Transboundary and Emerging Diseases
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ORIGINAL ARTICLE

Avian Influenza H5N1 Surveillance and its Dynamics in Poultry in Live Bird Markets, Egypt

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Keywords:
Egypt; H5N1 HPAI; live bird markets; prevalence; surveillance

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Received for publication June 6, 2015

doi:10.1111/tbed.12440

Summary

H5N1, a highly pathogenic avian influenza (H5N1 HPAI), is an endemic disease that is significant for public health in Egypt. Live bird markets (LBMs) are widespread in Egypt and play an important role in HPAI disease dynamics. The aim of the study was to evaluate the H5N1 HPAI prevalence in representative LBMs from 2009 to 2014, assess the effects of other variables and evaluate past outbreaks and human cases. It was found that ducks and geese are high-risk species and that the prevalence of H5N1 HPAI was higher immediately after the political crises of 2011. The end of a calendar year (June to December) was a high-risk period for positive samples, and the risk in urban LBMs was twice the risk in rural LBMs. Winter and political unrest was associated with higher H5N1 HPAI prevalence. Both human and poultry populations will continue to rise in Egypt, so continued poultry outbreaks are likely to be linked to more human cases. LBMs will continue to play a role in the dynamics of poultry disease in Egypt, and there is a need to reorganize markets in terms of biosecurity and traceability. It may also be beneficial to reduce inter-governorate inter-regional movements associated with poultry trade through promotion of regional trade or in the alternative provide sanitary features along the poultry market chain to reduce the speed of H5N1 HPAI infections. Policy formulation, design and enforcement must be pro-poor, and consideration of the sociocultural and economic realities in Egypt is important. The LBMs provide ideal platforms to carry out sound surveillance plans and mitigate zoonotic risks of H5N1 HPAI to humans.

Introduction

In 2007, Egypt's standing poultry population was approximately 218 million birds (Ibrahim et al., 2007). The poultry industry in Egypt currently produces about 750 million to one billion birds annually (Kandeel et al., 2010; Ali et al., 2013). The exact population of household poultry is unknown; however, an estimated 4 to 9.5 million households raise poultry in confined spaces in houses, on rooftops or free range, with few, if any, biosecurity measures (CAPMAS, 2006; Fasina et al., 2012). This household poultry and the live bird markets (LBMs) may have played roles in the recent surge of zoonotic infection in view of the above considerations and because LBMs are critical links for commercial, small-scale household farms, slaughter houses, producers, traders and consumers (Ibrahim et al., 2007).

Poultry is critical to Egyptians' livelihoods, agricultural economy and food security (Elnagar and Ibrahim, 2007). The Egyptian poultry sector appears to be a combination of a very developed traditional and a modern farming system (FAO, 2007b). These two distinct production scales and systems as well as the range of the intermediate types have been conveniently grouped by FAO into four operational sectors (1–4) based primarily on the scale of production and level of biosecurity (FAO, 2004). Briefly described, the Sector 1 is an industrial integrated system with high-level biosecurity where birds/products are marketed commercially. It comprises of integrated broiler and breeder farms with clear production goals and clearly defined and implemented standard operating procedures for biosecurity. The Sector 2 is commercial poultry production system with moderate to high biosecurity, birds/products are usually marketed commercially, and strict prevention of contact with other poultry or wildlife exists. Sector 3 is commercial poultry production system with low to minimal biosecurity, and birds/products usually enter the live bird markets (e.g. caged layer farm with birds in open sheds or a farm with semi-intensive operation). The Sector 4 consists of village, household or backyard production with minimal biosecurity, and birds/products consumed locally or may be sent to the LBMs (Food and Agriculture Organisation of the United Nations (FAO), 2004).

Generally, poultry production in Egypt can fit into the four main sectors described above. While the industrial system of poultry primarily includes the following: Sectors 1 and 2: large-scale very intensive broiler, layer, parent and grandparent farms, often with strict biosecurity measures; Sector 3: majority of the intensive small- to medium-scale commercial broiler and layer farms with poor or no biosecurity (El-Zoghby et al., 2013), the household production system where poultry of all species are raised for both family consumption and trade; and biosecurity is poor and is often referred to as Sector 4 (Food and Agriculture Organisation of the United Nations (FAO), 2007b).

