and (ii) a functional differentiation of critical nodes (distinguished on the basis of distance between infected sites). If geo-referenced data on all susceptible sites –farms, in this study– were available, these variables could, potentially, be measured before epidemics occur.

While the validity of *epidemic nodes* and *infective links* was supported, their limitations should not be ignored. *Infective links* assume that connectivity remains constant over the course of an epidemic, which is unlikely [27]. While *epidemic nodes* detected 'along-road' disease clusters even if they were not independent –an advantage over classic approaches [70]–, they depend on a circular assumption, which is not realistic. To improve such constructs, future studies may consider non-circular and non-Euclidean metrics, such as *road segments* and the *road length* associated with each node –here assessed but only partially evaluated.

While disease spread may be mediated by other means, rapidly disseminating epidemics seem to require pre-existing connecting networks. The integration of John Snow's approach interventions neither applied on the host nor imposed on the pathogen, but centered on connectivity– with network analysis, seems to be feasible.

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Supplementary Data (S1)

SUPPORTING INFORMATION

Description of the data

In order to measure connectivity, four constructs were created:

(i) epidemic nodes (EN, Table 1);

(ii) infective links (IL, Table 2);

(iii) the 'rank of epidemic nodes' (RENs, Table 3); and

(iv) proximity (Table 4), which measured the distance between pairs of RIRs.

For brevity, the FMD data on RENs and proximity (available upon request) are not reported.

In addition, a construct on neighbors was created, which is described in the main text.

The dynamic nature of ENs is documented in Figures 1A and B of SI: they show that the geographic location of EN centroids differed markedly, in both epidemics, between the first and the second TC.

Table 1. Determination of epidemic node radius

FMD*									
Epidemic day	Cases inside 5-km nodes	Cases outside 5-km nodes	Cases inside 7.5-km nodes	Cases outside 7.5-km nodes	Cases inside 10-km nodes	Cases outside 10- km nodes	Case % within 5-km nodes	Case % within 7.5-km nodes	Case % within 10-km nodes
1-3	1	5	1	5	2	4	16.7	16.7	33.3
4-6	15	15	18	12	20	10	58.3	70.8	75
7-60	227	345	408	170	466	106	39.1	70.8	82.3
Ratio TC II/TC I							3.49	4.24	2.25

AI H5N1*									
Epidemic week	Cases inside 22-km nodes	Cases outside 22-km nodes	Cases inside 31-km nodes	Cases outside 31-km nodes	Cases inside 34-km nodes	Cases outside 34-km nodes	Case % within 22-km n.	Case % within 31-km n.	Case % within 34-km n.
1	0	5	3	2	4	1	0	60	80
2	1	5	4	2	5	1	16.6	66	83.3
3	2	5	5	2	6	1	28.5	71.4	85.7
4	5	11	10	6	12	4	31.2	62.5	75.0
5	11	27	21	17	24	14	28.9	55.2	63.1
6	22	37	37	22	40	19	37.2	62.7	67.7
7	25	42	40	27	44	23	37.3	59.7	65.6
8	30	44	46	28	50	24	40.5	62.1	67.5
9	32	46	49	29	53	25	41.0	62.8	67.9
11	32	50	50	32	54	28	39.0	60.9	65.8
12	35	53	54	34	58	30	39.7	61.3	65.9
15	38	58	58	38	62	34	39.5	60.4	64.5
21	43	67	63	47	68	42	39.0	57.2	61.8
24	44	69	65	48	70	43	38.9	52.8	61.9
Ratio TC II/TC I		-					No data in TC I	1.1	1.0

*Cases (inside or outside) specified circles report cumulative counts.

Table 2. Infective links: number, number of links per node, and time of activation

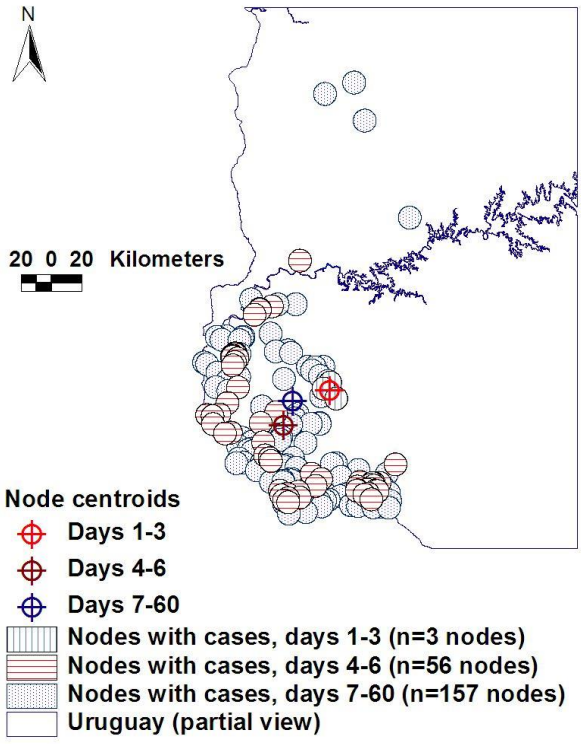
Infective links	FMD epidemic	AI H5H1 epidemic
TC I infective links	12903 (2.7 % of all links)	9499 (25.4% of all links)
TC II infective links	261945 (54.6 % of all links)	1320 (3.5% of all links)
TC I and II infective links	274848 (57.3% of all links)	10819 (29% of all links)
Post TC II infective links	204560 (42.7 % of all links)	26529 (71% of all links)
Time involved to activate TC I- II infective links	10 % of the total (6/60 days)	2..5% of the total (4/162 days)
Nodes involved in TC I*	3	2
Nodes involved in TC II*	56	2
Nodes involved after TC II*	157	15
Inf. links/node (TC I)	4301	4750
Inf. links/node (TC II)	4678	660
Inf. links/node (post TC II)	1303	1769
Inf. links/node (TC I-II)	4489 (244.5% higher than post TC II)	2705 (52.9% higher than post TC II)

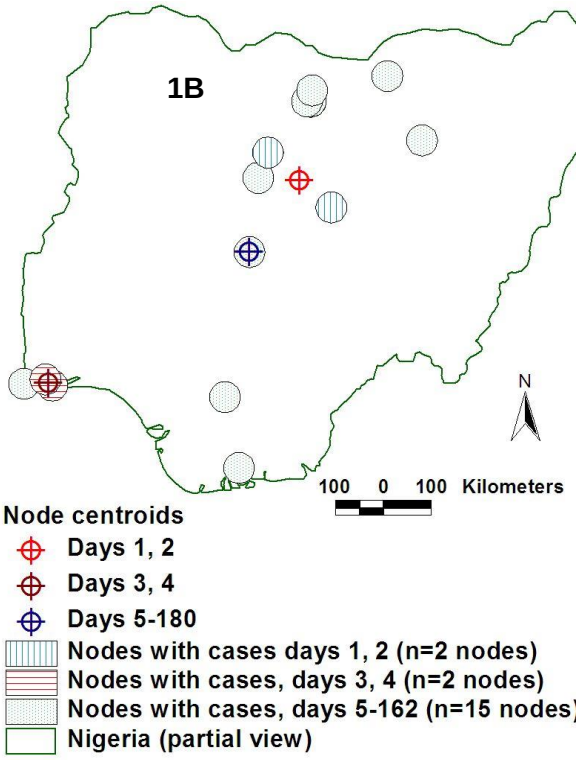
*: These numbers may include nodes that contributed cases in earlier or later TCs.

Table 3. AI H5N1 infective links (IL), ranked epidemic nodes (RENs), and Euclidean distance between pairs of road intersection areas (RIAs)*

REN # (number of ILs)	REN 1 (3847)	REN 2 (1946)	REN 3 (1848)	REN 4 (1328)	REN 5 (981)	REN 6 (669)	REN 7 (231)	REN 8 (225)	REN 9 (100)	REN 10 (90)
Distance between RIAs (km)										
RIA 1	--									
RIA 2	240.2									
RIA 3	184.1	170.8								
RIA 4	203.5	343.2	192.5							
RIA 5	717.1	806.9	687.9	517.3						
RIA 6	342.8	166.6	324.2	472.1	975.8					
RIA 7	452.3	646.7	504.5	318.2	393.5	751.6				
RIA 8	576.7	776.2	616.9	451.1	466.8	879.9	158.5			
RIA 9	402.0	602.1	438.9	281.1	472.2	684.3	84.1	186.1		
RIA 10	241.1	248.6	337.2	438.6	951.8	172.2	671.4	795.6	607.1	--
Median	342.8	343.2	337.2	343.2	687.9	472.1	452.3	576.7	438.9	438.6

* This table shows the distance between pairs of road intersection areas (RIAs). RIAs are identified based on the decreasing number of infective links (ILs) crossing over RIAs, e.g., RIA #1 (left column) was crossed by the highest number of ILs (shown on the top row) and RIA# 10 was crossed by the lowest number of ILs. Because not all RIAs are epidemic nodes (only those that include epidemic cases are), RIAs are here distinguished from ranked epidemic nodes (RENs, top row). The exception that shows the difference between RIAs and RENs is RIA #9: this was a road intersection area that did not include cases. The graphic version of this table is shown in Figure 7a where, to highlight the role of epidemic nodes, the data are expressed as RENs, i.e., RIA # 9 is not plotted in Figure 7a, where only RENs (ranked epidemic nodes or road intersection areas that included cases) are shown.


N
20 0 20 Kilometers
Node centroids
Days 1-3
Days 4-6
Days 7-60
Nodes with cases, days 1-3 (n=3 nodes)
Nodes with cases, days 4-6 (n=56 nodes)
Nodes with cases, days 7-60 (n=157 nodes)
Uruguay (partial view)



SI Figure 1. Number and location of epidemic nodes and centroid of epidemic nodes per epidemic days. **A**: FMD. **B**: AI H5N1.

