

Pro and Retrospective Epidemiological Situation of Avian Influenza in Egypt

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Pro and Retrospective Avian Influenza Situation In Egypt, through: Quantitative Observational Studies including: Officially available collected data: GOVS report, 2015, CAHO teams established that cover all Egyptian governorates by March 2015, Surveillance activities in Egypt include active and passive surveillance systems {where, The data collection addressed the poultry production systems according to the scope of production (grandparent, breeder, layer, broiler, nursery and household) } –Sampling and 3. Data collection. B. Phylogenic and Genetic Changes of H5 N1 and H9N2 in Egypt.

Meta-analysis Evaluation: To assess heterogeneity in meta-analysis: Q statistic OR I^2 index. Total numbers of 70130 samples (cloacal and tracheal swabs) were taken by a team of veterinarians from the different poultry sectors (poultry farms, backyard, LBM) all over the years 2010, 2011, 2012, 2013, 2014 and 2015.

The extensive circulation of Highly Pathogenic (HP) H5N1 Avian Influenza in Egypt in poultry since 2006 resulted in the emergence of distinct clades with the recent identification of a further clade: 2.2.1.1. Genetic characterization of Egyptian H9N2 viruses-Analysis of the haemagglutinin (HA) phylogenetic tree identified that the viruses are fall within the A, B and C groups.

Results of meta-analysis carried out on the pro and retrospective epidemic studies in Egypt revealed that; the studies had wide variations according to Cochran's Q statistic and Higgins and Thompson's I^2 . The proportion of total variability explained by heterogeneity showed a range of low to moderate precision.

Keywords: Pro and Retrospective; Quantitative Observational Studies; Phylogenic; Meta-analysis.

Introduction

Avian influenza viruses (AIV) are devastating diseases of poultry firstly observed in Italy in 1878 and was known as "Fowl Plague [1]. The genome of AIV consists of 8 gene segments where each segment represents an independent replication unit encoding one or more proteins. According to the surface glycol-proteins, the haemagglutinin (HA) and the neuraminidase (NA), 16 HA and 9 NA of IAV have been isolated from birds. A central dogma of influenza virus is that the wild birds are the reservoir for all IAV subtypes. The transmission of IAV from wild birds to domestic poultry occurs frequently [2] [3]. Another feature for IAV is the constant minor changes due to errors induced by the viral RNA-dependent RNA polymerase during replication inside the infected cells. The gradual changes (antigenic drift) in the

antigenic sites or in the receptor binding domain enable the virus to escape from the vaccine-induced immune response or to expand the host range, respectively [4].

Avian Influenza is one of the major diseases of importance in Egypt. Egypt has been declared endemic with HPAI since 2008 two years after the disease introduction in February 2006. The unprecedented spread of H5N1 high pathogenicity avian influenza virus (A/H5N1) from Asia to Africa in 2005 was considered as a global epidemiological twist [4].

Pandemic influenza is caused by new human influenza A virus which arises due to genetic reassortment of animal influenza viruses or direct intra-species transmission and has global public health significance. [6]. The segmented nature

of AIV allows the swapping of gene segments (reassortment or shift) between different influenza subtypes that infect the same cell. The resultant IAV reassortants differ compared to their parental viruses regarding virulence, adaptation and/or pathogenesis. Emergence of a novel reassortant virus in immune-naive human populations may result in a pandemic with severe mortality [7].

Egypt is considered a hotspot for the evolution of a pandemic potential virus either via antigenic drift of the H5N1 to increase its adaptation to humans [8] or H9N2 [9] or through reassortment with other IAV subtypes, especially H3N2 virus [10].

Conversely, the recent low pathogenic avian influenza viruses (LPAIV) H7N9 in China and Malaysia showed no clinical signs in poultry but it killed 112 out of 355 (»32%) confirmed laboratory human cases since February 2013. Exceptionally, the evolving HPAIVH5N1 since 1997 caused devastating outbreaks in poultry and wild birds in several countries and it was able to kill 393 out of 667 (»59%) infected humans [11].

The estimated **loss** of the Egyptian poultry industry after the first emergence of highly pathogenic avian influenza (HPAI) H5N1 in February 2006 was 1 billion US\$ and affected the income of 1.5 million people whose livelihoods depended on poultry. Although many countries successfully eradicated the HPAIV H5N1 from poultry, Egypt, China, Vietnam, Bangladesh, Cambodia and Indonesia were declared as H5N1-endemic countries [12] [13].

Because of the pressure for timely, informed decisions in health and clinical practice and the explosion of information in the scientific literature, research results must be synthesized. Meta-analyses are increasingly used to address this problem, and they often evaluate observational studies [14].

Principles of evidence-based methods to assess the effectiveness of health care interventions and set approach to identifying, appraising, synthesizing, and (if appropriate) combining the results of relevant studies to arrive at conclusions about a body of research, has been applied with increasing frequency to randomized controlled trials (RCTs), which are considered to provide the strongest evidence regarding an intervention [15] [16] [17].