The occurrence of highly pathogenic avian influenza subtype H5N1 (H5N1 HPAI) was officially confirmed in Egypt in February 2006, and the disease spreads widely in 15 governorates within a month (OIE, 2008; Kandeel et al., 2010). To date, HPAI outbreaks have been reported in at least 1101 commercial poultry farms, and in 1806 locations in the household poultry production sector, H5N1 HPAI has thus become endemic in Egypt (World Organisation for Animal Health (OIE), 2008; FAO, 2013; EMPRES i, 2014). A total of 336 confirmed human cases of A/H5N1 and 99 human deaths related to A/H5N1 infection have been reported until 31 March 2015 (WHO, 2015; Ministry of Health, Egypt, unpublished data). Specifically, in the last 29 months as at end of June 2015, the number of human cases has been growing exponentially in Egypt. Similar surge has been reported by the Egyptian veterinary authorities. Between November 2014 and 30 April 2015, a total of 165 human cases were reported and 48 deaths were confirmed by the national health authorities, the highest number of human cases ever reported by any country over a similar period (Arafa et al., 2015; WHO-EMRO, 2015). Because H5N1 HPAI is endemic in Egypt, it is unlikely that the virus will be eliminated from the country in the short term, considering current production systems (FAO, 2011). Hence, H5N1 continues to limit the optimization of poultry economies and poses a serious public health threat in the country (Ibrahim et al., 2013).

Multispecies, multi-age live poultry marketing in Egypt is practiced at live bird markets (LBMs), in poultry shops and through peddlers in Egyptian cities and villages. Fresh carcasses may be available at poultry shops. The poultry meat trade in Egypt relies strongly on LBMs, partly because of consumers' long-standing cultural preference for fresh poultry meat (Abdelwhab et al., 2010).

LBMs are important outlets in rural and urban settings for the purchase and trading of birds, so they are prominent nodes in the HPAI risk pathways (Ali et al., 2013). Fournié et al. (2011) have confirmed that live bird trade in H5N1 HPAI-endemic areas is a major pathway in spreading the disease. These LBMs are a continuing source of influenza viruses, because of the dense concentration and high turnover of birds presented at the markets. Sources of contamination, transmission, virus amplification and reservoirs roles for HPAI have also been associated with such markets (Webster, 2004; Abdelwhab et al., 2010; Samaan et al., 2011).

The exact number LBMs in Egypt is unknown (Ali et al., 2013), but it is estimated that there are more than 2000. There are no dedicated or separate locations for LBMs, so they operate on narrow, crowded streets or in open markets where other commodities are also displayed for sale (Fasina et al., 2016). Weekly LBMs are held in most parts of the country. There are urban LBMs at district level, and scattered rural LBMs serve groups of villages at subdistrict level. Whereas these LBMs operate daily or weekly, other type of LBMs apart from these traditional LBMs is the retail shop. All categories (big, small, daily and weekly) of LBMs in Egypt operate with minimal to no biosecurity standards, and veterinary inspections are rarely implemented (Abdelwhab et al., 2010).

Despite the fact that H5N1 HPAI is endemic in Egypt, and LBMs are probable important facilitators of infections and transmission of H5N1 HPAI, so far, only one study has investigated the LBM-associated virus in detail to elucidate LBMs' role in the epidemiology of the disease (Kayali et al., 2014). The objective of the present study was to assess the prevalence of the H5N1 HPAI virus in the Egyptian LBMs and to ascertain variables associated with viruses isolated in samples collected from the LBMs from 2009 to 2014.

Materials and Methods

The study areas and surveillance procedures

Three phases of H5N1 HPAI surveillance were carried out in LBMs from 2009 to 2014. A total of 257 LBMs were randomly selected and visited in 24 governorates (10 in governorates in Upper Egypt (southern part of the country) and

14 in governorates in Lower (northern) Egypt). Of these, 96 LBMs were urban, and 161 LMBs were rural. All selected locations were recruited based on computational randomization and proportional representations from a list of available LBMs in each governorate.

Poultry species considered eligible for sampling were chickens, ducks, geese and turkeys. A total of 4134 pooled samples (tracheal and cloacal) were collected from approximately 16 000 birds. The epidemiological unit of sampling was a pool of 5–10 samples from the same bird species collected in the same live bird shop. For example, in a shop that sold chickens and ducks, tracheal and cloacal samples were collected individually from each bird and placed in the separate transport medium. In the laboratory, 5 pooled samples of chicken tracheal swabs from the same shop formed a unit, and 5 pooled samples of duck tracheal swabs formed another unit.

