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

Folorunso O. Fasina[1,2]; Patrick M. K. Njage[3]; AbdelHakam M. M. Ali[4]; Jobre M. Yilma[5]; Dauda G. Bwala[1]; Ariel L. Rivas[6]; Arjan J. Stegeman[2]

[1]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

[2]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalenaan, the Netherlands

[3]Department of Food Science, University of Pretoria, Hatfield, South Africa

[4]Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), El-Seka El-Baeda St., Abbasia, P.O. Box 131, Cairo, Egypt

[5]Emergency Centre for Transboundary Animal Diseases (ECTAD), FAO, Al Eslah El Zerai Street, Dokki-Cairo, P.O. Box 2223, Cairo, Egypt

[6]Center for Global Health, Internal Medicine, School of Medicine, University of New Mexico, Albuquerque, New Mexico, USA

Address correspondence to:

Fasina Folorunso Oludayo,

Department of Production Animal Studies,

Faculty of Veterinary Science,

University of Pretoria, Onderstepoort 0110,

Tel: +27 84 958 0042

E-mail: dayo.fasina@up.ac.za

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

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?

Materials and Methods

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]).

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 regard 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 aerosolized 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 utilized the multistage model for the study based on biological premises that consider epidemiological data with increasing potential as time progresses (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).

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 $\leq 73^{O}C$ (Worsfold and Griffith, 1997; FAO/WHO, 2001).

The probability distributions included the following input parameters:

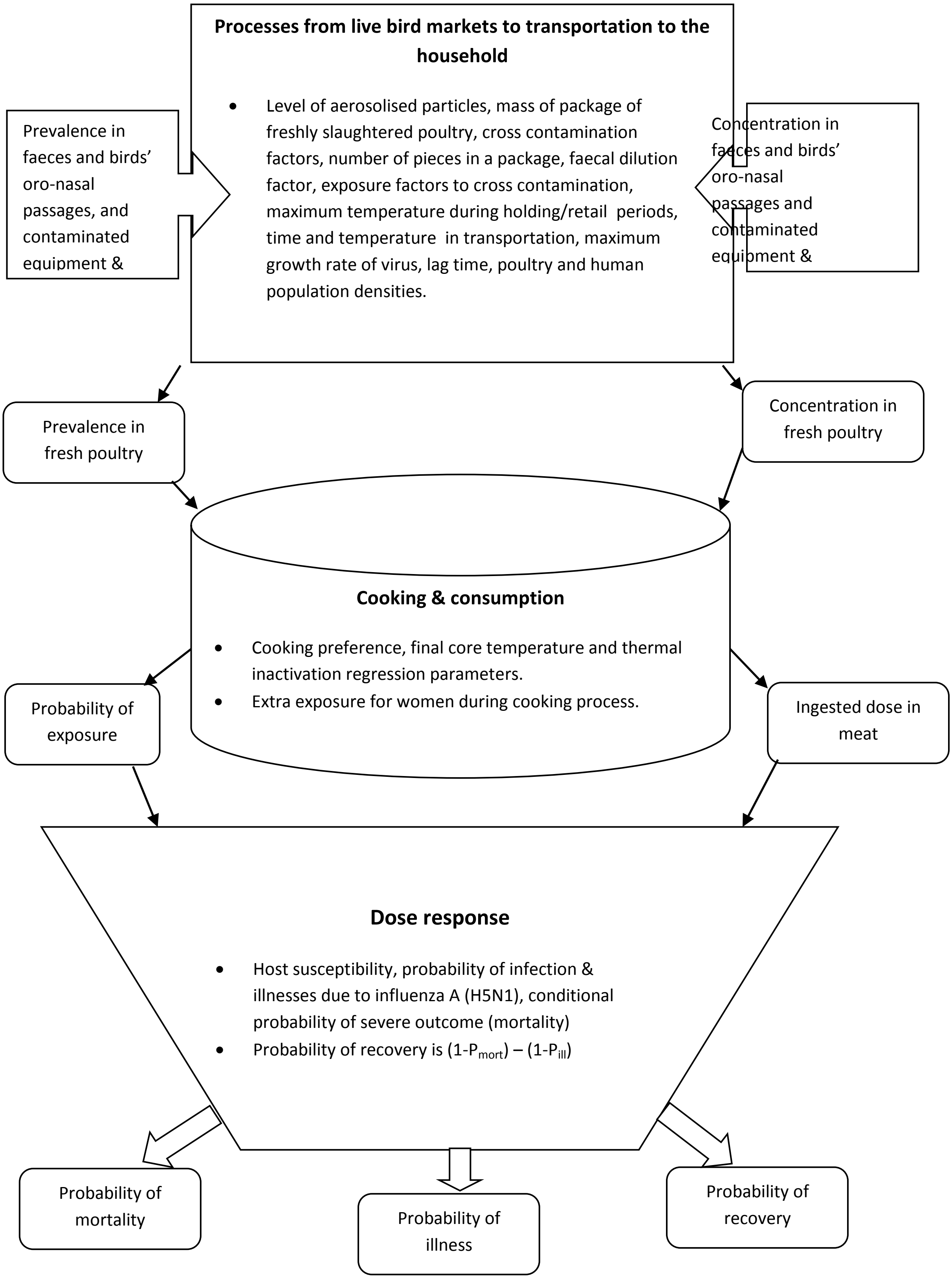
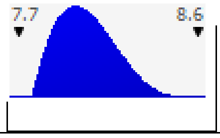
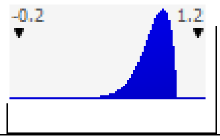
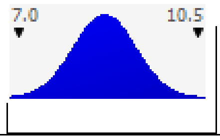
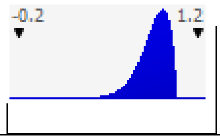
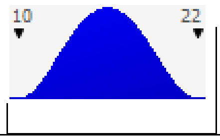
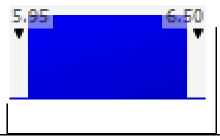
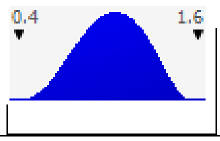
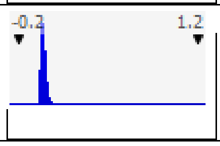
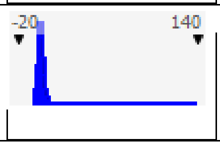
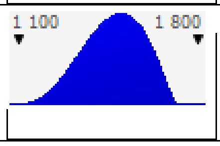
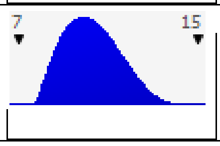
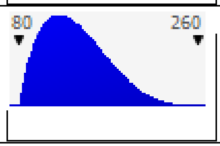
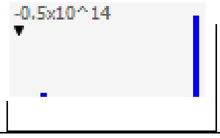
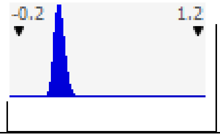
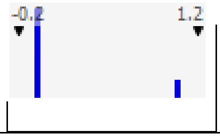
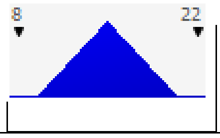
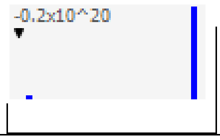
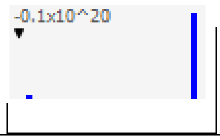
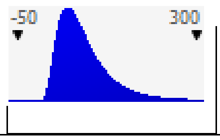
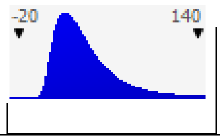
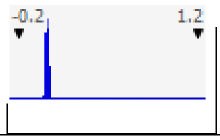
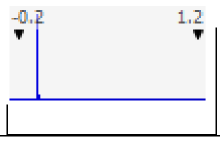
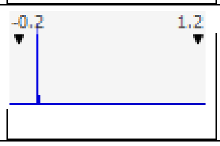
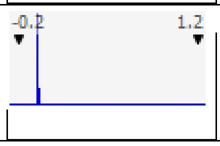
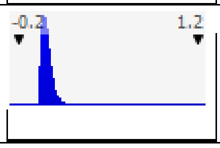


Figure 1. Food (chicken) production processes and risk pathways for human infection with influenza A (H5N1) through food borne or aerosolised routes.