Chapter 7

Development of disease-specific, context-specific surveillance models: Avian Influenza (H5N1)-related risks and behaviours in African countries

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Development of disease-specific, context-specific surveillance models: Avian Influenza (H5N1)-related risks and behaviours in African countries

Impacts

- A substantially higher number of human infections with HPAI H5N1 may have occurred than have been reported in most African countries since 2006. Such risks may be due to under-reporting, lack of awareness, and censoring effects.
- Because food-borne contamination can lead to potential human infection, abattoir-based chicken surveillance is recommended to accompany farm and live-bird-market based investigations.
- Whether classified as HPAI H5N1-infected or -free, African countries are likely to benefit from disease surveillance conducted on farm animals and their products.

Summary

Avian influenza virus (H5N1) is a rapidly disseminating infection that affects poultry and, potentially, humans. Because the avian virus has already adapted to several mammalian species, decreasing the rate of avian-mammalian contacts is critical to diminish the chances of a total adaptation of H5N1 to humans. To prevent the pandemic such adaptation could facilitate, a biology-specific disease surveillance model is needed, which should also consider geographical and socio-cultural factors. Here we conceptualized a surveillance model meant to capture H5N1-related biological and cultural aspects, which included food processing, trade, and cooking-related practices, as well as incentives (or disincentives) for desirable behaviours. This proof-of-concept was tested with data collected from 378 Egyptian and Nigerian sites (local [backyard] producers/ live bird markets /village abattoirs/ commercial abattoirs and veterinary agencies).

Findings revealed numerous opportunities for pathogens to disseminate, as well as lack of incentives to adopt preventive measures, and factors that promoted epidemic dissemination. Supporting such observations, the estimated risk for H5N1-related human mortality was higher than previously reported.

The need for multi-dimensional disease surveillance models, which may detect risks at higher levels than models that only measure one factor or outcome, was supported. To develop efficient surveillance systems, interactions should be captured, which include but exceed biological factors. This low-cost and easily implementable model, if conducted over time, may identify focal instances where tailored policies may diminish both endemicity and the total adaptation of H5N1 to the human species.

Keywords: avian influenza; HPAI H5N1, Africa, food-borne infection, Monte-Carlo simulation.

7.1 Introduction

Highly pathogenic avian influenza virus H5N1 is endemic in Bangladesh, China, Egypt, Indonesia, Viet Nam, and eastern India (FAO, 2011; FAO, 2013). Because recent reports have indicated that the virus is now causing multiple outbreaks in Nigeria (OIE, 2015), endemicity may soon include more countries.

Unless humans are exposed to or consume infected poultry, H5N1 is not viewed as zoonosis (Fournié et al., 2013; Patel et al., 2014; Van Kerkhove et al., 2011). However, zoonotic dissemination may occur if bird-mediated (viral-human) contacts promote the full adaptation of the virus to humans (Van Kerkhove et al., 2011).

Unfortunately, this avian virus has already adapted to mammals: ferrets can be infected (Herfst et al., 2012; Imai et al., 2012), and seropositive guinea pigs and cats have been found (Zhou et al., 2015; Li et al; 2015). In addition, reassortant viruses bind to a human-type receptor (Li et al., 2015). Such facts are highly disturbing: a few additional mutations would suffice for the avian H5N1 virus to become fully adapted to humans –an event that could result in a pandemic (Morens et al., 2012).

Measuring the complexity of disease-related interactions may uncover avian-mammalian contacts. To that end, surveillance systems should be designed to be not only biologically specific (adjusted to capture the biology of a specific pathogen) but also tailored to measure associated (geographical, social, commercial) conditions that may prevent (or promote) disease dissemination. If valid, such a surveillance model could both determine the actual prevalence of morbidity and mortality and evaluate the efficacy of measures meant to disrupt viral-human contacts –a critical piece in efforts aimed at preventing zoonoses.

To develop a H5N1-specific surveillance model adjusted both to African conditions, two elements may be considered: (i) poultry is a major food item in Africa, and (ii) H5N1 infected poultry may reach humans (de Jong et al., 2005; Fasina et al., 2009; Kandeel et al., 2010; Ali et al., 2013). Hence, instances associated with exposure to or consumption of H5N1-infected poultry, may be investigated such as: (i) food markets, (ii) food transportation, (iii) commercial practices, (iv) culture (food-cooking related behaviours), and (v) demographics (subgroups with higher mortality risks, e.g., young pregnant women) (Areechokchai et al., 2006; Greiner et al., 2007; Fasina et al., 2010; Van Kerkhove et al., 2011).

The rationale of such model is that, if a more complex construct was built (such that more interactions were captured and they were functionally related), such model could better monitor avian-human contacts, detecting both desirable and undesirable inputs and/or outcomes. For instance, it could identify why and/or how, over time, improvements (or undesirable changes) occurred at specific geographical sites, points, facilitating replications of successful experiences (or prompt and targeted remedies).

To build a model that accounts for both predictors and outcomes, two sets of factors could be considered: (i) 'early' or preceding behaviours (those that occur before health outcomes are observable), and (ii) 'late' health events or outcomes. One example of 'early' behaviours could be those that refer to food production/transportation/processing. One set of 'late' factors are laboratory-based information on viral survival and infectiousness in poultry. Such research-based 'late' data can be used to predict human mortality.

Other early behaviours could include: (i) household poultry producers; (ii) village slaughter sites; (iii) live bird markets, (iv) the presence or absence of open contacts (non-compartmentalized communications, such as those that both transport eggs, and also carry chickens ready for the market); (v) the number and professional quality of food inspectors, and (vi) economic conditions that prevent (or promote) hygiene and/or epidemic control (e.g., incentives to sell or not to sell H5N1-contaminated chickens). Together, these indicators could inform on: (i) hygiene, and (ii) the presence (or absence) of procedures that prevent contaminations among the various sub-sectors of the poultry-related food system.

Late (health-related) outcomes could be viewed as interactions that lead to human mortality risks. Such interactions may be mediated by: (i) concentration and prevalence of HPAI H5N1 virus in poultry meat, (ii) concentration and prevalence of the virus in poultry faeces and/or the intestinal content (purge) of slaughtered chickens, (iii) the proportion of virus transferred to meat through contamination, (iv) the average volume of a cut piece of meat, (v) the average number of pieces in a food package, (vii) the final concentration of HPAI H5N1 virus in freshly cut poultry, (viii) the presence of virus in undercooked chickens, and (ix) the average volume of poultry eaten by an adult or a child.

To assess external validity, two countries that differed markedly in geographical, ethnic, and linguistic attributes were evaluated. Such model was investigated in Egypt and Nigeria, between 2008 and 2010, in 378 sites or interviews that included: (i) 174 live bird markets, (ii) 191 household poultry producers, (iii) 5 local/village butchers (where ≤ 100 birds were slaughtered every day); (iv) one commercial abattoir (where up to 30,000 birds were slaughtered every day); and (v) seven professional (veterinary) authorities. Geographically, the study included 15 districts in 3 Egyptian governorates, and 174 Nigerian markets (Anon, 2008; Pagani et al., 2008; Fasina et al., 2011). Through a combination of documents recorded as pictures and interviews (qualitative data), which were then summarized in quantitative terms adjusted to geographical/behavioural/economic dimensions, the basic question asked was: does a disease surveillance model that includes two or more predictors result in the same or different mortality risk than simpler ones?

7.2 Materials and Methods

7.2.1 The sites

Egyptian sites - Field evaluation: Fifteen Districts located in 3 Egyptian governorates were visited between June and November 2010. A total of (i) 191 household poultry producers, (ii) 5 local/village butchers (where ≤ 100 birds were slaughtered every day); (iv) one commercial abattoir (where up to 30,000 birds were slaughtered every day); and (v) seven professional (veterinary) authorities were visited for observations, surveys or interviews. Photographic documentations were done to serve as checklist for qualitative and quantitative data. These were backed by other documents (Ali et al., 2013; Edmunds et al., 2013).

Nigerian sites -live bird markets (LBM): Document analysis on 174 Nigerian live bird markets (LBM) were based on previous works (Pagani et al., 2008; Anon, 2008). When such sites were visited, qualitative data on biosecurity were collected (pictures). Such data were ranked as: very good (4); good (3); good/poor (2); poor (1); or very poor (0). Composite biosecurity scores were then expressed as percentages of all sites visited and adjusted to market categories (i.e., a daily market [the type that and provides the daily supplies consumed by households] or a weekly market [the market that supplies retailers]).

7.2.2 Risk assessment framework

Risk assessment is a process whereby probable outcomes are estimated and recommendations are made based on data collection and analysis of potential exposure to a hazard. Typically, risk assessments include at least three factors: (i) risks, (ii) hazards, and (iii) exposure. One example of a risk is the possible disease, if not also death, that follows after humans are exposed to Influenza A (H5N1) virus-infected chickens. However, with adequate measures, this risk can be prevented. One example of a hazard is the slaughter process: when processing poultry suspected to be contaminated, a proper slaughtering process may eliminate or prevent risks. Slaughtering provides a focal point at which hazards should be identified promptly and stopped from further dissemination into the human food chain (Box A).

Box A

Definitions of hazard, hazard identification, risk assessment, exposure assessment, dose-response model and risk characterization with regards to avian influenza A (H5N1) ingestion by humans

Hazard: Infectious influenza A (H5N1) virus in food that may have arose through farm-based infection of poultry or contamination during the slaughter/processing.

Hazard identification: Human infection with influenza A (H5N1) virus through aerosol/ingestion is identified through clinical symptoms or sero-epidemiological/virological assessment.

Risk assessment: The determination of the probability that an adverse effect of influenza A (H5N1) (morbidity or mortality) will result from a defined exposure through naso-oral route.

Exposure assessment: Inadvertent exposure is likely through visiting the live bird markets, during slaughtering, scalding, de-feathering, processing, the cutting and cooking process and/or through the consumption of partially cooked meat. Infection occurs through inhalation of aerosolised virus particles, contact with human mucosa and wound surfaces, and certain behaviours that facilitate oral uptakes including licking of fingers, accidental splashing of juices from raw contaminated product, tasting of partially cooked meat and cross-contamination of foods not likely to be cooked which could result in deposition of the virus in the oral cavity (Bauer et al., 2010).