Meta-analysis is a quantitative, formal, epidemiological study design used to systematically assess previous research studies to derive conclusions about that body of research. Outcomes from a meta-analysis may include a more precise estimate of the effect of treatment or risk factor for disease, or other outcomes, than any individual study contributing to the pooled analysis. The examination of variability or heterogeneity in study results is also a critical outcome. The benefits of meta-analysis include a consolidated and quantitative review of a large, and often complex, sometimes apparently conflicting, body of literature. The specification of the outcome and hypotheses that are tested is critical to the conduct of meta-analyses, as is a sensitive literature search [18].

The aim of the present study is to understanding the epidemiological situation of Avian Influenza in Egypt including and to evaluate the retrospective observational studies, through the principles of evidence-based methods.

East, then Europe in the summer of 2005, and later to Africa [9].

Egypt experienced the disease since the first introduction of highly pathogenic Avian Influenza HPAI H5N1 in 2006. The virus widely extended in very short time and infected commercial production sectors and backyards [10] [11].

Intensive poultry production systems in which a continuous and easily accessible source of susceptible hosts are incomplete vaccination coverage that has allowed field strains to reassort with vaccinal strains [12].

In this study we have presented some ecological aspects of the isolated highly pathogenic avian influenza virus in Egypt and the effect of some physical and chemical agents on its activity.

Materials and Methods

Materials

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1. Officially available collected data:

- **Positive infected human cases with H5N1HPAI** (Year, Month, Governorate, District, Status, Sex, Age, Contact with sick or dead birds).

- **Infected farm details 2010- till January 2015:** Year, Month, Governorate, District, Species, Breed, and Age (**H5N1 Poultry outbreaks February 2006- 2015 ,H9N2 Poultry outbreaks 2014-2015 and Published Data**)

2. Sampling. Total number of 70130 samples (cloacal and tracheal swabs) was taken by a team of veterinarians of **GOVS** from the different poultry sectors (poultry farms, backyard, LBM) all over the years 2010, 2011, 2012, 2013, 2014. The swab is used to collect a tracheal or cloacal sample from poultry according to [19]. Number of swab samples depends on the size of the population; as many as 5 birds were sampled per flock. Birds were randomly selected except in case of presence of sick or dead birds they were collected. Samples were chilled in ice box until delivered to the laboratory (within 24 hours) and were stored at -80°C until used [20].

Softwares used in data analysis:

Winepiscope for calculation of the sample size

(<http://www.clive.ed.ac.uk/cliveCatalogueItem.asp?id=B6BC9009-C10F-4393-A22D-48F436516AC4>).

Epi info 7 (CDC,2015)

SPSS 21

MiniTAB (2011)

GIS geographical information system

Materials required for RRT-PCR

RNA extraction

RNA extraction done according to QIAamp Viral RNA Mini Kit that supplied from (Qiamp viral RNA mini Kit. GmbH, Hilden, Germany) Commercial licensed kit Catalogno. 52904.

MicroAmp® Optical 8-Tube Strip, 0.2 ml, Catalog number 4316567.

Reagents and volume of RRT-PCR reaction mix for M and H5 genes of H5N1 subtype according to [21].

Meta-analysis Evaluation

Assessing heterogeneity in meta-analysis: Q statistic OR I^2 index

The I² statistic describes the percentage of variation across studies that are due to heterogeneity rather than chance. I² = 100% x (Q-df)/Q. I² is an intuitive and simple expression of the inconsistency of studies' results. If there is very little variation between trials then I² will be low and a fixed effects model might be appropriate. With fixed effects all of the studies that you are trying to examine as a whole are considered to have been conducted under similar conditions with similar subjects.

Cochran's Q statistic:

$$Q = \sum w_s(\hat{\beta}_s - \bar{\beta})^2$$

where $w_s = 1/se(\hat{\beta}_s)$, and $\hat{\beta}$ is a weighted mean of the $\hat{\beta}_s$.

Used to test whether all studies are evaluating the same effect, but has low power

Higgins and Thompson's I²:

$$I^2 = (Q - df) / Q \times 100$$

A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity

-low heterogeneity: I² <25%

-moderate: 25%-75%

-high: >75%

Soft ware Meta-test

Statistical analysis

The collected data from 25 governorates for different types of surveillance systems applied in Egypt all over the different poultry sectors were subjected to descriptive and statistical analysis using Chi-square and Z-test to analyze difference and correlation of H5 and H9 infections in poultry sectors detected by different surveillance systems using SPSS and mini-tab software.