These samples were initially screened for the matrix gene. Positive pooled units were then re-evaluated individually (each aliquot in the constituent that contributed to the pool was considered) and tested separately for the H5 and N1 genes. The cloacal swabs were treated similarly. All swab samples were transported to, and analysed at the National Laboratory for Veterinary Quality Control on Poultry (NLQP) Dokki, Cairo, for confirmation of A/H5N1 diagnosis by RT-PCR, as described by Spackman et al. (2002).

Supportive epidemiological information on the sample types, sampling locations and dates, species of birds, clinical signs observed (if any) and sectorial origin of birds was collected using a pre-designed and tested checklist. In addition, comprehensive data on the reported poultry outbreaks (n = 3003) and human cases (n = 211) between 2006 and 2014 were obtained from Egypt's databank, the Emergency Centre for Transboundary Animal Diseases (ECTAD), FAO. An outbreak in this situation was defined as a case of an unusual increased mortality in a farm or LBM, wherein sample was collected and positive detection of H5N1 antigen or antibody was made or virus was isolated. Data on the 2012 human populations and land areas of governorates in Egypt were obtained from online sources (World Bank, 2015). Data on the 2010 poultry densities were estimated using the 2005 poultry population data and the livestock growth rates for each governorate (FAO, 2015). Population densities were then calculated by dividing the human or poultry populations by the total land area per governorate.

Prevalence estimation

HPAI prevalence, based on the pooled samples, was determined according to the following parameters of interest: (i) LBM types (urban or rural), (ii) LBM region (Upper or Lower Egypt), (iii) species (chickens, ducks, geese or turkeys), (iv) season [autumn (October–November), spring (March–May), summer (June–September) or winter (December–February)], (v) year and month(s) of isolation, (vi) governorates from which isolates originated, and (vii) origin of birds (household or commercial production sectors). The prevalence (Pr) was determined using the following equation: $Pr = \frac{nP.T}{N.T}$.

Where Pr = prevalence, nP = number of positive pooled samples for H5N1 HPAI for a given factor, T = time (in month, year or season as applicable) and N = total number of tested pooled samples for the same factor.

Data management and analysis

All the data were entered into Microsoft Excel® and filtered. STATA v 9.0 software (Stata Corp, College Station, Texas) was used to perform the statistical analysis. The result of the RT-PCR test using two categories (positive or negative and 95% confidence intervals) was used as the dependent variable, and all other factors were tested as explanatory variables (Table 1). The individual and confounding effects of the factors listed in the previous section were assessed using binary logistic regression. $P < 0.05$ was considered statistically significant.

A fitted linear regression model was used to identify the relationship between outbreaks in poultry as a single predictor variable (xj) and human cases as a response variable (y) if all the other predictor variables in the model were constant. A pairwise correlation was conducted, using a Bonferroni-adjusted significance level of $P = 0.05$ to assess the level of correlations of data for the two variables between 2006 and 2014. Similar analyses were conducted to correlate the relationship between poultry population densities as a single predictor variable (xj_2) and human population densities as the response variable (y_2) in order to determine whether one influences the other positively or otherwise.

Results

Prevalence of H5N1 HPAI and odds of isolation

At least 3003 poultry outbreaks and 211 human cases of avian influenza H5N1 have been reported since 2006. The number of poultry outbreaks and the number human cases differ per year (Table 1). More than a quarter (28.47%) of all pooled samples (n = 4134) were obtained in 2009, 5.56% were from 2012 to 2013 and 65.97% in 2014 (Table 2). Chickens accounted for 30.58% of the samples, ducks for 30.79%, geese for 18.80% and turkeys for 19.84% (Table 2). Approximately 64% of the samples originated from Lower (northern) Egypt, and samples from Upper (southern) Egypt made up the remaining 36.04% (Table 2). A total of 3.68% (n = 152 pools) were positive,

Table 1. Descriptive statistics of human cases and outbreaks in poultry, 2006–2014, Egypt

Chronological representations of reported outbreaks in poultry and human cases, 2006–2014									
Year	2006	2007	2008	2009	2010	2011	2012	2013	2014
Outbreaks in poultry	1007	282	115	176	476	386	97	98	366
Cases in human	18	25	8	39	29	39	11	4	38

Variable	Total number	Mean/year ± SD	Minimum case/year	Median case/year	Maximum case/year
Human cases	211	23.4 ± 13.8	4	25	39
Outbreaks in poultry	3003	333.7 ± 288.2	97	282	1007

Since the beginning of 2015 until 31 March, a total of 189 outbreaks in poultry and 131 human cases have been reported.

while 96.32% (n = 3982 pools) of all samples tested negative (Table 2).