Dose-response model: This refers to the magnitude of exposure and the degree/frequency of responses or adverse effects. We utilised the multi-stage model for the study based on biological premises that consider epidemiological data with increasing potential as time progress (Altshuler, 1981).
Risk characterization: The estimated adverse health effects predicted to occur in the population in response to foodborne infection with influenza A (H5N1) and a summary of assumptions and sources of uncertainties (Altshuler, 1981; Bauer et al., 2010).

Because disease dissemination is proportional to the number of contacts with contaminated food and the average viral concentration of each exposure, exposure-related factors should also be considered in risk assessments. An exposure to a pathogenic agent usually occurs in a dose-response relationship. Such relationship describes the likelihood and severity of adverse health consequences (the responses) in relation to the amount of the pathogens. In this case, an exposure to a low level of influenza A (H5N1)

virus may present with serological response but will not lead to clinical outcomes. However, with a higher dose, the same virus may lead to clinical outcomes and possibly death (Box A).
Risks were assessed with two models. One focused on food. The second focused on one demographic subset (pregnant women).

7.2.3 Food-related risks - Parameters and assumptions

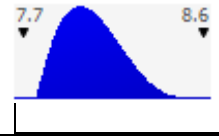
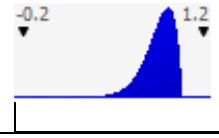
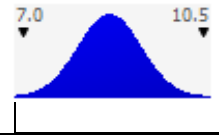
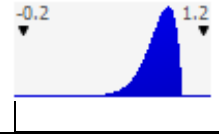
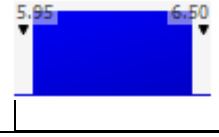
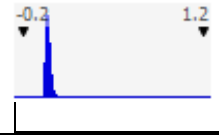
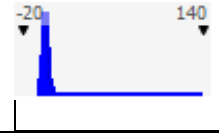
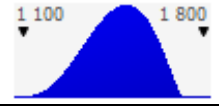
Based on the literature, input variables were functionally related and characterized as shown in Figure 1 and Table 1:

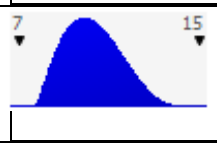
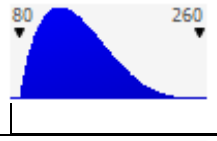
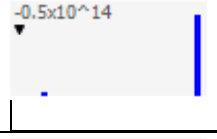
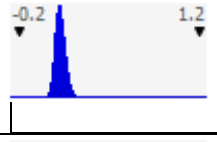
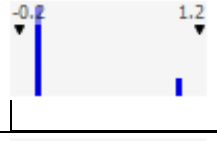
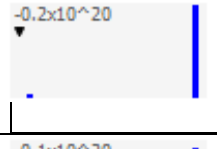
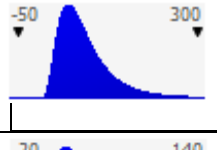
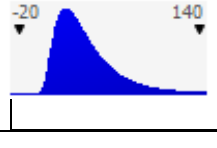
1. The concentration of Influenza A (H5N1) in meat is within the low to high infective dose (Log10 EID_{50} (10^7.8 -10^8.5) and, in faeces, 10^8.7 (Doyle et al., 2007; Swayne and Beck, 2005; Thomas and Swayne, 2007).
2. At least 90 % of the contaminated meat will contain infectious virus (Das et al, 2008; Swayne and Beck, 2005; Van Reeth, 2007).
3. In the absence of biosecurity, contamination will be carried out across all steps –from delivery of live birds, to delivery to homes for cooking.
4. Each processed chicken weighs between 1.13 and 1.7kg and will be cut into 8 – 14 pieces (USDA-AMS, 2008; ACMF, 2014). On average, adults eat 2 pieces/meal and children one piece/meal.
5. Fifteen percent of the meat is undercooked, i.e., with average cooking temperature ≤73^{O}C (Worsfold and Griffith, 1997; FAO/WHO, 2001).

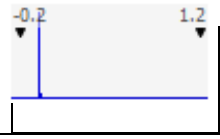
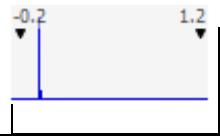
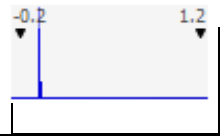
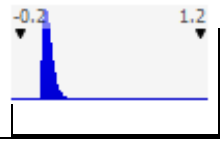
The probability distributions included the following input parameters:

- RiskBinomial: Input values: n= number of trials; P= the probability that each trial is successful (the desired event is achieved);
- Riskbeta: Input parameters: n= number of trials; number of successes X
- Riskbeta (alpha1, aplha2): p = Beta(x +1, n − x +1)
- Risk Poisson: Input is parameter lambda λ X t, average number of events per unit interval of space or time
- Uniform (minimum, maximum): Input: the minimum and maximum values of the variable
- Beta PERT: distribution: Input minimum, most likely, maximum values
- Triangular distribution: Inputs: minimum, most likely, maximum
- Normal distribution (inputs described by the mean and standard deviation).

Table 7.1: Input parameters for the process risk model for Influenza A (H5N1) food-borne infection

Maximum	Mean	Minimum	Function	Graph	Name
8,5	8,05	7,8	RiskPert		Concentration of HPAI in meat from infected animal
1	0,8333333	0	RiskBeta		Prevalence of HPAI in meat from infected animal
+∞	8,7	-∞	RiskNormal		Concentration of HPAI in faeces
1	0,8333333	0	RiskBeta		Prevalence of HPAI in faeces
21,4	16,01667	10,7	RiskPert		Factor for cross-contamination of carcasses (ratio of infected to clean carcasses)
6,45	6,225	6	RiskUniform		Fraction of HPAIV in the purge i.e fraction of C_A
1,49	1,028333	0,48	RiskPert		Log10 of the Proportion of transferred from meat to surface
1	0,0373134 3	0	RiskBeta		Occurrence of cross contamination
132	4,925373	0	RiskBinomial		BinomialDist (1;Pcross)
1700	1471,667	1130	RiskPert		Mass of a cut (g)

14	10,33333	8	RiskPert		Number of pieces in a package
250	139,8333	89	RiskPert		Weight (g) of a retail package of fresh cut poultry (FCP)
+∞	2,38595E +14	0	RiskPoisson		Concentration of HPAI in contaminated FCP
1	0,154545 5	0	RiskBeta		Prevalence of HPAI in undercooked meat
1	0,154545 5	0	RiskBinomial		Occurrence of HPAI in undercooked meat
20	15	10	RiskTriang		Fraction of the virus surviving in the protected area
+∞	1,87221E +20	0	RiskPoisson		Ingested dose per adult
+∞	9,36104E +19	0	RiskPoisson		Ingested dose per child
+∞	84	0	RiskLognorm		Mass of poultry consumed per meal (adult)
+∞	42	0	RiskLognorm		Mass of poultry consumed per meal (child)

1	0,06543967	0	RiskBeta		Probability of hospitalisation given pregnancy
1	1,19998E-05	0	RiskBeta		Probability of illness for below age 19-19years
1	2,49995E-05	0	RiskBeta		Probability of illness for age 20-64
1	0,000670987	0	RiskBeta		Probability of illness for age >64
1	0,06122449	0	RiskBeta		Probability of mortality in pregnant women