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

Table 1: Input parameters for the process risk model for Influenza A (H5N1) food-borne infection

Serial number	Maximum	Mean	Minimum	Function	Graph	Name
1.	8,5	8,05	7,8	RiskPert		Concentration of HPAI in meat from infected animal
2.	1	0,8333333	0	RiskBeta		Prevalence of HPAI in meat from infected animal
3.	+∞	8,7	-∞	RiskNormal		Concentration of HPAI in faeces
4.	1	0,8333333	0	RiskBeta		Prevalence of HPAI in faeces
5.	21,4	16,01667	10,7	RiskPert		Factor for cross-contamination of carcasses (ratio of infected to clean carcasses)
6.	6,45	6,225	6	RiskUniform		Fraction of HPAIV in the purge i.e fraction of C_A
7.	1,49	1,028333	0,48	RiskPert		Log10 of the Proportion of transferred from meat to surface
8.	1	0,03731343	0	RiskBeta		Occurrence of cross contamination
9.	132	4,925373	0	RiskBinomial		BinomialDist (1;Pcross)
10.	1700	1471,667	1130	RiskPert		Mass of a cut (g)
11.	14	10,33333	8	RiskPert		Number of pieces in a package
12.	250	139,8333	89	RiskPert		Weight (g) of a retail package of fresh cut poultry (FCP)

13.	+∞	2,38595E+14	0	RiskPoisson		Concentration of HPAI in contaminated FCP
14.	1	0,1545455	0	RiskBeta		Prevalence of HPAI in undercooked meat
15.	1	0,1545455	0	RiskBinomial		Occurrence of HPAI in undercooked meat
16.	20	15	10	RiskTriang		Fraction of the virus surviving in the protected area
17.	+∞	1,87221E+20	0	RiskPoisson		Ingested dose per adult
18.	+∞	9,36104E+19	0	RiskPoisson		Ingested dose per child
19.	+∞	84	0	RiskLognorm		Mass of poultry consumed per meal (adult)
20.	+∞	42	0	RiskLognorm		Mass of poultry consumed per meal (child)
21.	1	0,06543967	0	RiskBeta		Probability of hospitalisation given pregnancy
22.	1	1,19998E-05	0	RiskBeta		Probability of illness for below age 19- 19years
23.	1	2,49995E-05	0	RiskBeta		Probability of illness for age 20-64
24.	1	0,000670987	0	RiskBeta		Probability of illness for age >64
25.	1	0,06122449	0	RiskBeta		Probability of mortality in pregnant women

- 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).

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.

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).

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.

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).

Figure 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 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 online Supporting information.

Table 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-P_{mort}) - (1-P_{ill})], where 1- P_{ill} = Population not infected and 1-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).