Based on the year of sampling, the odds of obtaining positive samples were highest from 2012 to 2013. These odds were 5 times as high as those for 2009 ($P < 0.0001$). The odds that ducks, geese and turkeys would be positive were, respectively, approximately 5 times, 4.5 times and 3 times higher than those chickens. Significantly, higher numbers of the samples taken from ducks, geese and turkeys were positive, compared the samples taken from chickens (Table 2).

The odds of positive recovery of H5N1 virus in the LBMs in Upper (southern) Egypt were 2.3 times those in Lower (northern) Egypt ($P < 0.0001$). In addition, the findings revealed that the odds of positive cases of H5N1 virus from poultry in LBMs are higher in winter than at any other period in Egypt (OR = 3.7; $P < 0.0001$). Comparatively, the odds of isolation in summer were 3.2, as opposed to 2.1 in autumn, compared to spring. The odds of finding positive H5N1 virus in oronasal and cloacal samples from rural LBMs were half those of finding them in samples from urban LBMs ($P = 0.0002$; Table 2).

The odds of isolating positive samples from suspected cases in the LBMs increased from June to December and January of the following years, periods between summer and winter. Peak isolation rates were obtained in August and September and again in December and January. The odds of obtaining positive isolations from household birds (OR = 1.7) and unknown sources (OR = 1.8) were higher than the odds of obtaining them from commercial poultry at the LBMs (Table 2).

Predictive relationship between poultry and humans, outbreaks and populations

A slightly positive relationship was found between the poultry outbreaks of H5N1 HPAI and human cases of avian influenza H5N1 in Egypt, with a correlation value of 0.21 ($P = 0.58$). In the period from 2006 to 2014, for every additional outbreak in poultry, the regression fit predicted an increase of 0.0102 human cases (Fig. 1). For populations, a positive relationship was noted between the 2010 poultry population densities and the 2012 human population densities in the governorates in Egypt, with a correlation value of 0.67 ($P < 0.0001$). Generally, in the governorates, the regression fit associated every additional increase in poultry population density with a 0.5816 human population density (Fig. 2). However, human and poultry population growth rates differ between governorates, but it is more probable that the increase in human population density influenced the poultry population density (Data S1).

Discussion

H5N1 HPAI was declared endemic in Egypt in 2008 (Food and Agriculture Organisation of the United Nations (FAO), 2013), where LBMs are highly vulnerable to contamination with H5N1 virus, because infected poultry populations may arrive in and enter the markets intentionally or inadvertently (Samaan et al., 2012). These markets are key role players in the marketing of household and commercial poultry, including processed products, and LBMs are found in all districts in Egypt. Poultry of various species and ages arrive in the LBMs from various sources, and the prevalence of poor hygiene and a lack of biosecurity make these LBMs hotspots of infection in the poultry production value chain in Egypt (Ali et al., 2013). This study examined the prevalence of H5N1 HPAI in Egypt between 2009 and 2014. It was hoped that it would enhance understanding of the prevalence and dynamics of H5N1 HPAI in Egyptian LBMs to support decision-makers to improve future risk-based surveillance and control interventions.

Although it is possible that the political crises of 2010 and early 2011 affected the outcomes of this study, their impact does not invalidate the study. At the time of the crises, veterinary surveillance on poultry farms and in LBMs may have broken down temporarily, and the prevalence H5N1 HPAI may have intensified. It is also possible that H5N1 HPAI spreads further in poultry farms and LBMs at the time. The rate of isolation in the year immedi-

Table 2. Distribution and prevalence of samples analysed in the 2009–2014 H5N1 HPAI live bird market surveillance (n = 4134)