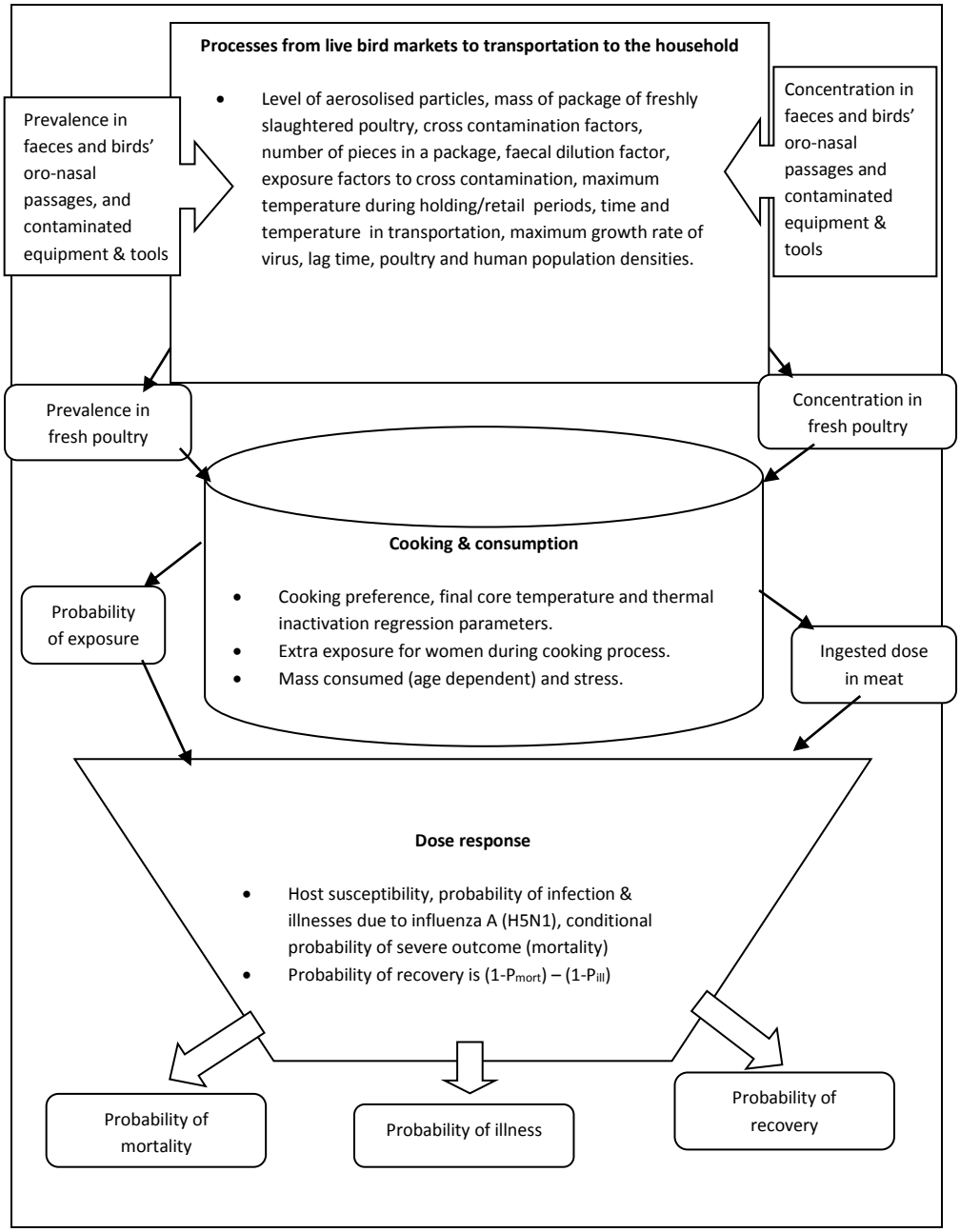


Figure 7.1. Food (chicken) production processes and risk pathways for human infection with influenza A (H5N1) through food borne or aerosolised routes.

NB: Pathway adapted from Cassin et al., 1998.

7.2.4 Pregnant women-related risks - Parameters and assumptions

In the second model, pregnant women were considered because, associated with their pregnancy-related impaired immunity and home cooking habits, they may possess higher risks of infection (WHO, 2007; Creanga et al., 2009). We assumed that between 1 and 25% of the pregnant women were infected and only half of them (between 0.5 and 12.5% of the pregnant women) would seek hospitalization. Such assumption was based on the 2008 Nigeria Demographic and Health Survey document (NPC & ICF, 2009), which indicated that 58% of pregnant women seek ante-natal hospitalization over a 5 year period. Therefore, 11.6% is the annual average of pregnant women that seek hospitalization in Nigeria, of whom 0.00058 (0.005*0.116) and 0.0145 (0.125*0.116) is the proportion of the women population that could be infected. Data on age categories used for pregnant women were estimated from the available demographic statistics (CIA, 2015). The obtained proportion of potentially infected pregnant women was introduced into the model built for the general population and adjusted appropriately.

7.2.5 Process risk modelling

All live bird markets (LBM) related processes were observed from delivery and trading of live birds, up to packaging and departure from the LBM. Home-related activities associated with increased risks of infection with influenza A (H5N1) virus were integrated based on the knowledge of the economic, cultural and sociological factors that prevail in African countries (see online Supporting information). Quantified estimates of risks were made for each sub-process based on available literature, integration of mathematical models where available or using the most likely scenario. Each exposure variable was estimated based on appropriate distribution models and a final multi-stage dose-response risk model was built as described by FAO/WHO (2001) and WHO (1995). The data were exported from Microsoft Excel® into the @Risk environment software package (Palisade Corporation, Ithaca, NY, USA), where 100,000 Monte Carlo simulations were run. The minimum, mean, maximum possible scenarios were calculated and the full model was subjected to sensitivity analyses. Demographics of the African countries affected by H5N1 were considered. To that end, the 2006 population data for Nigeria and 2013, or 2014 population estimates for Egypt, Sudan and Ghana were used. For other countries, the 2010 UN's World Population Prospects were used (UN-ESA, 2010).

7.3 Results

While the two investigated countries differed geographically, socially, and linguistically, they revealed similar information (Figure 2). Table 2 summarizes such findings as:

(i) no compartmentalization (each element of the system [a producer, a distributor, a buying market] had numerous and abundant contacts with all other elements, facilitating disease dissemination);
(ii) no hygiene;
(iii) dual use of the same distribution system (the same people/vehicles that distributed eggs also picked up chickens ready for the market);
(iv) no training/education on basic preventive behaviours;
(v) very limited (or no) supply of such services (only commercial abattoirs had veterinary inspectors);
(vi) no incentives to prevent contaminations/disease dissemination;
(vii) incentives to spread diseases (selling infected chickens was tacitly promoted).

Field evaluations revealed several anomalies (Figure 2). The composite mean biosecurity score was dismally low (25.00 – 25.74%, Table 2). Thus, poultry products seemed to possess high risks of contamination. Such inference was further supported by poor or non-existent veterinary services at LBMs (Table 2). Consequently, sick birds were likely to contaminate processed carcasses, and get access to the food chain outlined in the risk model.

Figure 7.2. A non-compartmentalized system that promotes disease dispersal

The same transportation system is used both to distribute eggs to small (backyard) producers and to pick up chickens ready to be processed and distributed to small (village) markets. No separation exists between the delivery of one-day old eggs (which are exposed to the environment) and the collection of chickens. Birds of different sources are kept together in slaughtering facilities and lacked water. No health inspection is performed at any one activity and no separation exist between the different compartments of the slaughter and processing sub-systems. Therefore, any infected animal has abundant opportunities to contaminate any other element of the system. Pictures like these were observed in both investigated countries.

Table 7.2: Review of data from 174 Nigerian markets to assess the risk of Influenza A (H5N1) food-borne infection

Variable*	Daily markets	Percentage score	Weekly markets	Percentage score
Monitor activities in the market by authorities	1	25	1	25
Document movement of poultry into or from the market	1	25	1	25
Availability of specifications for vehicles carrying birds	1	25	1	25
Formal training of operators	1	25	1	25
Location of market is appropriate to prevent/reduce human contacts	1	25	2	50
Poultry market separated from other stands	1	25	1	25
Reduce density for birds in cages	2	50	2	50
Separation of birds by age/class	2	50	2	50
Other animals traded in the market	1	25	1	25
Wild animals traded in the market	2	50	2	50
Mandatory routine disinfections of the market	2	50	2	50
Restriction of movement of operators from market to market	1	25	0	0
Availability of clean water	2	50	2	50
Availability of hot water	1	25	1	25
Access to facility to wash hands and shoes	1	25	1	25
Safe disposal of carcasses	2	50	2	50
Safe disposal of wastes	2	50	2	50
Disinfection of infrastructure and equipment	2	50	2	50
Disinfection of premises	1	25	1	25
Availability of storage facilities	1	25	1	25
Keeping of new arrivals separated from old stock	2	50	2	50
Water delivery system in place in the market	1	25	1	25
Food delivery system in place in the market	1	25	1	25
Cleaning of cages done routinely	1	25	1	25
Disinfection of cages done routinely	1	25	1	25
Prohibition of sharing of cages and other equipment	3	75	3	75

Disinfection of shared equipment	1	25	1	25
Traceability of origin of birds being sold	2	50	3	75
Certification system in place for transport of birds	1	25	1	25
Rest period between batches of birds	0	0	1	25
Disinfection of equipment used for slaughtering	2	50	2	50
Hands washing after slaughter	1	25	1	25
Composite mean score ± Standard Error (95% Confidence Interval)	**25.00±3.01 (18.99; 31.01)**		**25.74±2.96 (19.82; 31.65)**	

Data were compiled from the work of Pagani et al., 2008 and Anon, 2008. The qualitative scores were translated into very good = 4; good = 3, good to poor = 2, poor = 1 and very poor = 0 and used to calculate the composite mean biosecurity score in percentages.

**Note that: Several items in the variable carried the value "0" were part of the analysis but excluded in the table here. Other items were also excluded in this Table for brevity. The complete list of all the 68 variables tested can be viewed as supplementary material in the Supporting information.*

Combining both input parameters and recent population data (which included 11 African countries where avian influenza H5N1 has infected poultry), a total of 15,159 human infections (min = 4,758; max = 66,087) were estimated (Table 3). Of those, up to 1,964 may have died (min = 15; max= 12,688; Table 3). Such analyses did not include pregnant women.

When pregnant women were investigated (and a 25% infection rate was assumed), a total of 84,308 infections were estimated (min = 39,198; max = 176,594, Table 3) and up to 10,342 may have died. Instead, if a 1% infection rate was assumed, 3,372 women may have become infected with influenza A (H5N1) and approximately 414 may have died (Table 3).

The majority of the infected persons likely originated from Nigeria (n = 5,484, $CI_{90\%}$ = 1,686; 24,861), Egypt (n = 4,033, $CI_{90\%}$ = 1,370; 16,129), and Sudan (n = 1,446, $CI_{90\%}$ = 431; 6,419; Table 3). Ghana, Cote d'Ivoire and Burkina Faso were also likely to have numerous infected individuals (Table 3). On average, each of the African countries considered may have had between 0.0033 and 0.005% of their population infected, and up to 0.0006% of the population may have died (Table 3). Pregnant women and persons ≥ 64 years of age had high infection and death risks (81-82% and 10-11% of the total infected persons, respectively, Table 3). In contrast, children (≤ 19 years) accounted for only 2.3% of all infected persons and 2.5% of all deaths. Factors associated with mortality were: (i) virus presence in undercooked meat, (ii) ingested virus dose, and (iii) the volume (weight) of poultry consumed (Table 1).

Table 7.3: Probabilities of numbers of infected persons based on national demographic in the different African countries affected directly by HPAI H5N1.