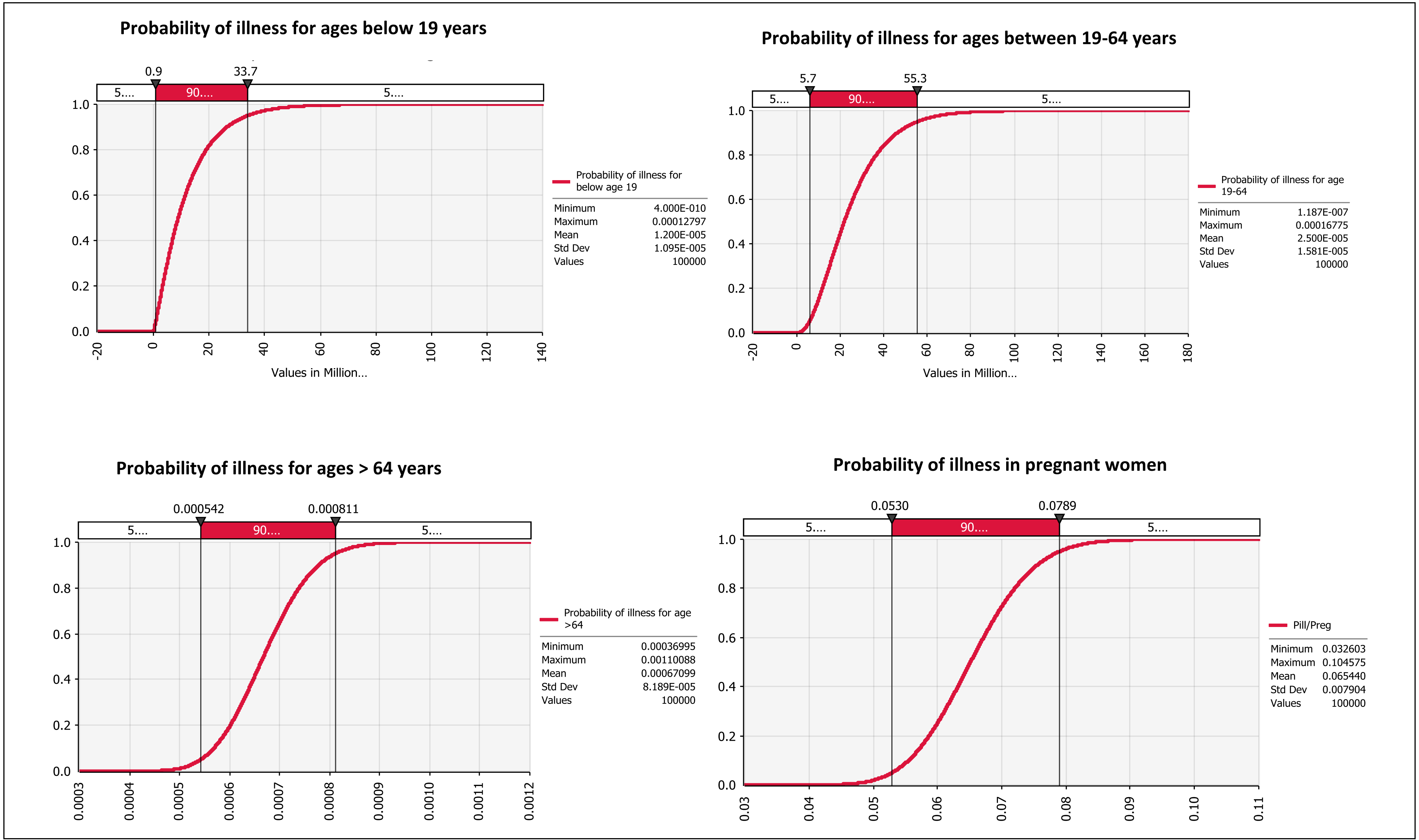


Figure 3: Probabilities of Illnesses with influenza A (H5N1) for the different age groups and pregnant women, Africa