Variables	Categories	Distribution of tested samples (% of total)	Positive samples (%)	$CI_{95\%}$ (Binomial Wald)	Odd ratio of positive samples[a]	P-value
Year of sampling	2009	1177 (28.47)	2.72	1.92–3.83	Ref.	
	2012–2013	230 (5.56)	12.17	8.51–17.08	5.0	<0.0001
	2014	2727 (65.97)	3.37	2.76–4.12	1.2	0.29
Species sampled	Chickens	1264 (30.58)	1.19	0.70–1.97	Ref.	
	Ducks	1273 (30.79)	5.58	4.44–6.98	4.9	<0.0001
	Geese	777 (18.80)	4.89	3.57–6.66	4.3	<0.0001
	Turkeys	820 (19.84)	3.42	2.35–4.91	2.9	<0.001
Regions	Lower Egypt	2644 (63.96)	2.53	2.00–3.21	Ref.	
	Upper Egypt	1490 (36.04)	5.71	4.63–7.01	2.3	<0.0001
Seasons	Spring	1131 (27.36)	1.50	0.92–2.41	Ref.	
	Summer	953 (23.05)	4.72	3.54–6.27	3.2	<0.0001
	Winter	1210 (29.27)	5.29	4.16–6.71	3.7	<0.0001
	Autumn	840 (20.32)	3.10	2.10–4.52	2.1	0.02
LBM types	Urban	1967 (47.58)	4.83	3.96–5.87	Ref.	
	Rural	2167 (52.42)	2.63	2.03–3.40	0.5	0.0002
Months	May	324 (7.84)	0.31	0.00–1.91	Ref.	
	January	193 (4.67)	5.70	3.11–10.02	19.4	0.0001
	February	211 (5.10)	0.47	0.00–2.91	1.5	0.79
	March	218 (5.27)	2.75	1.13–6.01	9.1	0.02
	April	657 (15.89)	1.52	0.79–2.82	5.0	0.09
	June	808 (19.55)	3.71	2.60–5.27	12.4	0.0005
	July	95 (2.30)	4.21	1.31–10.67	14.1	0.01
	August	57 (1.38)	7.02	2.28–17.18	24.0	0.002
	September	73 (1.77)	9.59	4.45–18.77	33.83	<0.0001
	October	641 (15.51)	2.81	1.75–4.43	9.3	0.005
	November	409 (9.89)	5.13	3.34–7.76	17.4	<0.0001
	December	448 (10.84)	8.71	6.41–11.70	30.7	<0.0001
Origin of birds	Commercial	1017 (24.60)	2.36	1.57–3.50	Ref.	
	Household	1710 (41.36)	3.98	3.14–5.02	1.7	0.02
	Not known	1407 (34.04)	4.26	3.32–5.46	1.8	0.01
Governorates	Gharbia	326 (7.89)	0.92	0.18–2.80	Ref.	
	Kafr el-Shiekh	230 (5.56)	0.87	0.03–3.32	0.9	0.97
	Fayoum	397 (9.60)	4.53	2.84–7.10	5.1	0.003
	Alexandria	74 (1.79)	0.00	–	NA	–
	Assiut	126 (3.05)	4.76	1.98–10.22	5.4	0.02
	Aswan	86 (2.08)	1.16	0.00–6.92	1.3	0.80
	Beni Suef	148 (3.58)	4.05	1.68–8.75	4.5	0.03
	Behera	180 (4.35)	0.00	–	NA	–
	Cairo	112 (2.71)	9.82	5.42–16.89	11.7	<0.0001
	Dakahlia	398 (9.63)	2.26	1.13–4.31	2.5	0.17
	Dumyat	56 (1.35)	3.57	0.28–12.82	4.0	0.18
	Giza	222 (5.37)	8.11	5.12–12.52	9.47	<0.0001
	Ismailia	142 (3.43)	2.11	0.44–6.30	2.3	0.33
	Luxor	52 (1.26)	9.62	3.75–21.04	11.3	0.002
	Matrouh	12 (0.29)	0.00	–	NA	–
	Menia	129 (3.12)	5.43	2.46–10.97	6.1	<0.01
	Monufia	361 (8.73)	5.54	3.57–8.45	6.3	0.0005
	Port Said	23 (0.56)	8.70	1.25–27.97	10.1	<0.05
	Qalubiya	277 (6.70)	4.69	2.69–7.94	5.3	0.005
	Qena	68 (1.64)	14.71	7.99–25.19	18.3	<0.0001
	Sharqia	384 (9.29)	0.52	0.02–2.01	0.6	0.56
	Sohaj	226 (5.47)	6.20	3.64–10.21	7.09	<0.001
	Suis (Suez)	69 (1.67)	0.00	–	NA	–
	Wadi El Gadid	36 (0.87)	0.00	–	NA	–

[a]Conditional maximum-likelihood estimate of odds ratio calculation was carried out using mid-P exact values.