		Probability of illness (n)					Probability of mortality (n)					Probability of recovery (n)
		Age 0-19	Age 20-64	Age >64	Pregnant women	Total	Age 0-19	Age 20-64	Age >64	Pregnant women	Total	
Nigeria (2006)	Mean ($CI_{95\%}$)	**884** (<1; 9423)	**1556** (7; 10444)	**3044** (1678; 4994)	**24654** (12282; 39397)	**30137** (13969; 64259)	**114** (<1; 1511)	**202** (<1; 2066)	**394** (<1; 1132)	**2987** (3; 23252)	**3696** (3; 27961)	**26441**
Egypt (2013)	Mean ($CI_{95\%}$)	**406** (<1; 4333)	**1152** (5; 7736)	**2475** (1364; 4060)	**18388** (9161; 29385)	**22422** (10531; 45514)	**53** (<1; 695)	**150** (<1; 1531)	**320** (<1; 920)	**2228** (2; 17343)	**2750** (3; 20488)	**19672**
Sudan (2014)	Mean ($CI_{95\%}$)	**174** (<1; 1854)	**496** (2; 3328)	**776** (428; 1273)	**6542** (3259; 10455)	**7988** (3690; 16910)	**22** (<1; 297)	**64** (<1; 658)	**100** (<1; 288)	**793** (<1; 6170)	**980** (<1; 7414)	**7008**
Ghana (2013)	Mean ($CI_{95\%}$)	**117** (<1; 1249)	**360** (2; 2418)	**684** (378; 1126)	**4757** (2370; 7603)	**5921** (2750; 12396)	**15** (<1; 200)	**47** (<1; 478)	**89** (<1; 255)	**576** (<1; 4487)	**727** (1; 5421)	**5194**
Djibouti (2010)	Mean ($CI_{95\%}$)	**4** (<1; 41)	**14** (<1; 91)	**20** (11; 32)	**210** (104; 336)	**247** (116; 500)	**<1** (<1; 7)	**2** (<1; 18)	**3** (<1; 7)	**25** (<1; 198)	**30** (<1; 230)	**217**
Cote dÍvoire (2010)	Mean ($CI_{95\%}$)	**97** (<1; 1033)	**273** (1; 1831)	**503** (277; 826)	**3773** (1880; 6030)	**4646** (2159; 9719)	**13** (<1; 166)	**35** (<1; 362)	**65** (<1; 187)	**457** (<1; 3559)	**570** (1; 4274)	**5351**
Benin (2010)	Mean ($CI_{95\%}$)	**46** (<1; 495)	**118** (<1; 791)	**178** (98; 292)	**1688** (841; 2697)	**2030** (937; 4276)	**6** (<1; 79)	**15** (<1; 157)	**23** (<1, 66)	**204** (<1; 1592)	**249** (<1; 1894)	**1781**
Togo (2010)	Mean ($CI_{95\%}$)	**29** (<1; 305)	**86** (<1; 575)	**138** (76; 226)	**1240** (618; 1982)	**1492** (694; 3089)	**4** (<1; 49)	**11** (<1; 114)	**18** (<1; 51)	**152** (<1; 1170)	**183** (<1; 1384)	**1309**
Burkina Faso (2010)	Mean ($CI_{95\%}$)	**89** (<1; 955)	**216** (1; 1448)	**243** (134; 399)	**3057** (1523; 4886)	**3606** (1658; 7687)	**12** (<1; 153)	**28** (<1; 286)	**31** (<1; 90)	**370** (<1; 2884)	**441** (<1; 3413)	**3165**
Niger (2010)	Mean ($CI_{95\%}$)	**91** (<1; 973)	**189** (1; 1270)	**229** (126; 376)	**2682** (1336; 4287)	**3192** (1464; 6905)	**12** (<1; 156)	**25** (<1; 251)	**30** (<1; 85)	**325** (<1; 2530)	**391** (<1; 3022)	**2801**
Libya (2010)	Mean ($CI_{95\%}$)	**35** (<1; 375)	**158** (1; 1058)	**279** (154; 457)	**2158** (1075; 3449)	**2630** (1230; 5339)	**5** (<1; 60)	**20** (<1; 209)	**36** (<1; 104)	**261** (<1; 2036)	**323** (<1; 2409)	**2307**
Africa	Mean	**1972**	**4618**	**8569**	**69149**	**84308**	**256**	**599**	**1109**	**8378**	**10342**	**73966**

Values in boldfonts are the mean number of individuals that were potentially infected, died or recovered. Probability of recovery = $[(1\text{-}P_{mort}) - (1\text{-}P_{ill})]$, where $1\text{-}P_{ill}$ = Population not infected and $1\text{-}P_{mort}$ = Population not infected + population infected without fatal outcome. Recovered persons may show sign of illness or may not have displayed classical respiratory signs of influenza virus infection (de Jong et al., 2005). The values in parenthesis behind the means are minimum and maximum values obtained at 90% confidence interval ($CI_{90\%}$). Note that the data used for pregnant women also considered women that seek hospitalization due to illness during pregnancy (Creanga et al., 2010; Thompson et al., 2010).

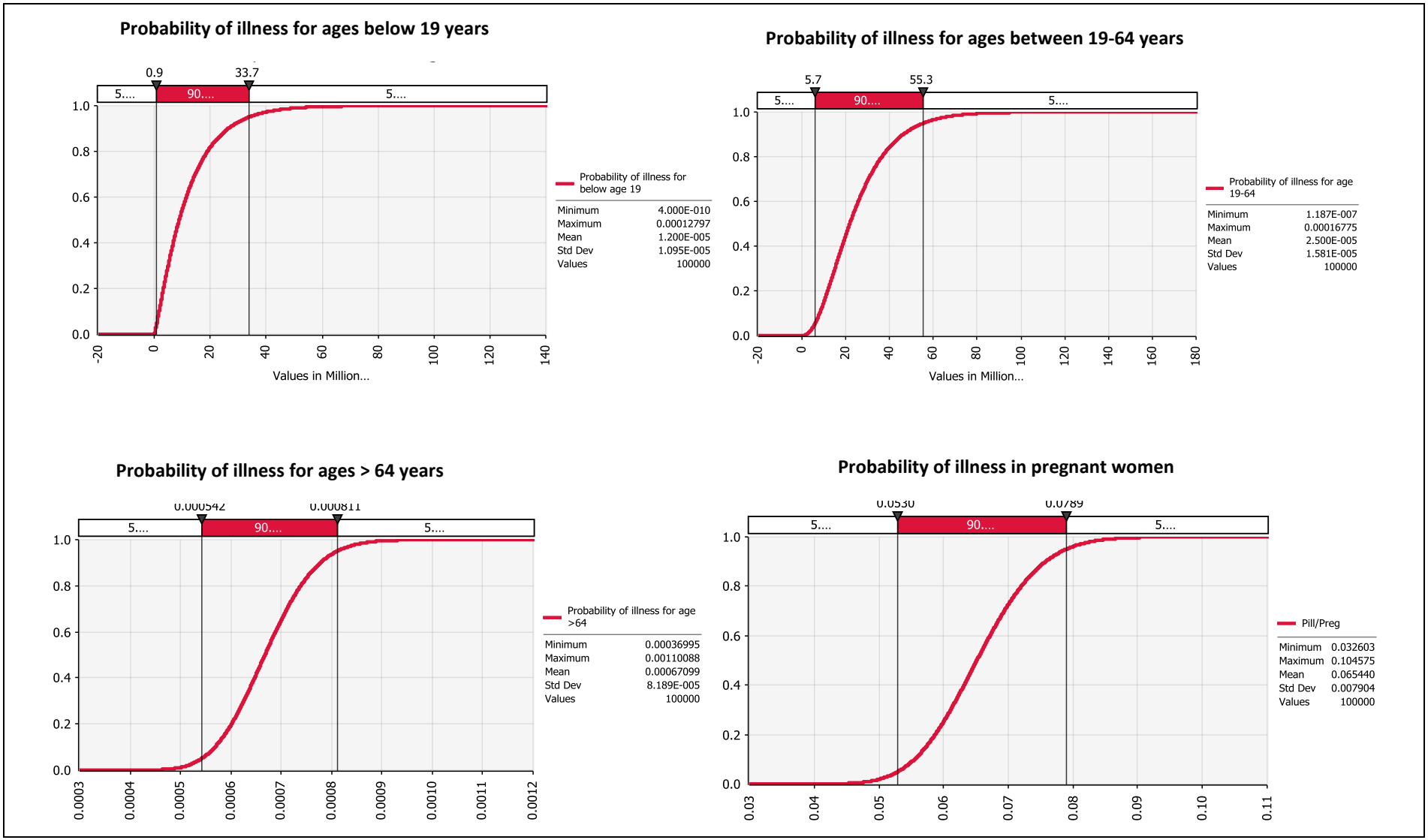


Figure 7.3: Probabilities of Illnesses with influenza A (H5N1) for the different age groups and pregnant women, Africa