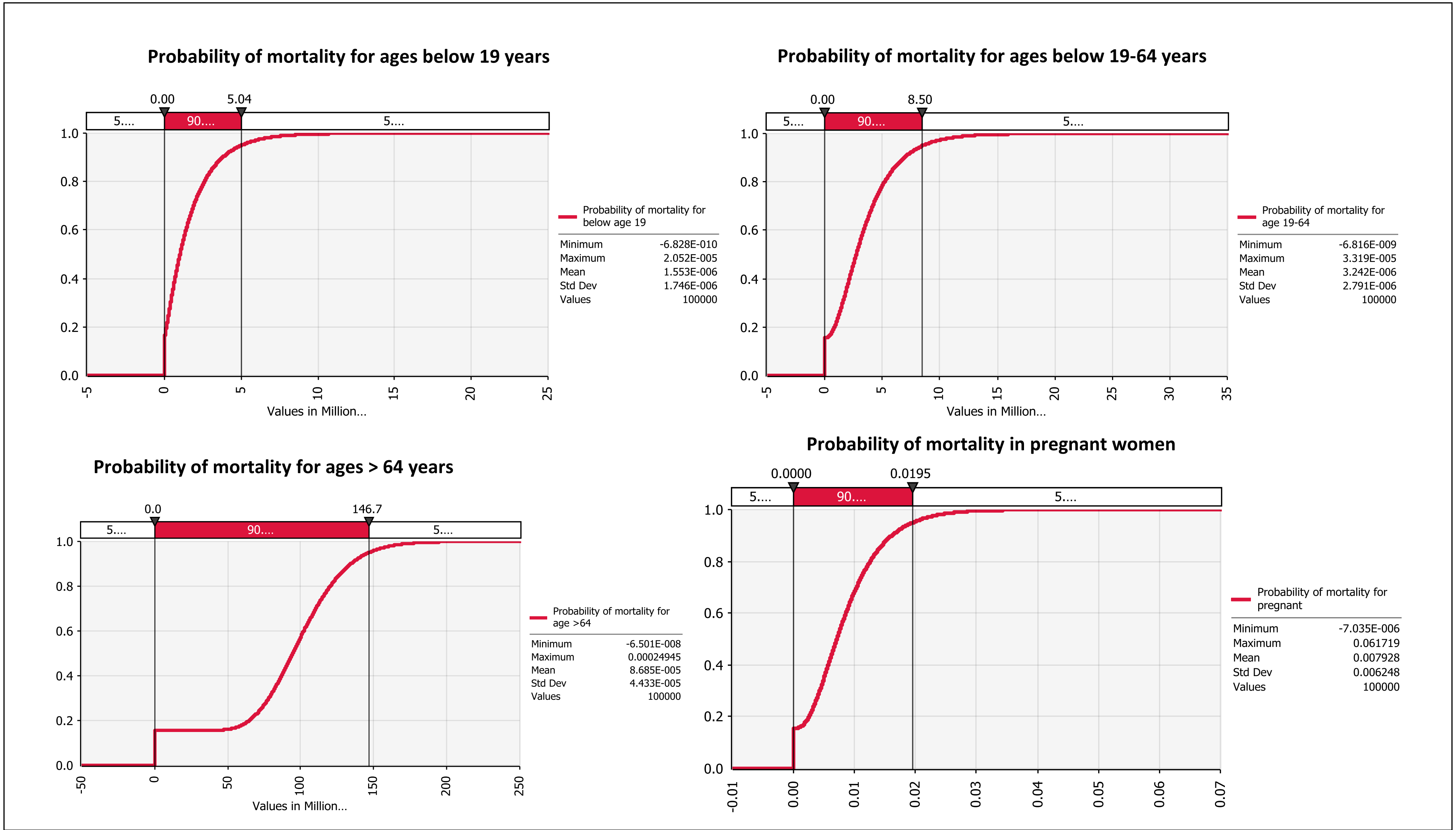


Figure 4: Probabilities of mortalities due to influenza A (H5N1) for the different age groups and pregnant women, Africa

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).

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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Trading

- Prevalence and concentration of Influenza A H5N1 in the LBM with direct or inadvertent human mucosal and wound contacts are determined by the sources of birds, level of aerosolised virus, faecal contamination, unkempt wound & unsanitary behaviours. These are influenced by temperature, humidity, virus strain, pH, dehydration, salinity and other microorganisms (Bauer et al, 2010; Scholtissek et al., 1985; Stallnecht et al., 1990; Shortridge et al., 1998; Lu et al., 2003b; Swayne and Halvorson, 2003; John and Rose, 2005; Fasina et., 2007).

Slaughtering

- Prevalence of concentration of Influenza A H5N1 posing risk to human during the slaughter process are influenced by the level of infection carried forward from the infected bird from trading, faecal contaminations, sources of birds, unsanitary behaviour, wound contaminations, behaviours supporting oral uptake like licking/eating during slaughter, season of slaughter, and contaminations from utensils, wipers and equipment (Luber et al., 2006; Bauer et al., 2010; Da Silva, 2014a, 2014b).

Scalding

- Prevalence of concentration of Influenza A H5N1 posing risk to human during the scalding process are influenced by the level of infection carried forward from the slaughtered infected /contaminated bird, temprature of water for scalding, unsanitary behaviour, contaminations from utensils, water quality, wipers and equipment (Arnold, 2007; Cohen et al., 2007; Nicholson, Groves and Chambers, 2005; Bauer et al., 2010; Da Silva, 2014b). Scalding temperature range between $50-58^{O}C$ and maximum of $\leq 65^{O}C$ for 4 minutes (insufficient to kill the virus). Underscalded carcasses are difficult to pluck and overscalded ones are cooked with torn flesh (Da Silva, 2014b.

Defeathering

- Prevalence of concentration of Influenza A H5N1 posing risk to human during the defeathering process are influenced by the level of infection carried forward from scalding of infected/contaminated bird. Unsanitary behaviour, contaminations from utensils, season, water quality aiding contamination, rinsing and wiping, and equipment will aid perpetuation of infection (Arnold, 2007; Cohen et al., 2007; Nicholson, Groves and Chambers, 2005; Bauer et al., 2010; Da Silva, 2014b). Humans may also get infected from infected contaminated feathers, aerosolised materials, and improperly discarded or accidentally discharged gut contents.