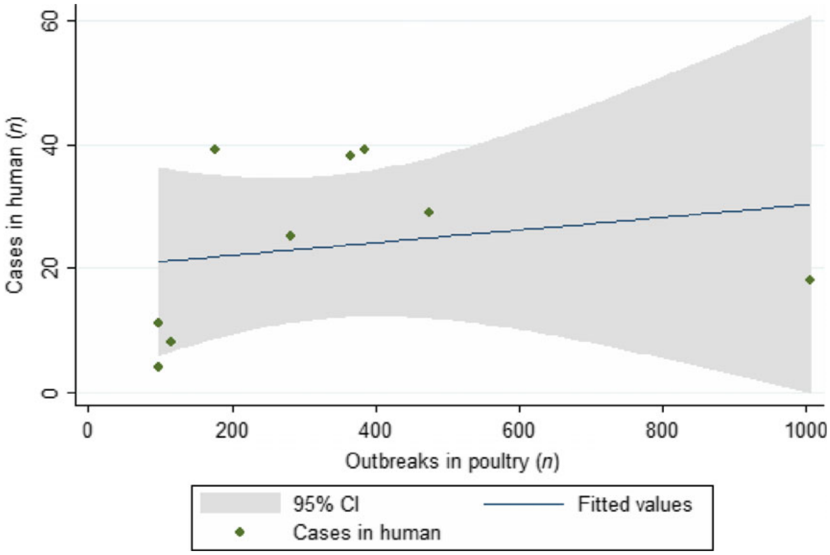

Fig. 1. Scatter plot with linear regression fit and a 95% confidence interval for reported poultry outbreaks and human cases, 2006–2014. Slope coefficient for outbreaks in poultry = 0.0102; *P* = 0.58. [Colour figure can be viewed at wileyonlinelibrary.com]

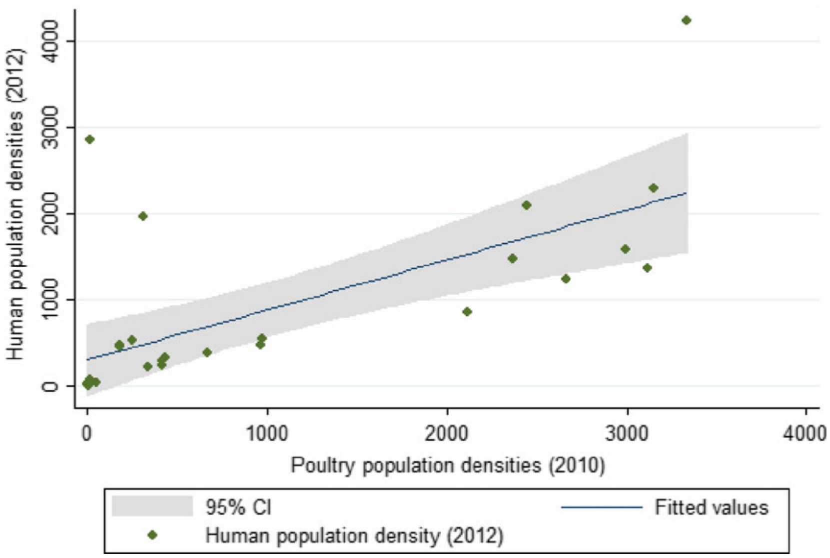

Fig. 2. Scatter plot with linear regression fit and a 95% confidence interval for 2010 poultry population densities and 2012 human population densities in governorates, Egypt. Slope coefficient for outbreaks in poultry = 0.5816; *P*-value < 0.0001. [Colour figure can be viewed at wileyonlinelibrary.com]

ately after the crises (2011–2012) was 4.5 times what it was in 2009. It is relevant to this study that Bakamanume (1998) and Kherallah et al. (2012) have previously confirmed the effect of conflicts and political unrest on the epidemiology of animal and human diseases.

Furthermore, because the Egyptian government still struggles with partial implementation of the 2010 Animal Health and Livelihood Sustainability Strategy which was aimed at culling mass vaccination and compensation for poultry farmers, and the human populations continue to live closely with poultry, LBMs continue to play a major role in the distribution and consumption of poultry, and government efforts at enforcement and compliance are somewhat weak; all these in addition to a relatively unstable political system make the control and eradication of H5N1 HPAI in poultry and humans in Egypt an arduous task (ProMed Mail, 2015).

While our results display similar trends to those of Kayali et al. (2014), the incidence rates were generally lower. Overall, many more chickens have been slaughtered and destroyed in Egypt, possibly due to the comparatively higher population of chickens (Hosny, 2007), but it seems that the other poultry species (ducks, geese and turkeys) are primary carriers of the virus – their role in contaminating other poultry is obvious (MoALR, 2010). In this study, the odds were higher that ducks and geese would be positive for H5N1 HPAI, and these species may incubate the infection without showing immediate clinical signs, becoming carriers for a relatively long time and inadvertently spreading infection among other species in LBMs. Chickens and turkeys succumb to infection with H5N1 HPAI more rapidly and display obvious clinical signs. The FAO has previously established that ducks and geese pose a very high risk of infection to household and small-scale poultry (Ali et al., 2013). In addition, it is probable that infected chickens in households were rapidly slaughtered and sold before they were presented at the market, as previous surveys have confirmed (Aly et al., 2012; Ali et al., 2013), and this may have significantly lowered the incidence in samples from chickens in the LBMs, while increasing household risks of H5N1 infection from poultry.