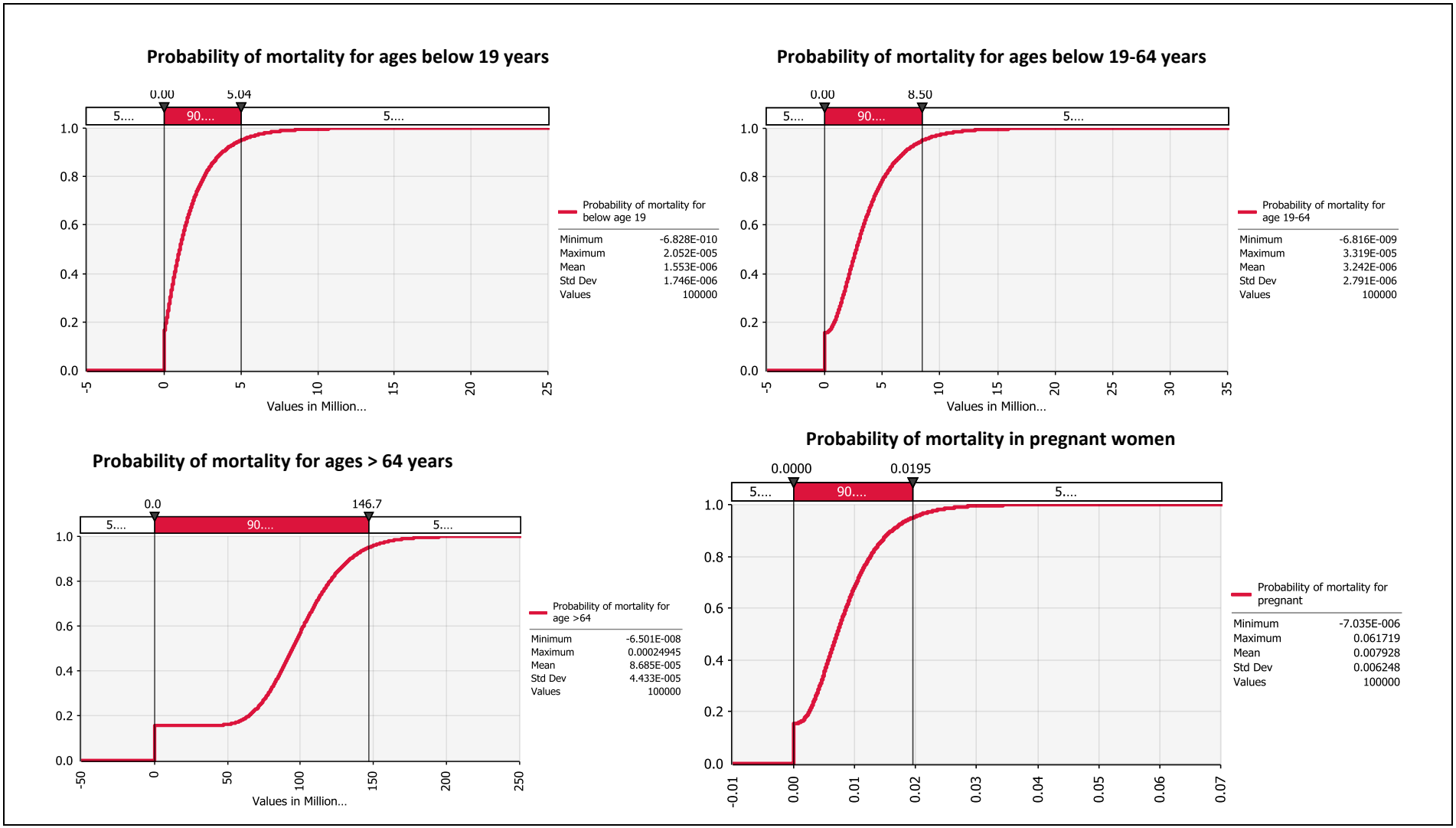


Figure 7.4: Probabilities of mortalities due to influenza A (H5N1) for the different age groups and pregnant women, Africa

7.4. Discussion

Risk assessment-related findings were based on assumptions that may influence the validity of this proof-of-concept. For example, when data on H5N1 were unavailable (due to scarcity of validated empirical data), parameters from other influenza A subtypes were utilized. Seasonal variations, which may be substantial (Robinson et al., 2013), were not considered. Such limitations do not modify the major message of this study: surveillance systems are critical to promote both health and safe nutrition, and to prevent zoonoses that may become pandemic. To build effective surveillance systems appropriate for viral influenza, the adaptation of the pathogen to other species should be considered.

Viral adaptations to new host species are known to depend on the frequency of interspecies contacts: the more contacts, the higher the chances of a full adaptation of avian influenza virus to humans. Should that occur, a pandemic cannot be ruled out.

The post-World War I pandemic provides an example of what a full adaptation of an avian virus to human hosts may generate: the virus disseminated by soldiers returning from the war killed up to ten times more people than humans deaths directly attributed to the war (Beran, 2008). Such example gives a reference on the magnitude of the problem today faced not only by African populations but also by the whole human species.

Such risk depends on how disease surveillance is conceptualized and then implemented. The available evidence suggests that many, if not most countries, are not well prepared for the challenge. Too many agencies, usually built along professional traditions, still lack centralized decision-making. Human health agencies tend to lack veterinary epidemiological expertise and animal health agencies may not possess human epidemiological expertise (Rabinowitz et al., 2012; Nichols et al., 2014).

Food biosecurity is a component of disease surveillance. To prevent a H5N1-mediated pandemic, the way poultry is produced, processed, transported, and commercialized is the first line of defense. Yet, biosecurity is not enough: public education campaigns aimed at promoting behavioural change are also needed. Countries affected by H5N1, such as Vietnam, have shown successful models on behavioural change (MARD and MoH, 2011). Such campaigns may consider food preparation methods (cooking, roasting, flame-grilling, barbequing, frying), which can reduce the risk of foodborne infection (Kayali et al., 2014).

Such campaigns may also focus on local or age-related aspects that influence risks.

For instance. teenagers, as well as pregnant women (and, consequently, young pregnant women) are suspected to be at high risk of infection and associated death (Jo-Trepka et al., 2007; WHO, 2007; Creanga et al., 2010; Oner et al., 2012).

Thus, interdisciplinary, research-based, surveillance systems are needed. While, potentially, the number of topics and disciplines may lead to an exorbitantly large number of combinations, a disease surveillance model that covers at least some inputs or early behaviours, and some health-related outcomes, such as this, is both broad enough to capture more information than classic models –which focus on clinical cases, a limited subset– and simple enough to be practiced at a low cost, and rapidly.

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Conflict of Interest

There are no conflicts of interest.

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The schematic risk pathway processes were colour coded. Those with red colour present with most risk comparatively, those in yellow colour with medium risk and those with green colour with low risk. It will be desirable to minimise risks at each process stage through the Hazard Analysis Critical Control Points (HACCP).

Supplementary Information 1: Schematic risk pathways and detailed contributors to each stage of the infection pathways for influenza A (H5N1)

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Appendix 2: Complete Table of the Review of data from 174 Nigerian markets to assess the risk of Influenza A (H5N1) food-borne infection

Variable	Daily markets	Percentage score	Weekly markets	Percentage score
Monitor activities in the market by authorities	1	25	1	25
Document movement of poultry into or from the market	1	25	1	25
Control movement of poultry into or from the market	0	0	0	0
Availability of specifications for vehicles carrying birds	1	25	1	25
Formal training of operators	1	25	1	25
Location of market is appropriate to prevent/reduce human contacts	1	25	2	50
Fencing and gates around the market	0	0	0	0
Poultry market separated from other stands	1	25	1	25
Ante and post-mortem inspection of birds	0	0	0	0
Access to para-veterinary services	3	75	3	75
Access to veterinary inputs	3	75	3	75
Garbage disposal services	0	0	0	0
All in-all out policy in the market	0	0	3	75
Segregation of customers and birds	0	0	0	0
Availability of cold chain	0	0	0	0
Quarantine for sick birds	0	0	0	0
Facilities for culling birds	0	0	0	0
Presence of a lab in the LBM	0	0	0	0
Presence of an incinerator in the LBM	0	0	0	0
Disinfection facilities for trucks	0	0	0	0
Reduce density for birds in cages	2	50	2	50
Active poultry sellers association	3	75	2	50
Separation of birds by species	0	0	0	0
Separation of birds by age/class	2	50	2	50
Control presence of wild birds	0	0	0	0
Control presence of pests	0	0	0	0
Other animals traded in the market	1	25	1	25
Wild animals traded in the market	2	50	2	50
Mandatory routine disinfections of the market	2	50	2	50

Restriction of movement of operators from market to market	1	25	0	0
Floor and walls are easy to clean	0	0	0	0
Presence of drains on the floor	0	0	0	0
Availability of clean water	2	50	2	50
Availability of hot water	1	25	1	25
Availability of toilets	0	0	0	0
Access to facility to wash hands and shoes	1	25	1	25
Access to facility to disinfect hands and shoes	0	0	0	0
Safe disposal of sick birds	0	0	0	0
Safe disposal of carcasses	2	50	2	50
Safe disposal of wastes	2	50	2	50
Good hygiene in the market	0	0	0	0
Good hygiene at slaughtering points	0	0	0	0
Disinfection of infrastructure and equipment	2	50	2	50
Disinfection of premises	1	25	1	25
Alternative use of disinfectants	2	50	2	50
Compensation mechanism in place for culled birds	1	25	1	25
Availability of storage facilities	1	25	1	25
Keeping of new arrivals separated from old stock	2	50	2	50
Enclosure to prevent escape	3	75	3	75
Improved cages present in the market	0	0	0	0
Water delivery system in place in the market	1	25	1	25
Food delivery system in place in the market	1	25	1	25
Cleaning of cages done routinely	1	25	1	25
Disinfection of cages done routinely	1	25	1	25
Prohibition of sharing of cages and other equipment	3	75	3	75
Disinfection of shared equipment	1	25	1	25
Traceability of origin of birds being sold	2	50	3	75
Certification system in place for transport of birds	1	25	1	25
Rest period between batches of birds	0	0	1	25
Policy is in place for unsold birds	3	75	2	50
Availability of processing facilities	3	75	2	50

Live in-dead out policy is in place	1	25	1	25
Improved packaging of slaughtered birds	1	25	1	25
Cleaning of equipment used for slaughtering	1	25	1	25
Disinfection of equipment used for slaughtering	2	50	2	50
Protective materials worn by slaughter/processing persons	0	0	0	0
Hands washing after slaughter	1	25	1	25
Hands disinfection after slaughter	0	0	0	0
Composite mean biosecurity score ± Standard Error (95% Confidence Interval)	**25.00±3.01 (18.99; 31.01)**		**25.74±2.96 (19.82; 31.65)**	

Data were compiled from the work of Pagani et al., 2008 and Anon, 2008. The qualitative scores were translated into very good = 4; good = 3, good to poor = 2, poor = 1 and very poor = 0 and used to calculate the composite mean biosecurity score in percentages.

*Note that: Several items in the variable that carried the value "0" which were excluded in Table 2 of the document were listed here. Similarly, all the items which were excluded previously for brevity were listed here. This is the complete list of all the 68 variables tested.

Chapter 8

General Discussion

This work explored the outbreaks of and factors associated with avian influenza H5N1 in humans and poultry of Africa. Focusing on Egypt and Nigeria, and using a combination of classical statistical and modelling tools; several lessons were learnt, which may be helpful in the future.