Evisceration

- Prevalence of concentration of Influenza A H5N1 posing risk to human during evisceration are influenced by the level of infection carried forward from defeathering. It is perhaps the most important sources of risk to processors of infected bird. Discharged gut content contains a high level of infectious virus and may contaminate carcass, tables and utensils directly. Unsanitary behaviour, contaminated utensils, wiping cloths, water quality, and equipment will aid perpetuation of infection (Cassin et al., 1998; Arnold, 2007; Cohen et al., 2007; Nicholson, Groves and Chambers, 2005; Bauer et al., 2010; Da Silva, 2014b).

Processing

- Prevalence of concentration of Influenza A H5N1 posing risk to human during further processing are influenced by the log concentration of virus shed above and the level of contamination. Washing of meat before and after evisceration, utensils, washing of tables, poor water quality, wiping cloths can all contribute to contamination. Surface areas of the carcasses are also opened up while cutting possibly with contaminated equipment and tools thereby aiding contaminants. Virus can continue to survive in the tissues up for 5-6 days post-slaughter. (Luber et al., 2006; Cohen et al., 2007; Nicholson, Groves and Chambers, 2005; Busquets et al., 2010; Bauer et al., 2010; Da Silva, 2014b; Indriani et al., 2010; Cogan, Bloomfield and Humphrey, 1999).

Packaging /Retailing

- Prevalence of concentration of Influenza A H5N1 posing risk to human during packaging/retailing are influenced by the log concentration of virus retained from the process above The final contribution will be dependent on the downstream and immediate transport for consumption, refrigeration (where feasible), and exposure of the packs to sulnight, pH, chemicals etc. (Logue and Nde, 2007; Bauer et al., 2010; Da Silva, 2014b; Indriani et al., 2010; Cogan, Bloomfield and Humphrey, 1999).

Transport

- Prevalence of concentration of Influenza A H5N1 posing risk to human during transportation is not significantly since it is expected to be done within a short time. It should be noted that many consumers in Africa prefer a freshly slaughtered and prepared carcass. Sociocultural observation shows that women will usually buy the birds for slaughter, pack it and travel home at the end of wirking day with about 30 minutes to 2 hours of travel time. Input is a factor of travel time to reach home. Most packages are transported piecemeal to meet the need of a family. At home, there is high likelihood that the meat is processed immediatelyfor consumption. (Ali et al., 2013a, 2013b; Bauer et al., 2010; Indriani et al., 2010;).

Refrigeration

- Socio-culturally, refrigeration is hardly practised in Africa particularly for smallcarcass originating from chicken or other smaller birds. Most consumers will prefer a freshly cut pieces and will proceed to cooking directly once the carcass reach the kitchen. In few cases, the more affluent individuals may have refrigerators and store a month supply of meat. However, where electricity is a challenge, and this further discourage the storage of meat for a relatively long time (Ali et al., 2013a, 2013b)

Cooking

- Prevalence and concentration of infectious Influenza A H5N1 in the cooked meat is dependent on the cooking preference, cooking time, final core temperature of $\geq 65^{O}C$, and the thermal inactivation regression parameters (FAO/WHO2002 doc, Ecosure, 2008, Cassin et al 1998; Doyle et al., 2007) . Traditional cooking method will inadvertently kill the virus but where meat is mildly roasted, marinated and prepared using fast-food preparation processes that does not consider relatively long cooking time, the risk of passing on of infectious agent (Influenza A H5N1 virus) is real.

Consumption

- Consumption present with minimal risk of infection save for the house wives that may taste meat during the cooking process to assess the degree of cooking. However, stressful conditions such as pregnancy, malnutrition, poverty, war situation, natural disaster are major issue that may drive an increase in food borne infection such as influenza A H5N1. Persons exposed to coagulated blood, blood puddings, chicken-and-duck blood soup and such food are also at higher risks (Chotpitayasunondh et al., 2004; De Jong et al., 2005; Hayden and Croisier, 2005; Doyle et al, 2007; Da Silva, 2014b).

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

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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 equipments	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 equipments used for slaughtering	1	25	1	25
Disinfection of equipments 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.