It is evident that both poultry and human densities are higher in Lower Egypt (Rabinowitz et al., 2012; Shakal et al., 2013), but it is not clear whether movement associated with trade towards Upper (southern) Egypt was responsible for the higher incidence of poultry infections in this location. Yupiana et al. (2010) have previously reported that the number of poultry outbreaks was negatively associated with poultry density, due to the transportation of sick birds from high to low poultry population density districts. Surveillance also tends to be more intense in Lower Egypt, near the central administration, and it is likely that farmers who are unsure of the health status of their flocks may send such birds to LBMs in governorates further away.

Because this surveillance is LBM-based, the likelihood of finding positive samples in LBMs depends on the presentation of infected poultry in the LBMs. Seventy-one per cent (71%) of households in Upper (southern) Egypt raise household poultry and mostly trade their outputs in the LBMs (Geerlings et al., 2007); household poultry has been identified as a major source of outbreaks (Kayali et al., 2014), it is highly likely that infected poultry from the households will reach the LBMs, and where *ante-mortem* inspections are not carried out, the result is not surprising. The role of trade and other associated factors does, however, require further evaluation.

In our analyses, the highest odds of isolating H5N1 HPAI and the highest prevalence were associated with winter. Sakoda et al. (2012) has previously found that winter was characterized by a higher incidence of cases of H5N1 in Japan, and similar results have been obtained in wild and domestic birds in Korea (Choi et al., 2013). Park and Glass (2007) have also indicated that the risk of avian and human influenza appears to be higher in winter in East and South-East Asia. Lower temperatures (in winter) in Egypt have also been positively associated with higher risk (Rabinowitz et al., 2012). Elsewhere, increased environmental temperature was found to be a predictor of reduced efficiency of influenza A transmission (Lowen et al., 2007) and survival (Chumpolbanchorn et al., 2006; Shahid et al., 2009; Paek et al., 2010). It should be noted that in the winter of 2014–2015, there was a steep increase in the number of human cases of H5N1 infections in Egypt (ECDC, 2015). LBMs could serve as important surveillance sites during high-risk HPAI seasons as access to both commercial poultry farms and household poultry sector is often difficult due in part to the fear of intrusive measures implemented by veterinary authorities during such operations.

In our evaluation, summer and autumn also displayed higher odds than spring. This finding is consistent with the study of Abdelwhab et al. (2010), who reported that the temporal pattern of the virus has changed in Egypt since 2009, with outbreaks now also occurring in the warmer months of the years. It is possible that the viruses circulating in Egypt have become established and have adapted to warmer environmental conditions (El-Zoghby et al., 2013).

It is probable that, because urban LBMs are responsible for the majority of the slaughtered poultry in Egypt, poultry is moved intensely and dynamically in these locations. Secondly, because these urban markets are likely to be more intensely surveyed than other locations (due to easy accessibility and convenience), it is likely that positive samples will be more regularly obtained in urban LBMs, compared to rural markets, as evidenced in this study. In addition, there are many on-site slaughtering points in urban markets, which operate on multiple poultry species, in confined spaces, compared with home slaughter, which is more prevalent in rural areas (Cardona et al., 2009; Kirunda et al., 2014). The government attempted to close urban LBMs in an effort to control the spread of H5N1 HPAI, but this measure has been counterproductive, because it led to more clandestine operations and the expansion of live poultry trade in the rural areas. Government legislation should not be top-down, but should carefully consider the sociocultural and economic realities of a country's citizenry (Ali et al., 2103).

More poultry is delivered to the markets around secular and religious feast dates, such as Ramadan or Christmas and Easter, so targeted maturation of poultry is aimed for these times of the year, from the Baladi system, to enable premium sales and satisfy annual trade patterns.

Previous reports have indicated that household/backyard poultry are major sources of the continuous outbreaks in Egypt (Kayali et al., 2014; Emergency Prevention System Global Animal Disease Information System (EMPRES i), 2014), and poultry from non-traceable sources are likely to be associated with free range and backyard systems more than with commercial sources. Our results have confirmed the previous observations; significantly, higher percentages (3.98% and 4.26%) of samples originating from household and non-traceable poultry respectively were positive for H5N1 HPAI compared with the commercial poultry. It is thus vital to reorganize the markets to maintain auditable accounts to make it possible to trace the sources of poultry delivered to the market. The lack of such a system has already been identified by Fasina et al. (2016).