The effectiveness of active and passive surveillance programmes in Nigeria and the identification of gaps in such programmes in developing economies particularly in Africa were assessed. It was worrisome that despite the huge numbers of air travels and large volumes of trade between African countries and China, and the joint warning by the WHO, FAO and OIE, the level of preparedness for possible avian influenza H5N1 epidemic on the African continent was mostly lacking (WHO/FAO/OIE, 2004; WHO-AFRO, 2005; WHO, 2005; Mwacalimba and Green, 2014). Moreover, many African countries have factors that severely constrained the effectiveness of pandemic preparedness. Due to limited to non-existing diagnostic capacity and surveillance in wild and migrating bird populations, live poultry markets and other high risk locations early warning for the 2006 outbreaks of HPAI H5N1 in Africa was severely hampered, particularly in Nigeria and Egypt (Joannis et al., 2006; Joannis et al., 2008). The introduction of the virus into the African poultry populations resulted in massive outbreaks and deaths in poultry covering a total of 11 countries (Nigeria, Egypt, Niger Cameroun, Sudan, Burkina Faso, Djibouti, Ivory Coast, Ghana, Togo and Benin) primarily between 2006 and 2009 (Cattoli et al., 2009; OIE, 2010). These outbreaks have originated from multiple introductions and resulted in several mutations in the viruses and they have claded into at least four known sublineages (I, II, III & IV) (Ducatez et al., 2006; Cattoli et al., 2009; Fusaro et al., 2009; Cattoli et al., 2011). Recently presence of the virus has been confirmed in Libya and there have been a resurgence of infections in 2015 in parts of West Africa including Nigeria, Niger, Ghana, Burkina Faso and Cote d'Ivoire (OIE, 2015).

A WHO-led post-outbreak evaluation conducted in Africa has revealed that though 46 countries in the continent claim to have pandemic influenza preparedness and response plans, in most countries these plans are incomplete regarding preparedness for avian influenza H5N1, because they lack information on planning and coordination, have poor prevention, containment and control strategies for avian influenza H5N1 and/or poor monitoring and assessment for the national pandemic preparedness (WHO-AFRO, 2010; WHO, 2011). Furthermore, the regional and national capacities for early detection, confirmation and characterization of epidemic and

pandemic threats were low and most countries do not have the capacity to conduct comprehensive risk assessments that identify populations and geographic areas at risk of epidemics (WHO-AFRO, 2010). Surveillance systems were unable to function as early warning systems; and linkages and collaboration between human and animal health sectors for a comprehensive approach to zoonoses were weak both at regional level and nationally (WHO-AFRO, 2010). In view of the many limitations mentioned above, it was not surprising that outbreaks were reported and became widespread in Africa. As at the time of writing this report, Egypt continues to report infection in human and poultry; and Nigeria, Niger, Ghana, Burkina Faso and Cote d'Ivoire have recently reported resurgence of infection in their administrative regions (OIE, 2015; Monne et al., 2015).

The problems at the human-animal interface for the transmission of some emerging zoonotic diseases, especially the H5N1 avian influenza revealed a lack of multi-sectoral coordination structures, lack of incorporation of technical expertise, lack of systems for sharing routine disease surveillance information and preparedness among countries, lack of timely responses to epidemics, as well as several underlying ecological, environmental and socio-economic factors (WHO-AFRO, 2010). Specifically, government agencies are usually built along the narrow much related professional views and often lack broad-based multidisciplinary-interdisciplinary and centralized decision-making. Human health agencies tend to lack veterinary epidemiological expertise and animal health agencies may not possess human epidemiological expertise (Rabinowitz et al., 2012; Nichols et al., 2014). In addition, public health programmes should be tailored to meet the local situations and take into consideration the agricultural practices and the people's perceptions.

While the challenges described above have been identified by African human- and animal health policy makers, the solutions are not far-fetched; there is a need to correct the identified limitations while carrying out sustained routine surveillance for avian influenza A and other influenza viruses in human and animals in Africa. Partial evidence of sustained surveillance has been seen in South Africa (Abolnik, 2010; Abolnik et al., 2010) and limited surveillance continues in the field in Egypt in the face of continuous outbreaks (Kandeel et al., 2010; El-Zoghby et al., 2011; Kayali et al., 2014). Whether other African countries plan and implement such routine programmes as part of the comprehensive animal-human health component is doubtful.

Trade implications may enhance the enforcement of the conduct of regular surveillance; for example, South Africa exports ostrich's leather to the European Union. While it may be argued that sustained surveillance is possible but extremely expensive, this work has demonstrated that carefully planned cheaper but modified surveillance programmes can be carried out

even in resource poor setting in Africa with immense benefits to human and animal health.

Sero-epidemiological surveys for avian influenza A (H5N1) antibodies in humans in the different African countries have yielded variable results, it seems unambiguous that the virus is not yet capable of efficient human to human transmission (Ortiz et al., 2007; Kandeel et al., 2010; Okoye et al., 2013; Kayali et al., 2014). However, in view of the following facts:

1. the virus is constantly mutating and may become effectively transmissible through airborne sources between mammals (Herfst et al., 2012);

2. closely matched molecular signatures have been found between birds and humans (Cattoli et al., 2009);

3. reassortant H5 viruses now bind to human-type receptor (Li et al., 2015);

4. and the virus can infect cats, ferrets, guinea pigs and other mammals (Herfst et al., 2012; Imai et al., 2012; Li et al., 2015; Zhou et al., 2015),

there is a need for continued monitoring for the H5N1 viruses in Africa where adherence to biosecurity principles is lacking and human-animal interactions are very frequent.

The Egypt household poultry production is peculiar because most household poultry are raised within the human habitations (See figure below) and the system facilitates intensive human-animal interactions, more than elsewhere in Africa and it is not surprising that the country recorded a large number of the outbreaks from Africa compared to the other countries. Egypt has since been declared as an endemically infected and many unreported human deaths may have been associated with H5N1 infections (FAO, 2011).

Figure. Human habitations with poultry building sandwiched in-between. Co-mingling of chickens, ducks, goats, turkeys and humans in human habitations are observed in Egypt.

While both the analyses of the human cases and deaths in Egypt, as well as the modelled food-related infections with influenza A (H5N1) have revealed that women appeared to be at a higher risk of infection and death than man, there will be a need to collect more underlying information supports and explains these findings with a view to implement actions to mitigate the increased risk. A previous study has associated the difference in outcomes of acute respiratory infections between men and women to differences in exposure (WHO, 2007). Women are more likely to be highly exposed due to their occupational roles, such as: being the primary household poultry producers, in-home slaughtering, defeathering and cooking procedures, caretakers and caregivers of infected individuals, marketers of infected poultry, as well as pregnancies with waned immunity, lesser access to healthcare facilities, and nosocomial infections (Fasina et al., 2007; Vong et al., 2009; Zhou et al., 2009).

Although the studies reported in this thesis have arrived at some valid outcomes, most of the results in human studies should be considered with some degree of caution because the analyses were based on reported infections and fatalities –and not on active surveillance. It is likely that unapparent infections have been missed, and individuals may have avoided hospitalisation (Kandeel et al., 2010; Okoye et al., 2013; Okoye et al., 2014). Because there is a general belief among backyard and rural farmers that there is little or no risk of infection from culling, defeathering, home slaughtering, and/or visiting infected premises, targeted biosecurity education will have to be intensified in Africa. Governments will need to back this type of education with prompt payment for compensation of lost stock. In view of the limited governmental resources, such plans may involve public-private (industry) partnership to fund such intervention. Structured compensation programmes do not exist in Africa at present.

Overall, the epidemiologic analysis revealed that low biosecurity standards and poor adherence to these principles contributed to the wide spread of HPAI H5N1 in poultry farms in Nigeria and Egypt. Because the farm-level risk factors for continued infection of poultry farms with HPAI H5N1 found in this work are inherently behavioural and can be modified by human decisions, farmers' education and carefully planned communications on biosecurity should be improved as done elsewhere (MARD and MoH, 2011). There is also a need to reorganise the animal agriculture industry in Africa. Peri-urban agriculture must be guided by strict adherence to principles of biosecurity. This can be achieved through (i) government involvement in animal agriculture; (ii) incentivising good farming practices that utilise and implement biosecurity rules; (iii) setting and enforcement of strict standards by the authorities for engagement in poultry farming in the rural and peri-urban areas of Africa.

In Egypt and Nigeria, the in-house poultry production systems and its intensity are fraught with challenges to animal disease control (Cristalli and Capua, 2007). The poultry networks system is highly connected at all administrative levels and both on-farm and community-mediated biosecurity will be vital to prevent and control disease. This work has established that biosecurity is less costly for the African countries than to allow the situation of avian influenza H5N1 to continue unabated.

In addition, live bird markets and poultry slaughter slabs/abattoir should also be reorganized. Pre-inspection slaughter will become necessary and test and slaughter policy may need to be implemented at the market level. Open slaughter, evisceration, improper disposal of visceral, flying feathers and contaminated equipment should be discouraged and incineration system must

be used installed in relatively big LBM while small LBM should be connected to these incinerators.

The avian influenza H5N1 epidemics in poultry and humans in parts of Africa have revealed that the interactions between biological factors and other inter-related network properties –physical and geographical- can supports connectivity-based model. The role of geographical factors has been confirmed in the spread of HPAI H5N1including roads, market networks, people and animal movements, river network and other such factors.

The important messages in this work remain that: (1). good surveillance systems are critical to control highly pathogenic avian influenza H5N1; and (2). biosecurity implementation and adherence at the farm, community and market-level will secure the future of poultry industry in Africa by reducing the risks of infections with avian influenza H5N1. Effort should be intensified to reduce human - animal contacts as more frequent contacts facilitate higher risk of infection with avian influenza H5N1 virus to humans.

Finally, it is important to note that:

1. The avian influenza H5N1 outbreaks experienced in Nigeria, Egypt and elsewhere illustrate a complex problem that affects not only African countries but have implications for the whole animal health system. These implications span human and animal health, food security, economic and policy.

2. Today, not only Egypt (where outbreaks affecting poultry and also humans are now endemic) and Nigeria (where the same situation may be supervening) but the larger poultry industry globally is at risk of avian influenza H5N1 and indeed other influenza viruses.

3. It is irrelevant to speculate on whether the 'probabilities' of a pandemic are low or high, the focus of all countries and development partners should be to jointly tackle the epidemics frontally in all countries and once and for all control and possibly eradicate them.

4. Because the virus can undergo genetic drift and shift, and has frequently mutated, the matter of eradication should be handled with some degree of urgency it desired. For example, currently, H5N1viruses now co-circulate with human and avian originated viruses like H5N9, H7N9, H5N9 and H9N2 in China; in the USA, the H5 viruses have infected 21 states with massive outbreaks in poultry and wild birds and in Egypt, new mutations have occurred and the virus has now transpired a large temporal space. These rapid actions will reduce the risk of further mutations and broader spread.

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Summary

Bird flu H5N1 has caused millions of deaths in poultry and is a zoonosis in humans. To control the viruses and prevent continuous circulation in poultry, an understanding of the epidemiology of the virus is needed. We evaluated the virus epidemiology in two affected African countries-Nigeria and Egypt to identify factors that played roles in the spread and to explore the potentials of the virus for becoming endemic in the poultry and human populations in Africa. The situation of influenza A H5N1 has been partially described in at-risk farms and live bird markets and collected samples were analysed to detect the virus.

The risk factors for HPAI H5N1 virus infection in poultry farms in Nigeria and in humans in Egypt during previous outbreaks include biosecurity-related factors such as receiving visitors on farm premises, purchase of live poultry/products and farm workers that live outside the premises. Because biosecurity is critical to reduce risk of infection, the effects of the implementation or neglect of biosecurity was assessed and people-related, environmental-related and other birds/animal-related risks exist that can be prevented through biosecurity. It is 8.45 times better to implement biosecurity than to do nothing.

The road networks also supported the epidemics in Nigeria because the disease clustered, shared nodes in the network and supported the infection to move from highly to poorly connected locations. Finally, models suggested that more human cases may have existed than those reported officially. Continuous monitoring of influenza viruses and implementation of measures that should reduce the burden of infection in Africa are necessary.

Nederlandse samenvatting

Vogelgriep H5N1 veroorzaakte miljoenen doden bij pluimvee en is een zoönose bij de mens. Om te voorkomen dat het oorzakelijk virus continu blijft circuleren bij pluimvee, is begrip van de epidemiologie van het HPAI Influenza A H5N1 virus noodzakelijk. In deze thesis wordt de epidemiologie van het virus in twee getroffen Afrikaanse landen, Nigeria en Egypte beschreven om factoren die een rol hebben gespeeld in de verspreiding te identificeren en om de mogelijkheden van het virus om endemisch te worden in de pluimveesector te beschrijven. Influenza A H5N1 is deels beschreven in risicogroepen en boerderijen en markten voor levende vogels, verzamelde monsters werden onderzocht op voorkomen van het virus.

Risicofactoren voor HPAI H5N1-virus infectie bij pluimveebedrijven in Nigeria en voor infectie bij de mens in Egypte omvatten biosecurity factoren: ontvangen van bezoekers in de stal, aankoop van pluimvee en pluimveeproducten, pluimveemedewerkers die buiten het terrein van het pluimveebedrijf wonen. Het effect van implementatie van biosecurity maatregelen werd onderzocht waarbij werd gekeken naar mens gerelateerde factoren, milieu gerelateerd factoren en andere vogels en dieren.. Ter voorkoming van vogelgriep bleek het 8,5 keer beter biosecuritymaatregelen te implementeren dan niets te doen.

In Nigeria heeft het wegennet de verspreiding van vogelgriep ondersteund, omdat de ziekte geclusterd bleek langs de knooppunten van dat newerk en de ziekte zich langs gedeelde knooppunten kon verplaatsen naar minder goed verbonden delen van het netwerk. Tenslotte bleek uit modellen dat mogelijk meer meer mensen geinfecteerd zijn geweest met het virus dan officieel gerapporteerd. Continue monitoring van influenzavirussen en de uitvoering van maatregelen ter preventive van verspreiding in Afrika zijn noodzakelijk.

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Scientific Abstract

Influenza A viruses have caused several devastating outbreaks in poultry with some zoonotic infections and deaths in humans. To control the viruses and their continuous circulation in poultry population, an understanding of the epidemiology of the viruses is needed. An evaluation of the situation of avian influenza A H5N1 in two African countries most affected by this virus was conducted. To that end, two aims were pursued: (i) to identify the factors that played important roles in the dissemination and circulation of the virus, and (ii) to explore the potentials of the virus from becoming endemic in the poultry populations in Africa. The analyses partly described the extent of H5N1outbreaks in Nigeria in at-risk and non-infected premises and live bird markets in the country. All samples (tracheal and cloacal swabs, parenchymatous tissues and sera) were analysed using virus isolation, reverse-transcription polymerase chain reaction and serology.

Primary data on influenza A H5N1 in human as recorded by the Egyptian government was collected and analysed using different variables and risk factors for HPAI H5N1 virus infection in poultry farms in Nigeria during 2006-2007 outbreaks in Nigeria were explored using the conditional logistic regression models. Factors that supported infections in new premises included: receiving visitors on farm premises, purchase of live poultry/products, farm workers that live outside the premises. Because biosecurity is important to reduce risks of infection in poultry farms, the effects and costs associated with the implementation or neglect of biosecurity, as well as the feasibility of implementation in the household poultry in Egypt were evaluated. Risks of particular importance for the household poultry were categorised into people-related, environmental-related and other birds/animal-related risks. Biosecurity measures were compatible in the household poultry and it is 8.45 times better to implement biosecurity than to do nothing against HPAI H5N1. The financial risk analysis was robust and withstood sensitivity analysis. The change that may occur in the course of the household poultry project was accommodated profitably.

The effect of road network and its contributions to the epidemics in Nigeria were evaluated to determine whether HPAI H5N1 exhibit properties of network theory. Spatial aggregation of cases (disease clusters), links among similar 'nodes' (assortativity), simultaneous activation of similar nodes (synchronicity), epidemic flows moving from highly to poorly connected nodes (directionality), and a Pareto analyses pattern were observed including synchronicity and directionality of spread properties.

An exploratory analysis was conducted on the probability of human infection through the oral contacts with A-H5N1 contaminated meat. Detailed quantitative risk assessment using predictive microbiology-process risk model revealed that up to 15,159 humans may have contracted HPAI H5N1 in Africa, with 1,964 deaths. These figures are higher than those officially reported. The model has limitations but bias associated with official epidemiological statistics which are often prone to underreporting, lack of awareness especially in the rural communities and censoring effects may also account for this discrepancy.

Finally, continuous monitoring of influenza viruses in Africa and implementation of measures that should reduce the burden of infection in Africa is necessary.

Curriculum vitae

Fasina, Folorunso Oludayo was born on 17th August 1972 in Ibadan, Nigeria. in 1998, he received the degree of Doctor of Veterinary Medicine (DVM) from the University of Ibadan, Nigeria. He did am MSc (Veterinary Science) *Cum Laude* at the Department of Production Animal Studies, University of Pretoria, South Africa in 2008 working on the molecular epidemiology of avian influenza H5N1 in Nigeria. He registered for the PhD programme at Utrecht University in the year 2009 and concurrently for another PhD programme at the University of Pretoria in 2010. In 2013, he obtained a PhD in Zoology (Molecular and classical epidemiology of African swine fever from the University of Pretoria. He has previously worked in the National Veterinary research Institute, Vom, Nigeria between 2001 and 2011 and joined the Pig Herd Health Unit of the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria in February 2011, where he works to date.

He has supervised 15 students to date several accolades and awards to his credit. He has published over 70 publications in peer-reviewed journals with the main focus on viral epidemiology, particularly those involving African swine fever and highly pathogenic avian influenza A H5N1.

List of publications included in the thesis

1. TM Joannis, CA Meseko, AT Oladokun, HG Ularamu, AN Egbuji, P Solomon, DC Nyam, DA Gado, P Luka, ME Ogedengbe, MB Yakubu, AD Tyem, O Akinyede, AI Shittu, LK Sulaiman, OA Owolodun, AK Olawuyi, ET Obishakin and **FO Fasina**. Serologic and Virologic Surveillance of Avian Influenza in Nigeria, 2006-2007. ***Eurosurveillance*** 13(42) (2008), 608-612, pii=19007; Article 5.Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19007.
2. **FO Fasina**, VI Ifende, AA Ajibade. Avian influenza A (H5N1) in humans: lessons from Egypt. ***Eurosurveillance,*** 15 (4) (2010), pii=19473. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19473
3. **Folorunso O. Fasina**, Ariel L. Rivas, Shahn P. R. Bisschop, Arjan J. Stegeman and Jorge A. Hernandez. Identification of risk factors associated with highly pathogenic avian influenza H5N1 virus infection in poultry farms, in Nigeria during the epidemic of 2006-2007. ***Preventive Veterinary Medicine***, 98 (2011), 204-208.
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5. Ariel L. Rivas, **Folorunso O. Fasina**, Almira L. Hoogesteyn, Steven N. Konah, José L. Febles, Douglas J. Perkins, J. Mac

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6. **Folorunso O. Fasina**; Patrick M. K. Njage; AbdelHakam M. M. Ali; Jobre M. Yilma; Dauda G. Bwala; Ariel L. Rivas; Arjan J. Stegeman. Development of disease-specific, context-specific surveillance models: Avian Influenza (H5N1)-related risks and behaviours in African countries. ***Zoonoses and Public Health***, March, 2015, doi: 10.1111/zph.12200.

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