Outbreaks of H5N1 HPAI in poultry have positively predicted human outbreaks in previous analyses (Rabinowitz et al., 2012). Our study has confirmed these prior findings. It is not known whether censorship and incomplete reporting of cases in humans and outbreaks in poultry affect these outcomes. However, because poultry and human population densities will continue to increase correspondingly (Fig. 2), and more outbreaks in poultry predicted more human cases (Fig. 1), it is possible that more human cases will be reported in the future if outbreaks of H5N1 HPAI in poultry are not controlled. Such a trend was already observed in poultry and humans from January to April 2015 (World Health Organization Regional Office for Eastern Mediterranean (WHO-EMRO), 2015). Shared environmental and other geotemporal factors have also been confirmed to be associated with infections in poultry (Rivas et al., 2010; Ahmed et al., 2011), and human zoonotic infections have been preceded by outbreaks in poultry (Rabinowitz et al., 2012).

Our sampling and analyses focused on the years 2009, 2012 to 2013 and 2014, but similar surveillance conducted in the period from 2010 to 2012 and that included LBMs, commercial farms, backyard flocks and abattoirs arrived at similar, or widely varied, results (Kayali et al., 2014). Although a previous evaluation of the LBM value chain indicated that slaughtered poultry at the LBMs represents a low-level risk for the spreading of H5N1 HPAI to poultry farms (Ali et al., 2013), the zoonotic risk to residents in household and consumers of poultry products through probable contaminations has been outlined by Fasina et al. (2016).

Our analysis is limited by the following factors: (i) the prevalence data and sample distribution were LBM-based, so it would be problematic to extrapolate the findings to other outlets, including commercial farms, to household poultry, and birds from peddlers, abattoirs and slaughterhouses, (ii) as the country has poor transparency for H5N1 HPAI reporting and issues with censorship (Food and Agriculture Organisation of the United Nations (FAO) (2007b, 2011), drawing a national parallel or wider implications from these results may be inaccurate, as there are no exact data on the true distribution of H5N1 HPAI in Egypt. However, we have made an effort to reduce bias and collect all possible outbreak information available to us in this analysis, (iii) the sample analyses were conducted based on pooled sampling, which may delay the time to the detection of particularly positive cases. Realistically, human resources and other resources are limited in developing economies, with many competing priorities. In this case, it was cheaper to conduct laboratory analyses on 4134 pools and do an additional 760 tests (152 positive pools * 5) than to conduct primary testing on 16 000 samples. Our sampling was positively skewed to the year 2014. Field-based sampling is often affected by factors beyond the control of researchers, as seen here. Conflict, wars, emergency situations and other such variables have a significant impact on field surveillance.

Conclusion

Egypt faces substantial challenges in achieving effective control over and eradicating the H5N1 HPAI virus. This situation poses a high risk, both that the disease will be perpetuated and that new virus strains with human influenza pandemic potential may emerge (FAO, 2007a). LBMs are the most critical points in the poultry value chain, because they link commercial, small-scale household farms, slaughter houses, producers, traders and consumers (Ibrahim et al., 2007). They also present an ideal location for surveillance to monitor the risk of avian influenza incursion and emergence, and the re-emergence and re-assortment of viruses.

Political instability and emergency situations affect both human and animal disease surveillance, with potentially dire consequences for the health of human and animal populations. H5N1 HPAI is still entrenched in the household and commercial poultry production sectors, and LBM-oriented solutions may assist in reducing continued zoonotic risks to humans and poultry.

Acknowledgements

This work was supported by the United States Agency for International Development (USAID) [grant number AID-263-IO-11-00001, Mod.#3] in the framework of OSRO/EGY/101/USA, through projects jointly implemented by the FAO, General Organization for Veterinary Services and NLQP. The authors express their sincere gratitude to USAID for its continued support in addressing the endemic HPAI situation in Egypt. FOF was supported by a Support for Smallholder Poultry Development Personal Development Grant (Associate Poultry Adviser) facilitated by International Network for Family Poultry Development (INFPD), the Food and Agriculture Organization of the United Nations (FAO) and the International Fund for Agricultural Development (IFAD) grant number GCP/INT/197/IFA.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Multivariable analyses of factors analysed for highly pathogenic avian influenza H5N1 in livebird markets, Egypt.