Primers used in RRT-PCR of M and H5 genes

Gene	Name	Type	Sequence (5' - 3')	Reference
M	M24	Forward	AGA TGA GTC TTC TAA CCG AGG TCG	(Spackman et al .2002) [22]
	M25	Reverse	TGC AAA AAC ATC TTC AAG TCT CTG	
	SEPRO	Probe	FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA	
H5	H5 LH1	Forward	ACG TAT GAC TAC CCG CAG TAT TCA	(Slomka et al., 2007) [23]
	H5 RH1	Reverse	AGA CCA GCC ACC ATG ATT GC	
	H5PRO	Probe	FAM- TCWACAGTGGCGTTCCTAGCA – TAMRA	

Results and Discussion

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Quantitative Observational Studies

Prevalence of AIV in poultry sectors (Descriptive analysis).

Total tested samples for AI in the period 2010-2014 for testing avian Influenza H5 and H9 were 75380

Total AI outbreaks in the period 2006-2015 were 1327

Between 18 January and 7 February 2015 a total of 76 H5N1 HPAI outbreaks were detected in 20 out of Egypt's 27 governorates namely Asyut (2), Behera (12), Beni-suef (3), Cairo (3), Dakahlia (2), Damietta (3), Fayoum (2), Gharbia (4), Giza (8), Ismailia (9), Kafr-el-sheikh (2), Kalyoubia (7), Luxor (1), Menia (3), Menoufia (2), North Sinai (2), Qina (4), Sharkia (5), South Sinai (1) and Al Wadi/Al Jadid (1) Governorates. A total of 66 outbreaks occurred in household poultry, 9 in commercial farms and 1 detected in a live bird market (LBM); 5 outbreaks occurred in vaccinated farms, the rest were in unvaccinated birds. Active surveillance detected 40 outbreaks including the cases in LBM, 9 outbreaks were reported through passive surveillance, and 27 outbreaks in unvaccinated households poultry (chickens, ducks and

turkeys) were reported by Community-Based Animal Health Outreach (CAHO).

Total H5 positive cases (Temporal distribution) in relation to Commercial Production Sectors using RT-qPCR 2006 – 2014

As shown in Table 1 , the total positive cases according to the Commercial Production Sectors were varied during 2010 to 2014. But, at 2010 and 2011 were the highest years having serious HPAI outbreaks. At the same time , the HPAI positive cases were flourished in the year 2010 (60,394) in farm and household sectors respectively. At 2011 were (54, 309 and 4) in farm, household and LBM sectors, respectively. On the other hand, the prevalence of H5 positive cases in farm, household, LBM, Nursery and Village sectors were 1.40%, 15.60%, 81.18%, 1.60%, 0.07% and 0.15%, respectively.

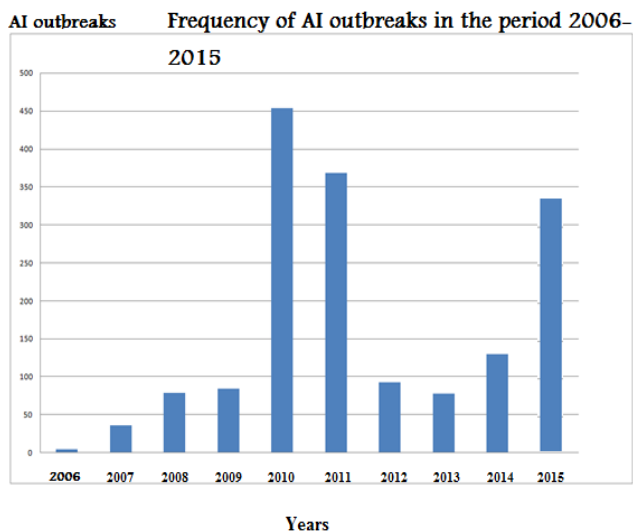


Fig. (1): Frequency of AI outbreaks in the period 2006-2015

Total AI outbreaks recorded in figure (1) during the period 2006-2013 revealed that ; frequencies of AI outbreaks in this period were: 5 (2006) ; 36 (2007) ; 79 (2008) , 84; (2009) ; 456 (2010); 368 (2011); 93 (2012); 78 (2013), and 130 (2014) respectively. Between 18 January and 7 February

2015 a total of 76 H5N1 HPAI outbreaks were detected in 20 out of Egypt’s 27 governorates namely Asyut (2), Behera (12), Beni-suef (3), Cairo (3), Dakahlia (2), Damietta (3), Fayoum (2), Gharbia (4), Giza (8), Ismailia (9), Kafr-el-sheikh (2), Kalyoubia (7), Luxor (1), Menia (3), Menoufia (2), North Sinai (2), Qina (4), Sharkia (5), South Sinai (1) and Al Wadi/Al Jadid (1) Governorates. A total of 66 outbreaks occurred in household poultry, 9 in commercial farms and 1 detected in a live bird market (LBM); 5 outbreaks occurred in vaccinated farms, the rest were in unvaccinated birds. Active surveillance detected 40 outbreaks including the cases in LBM, 9 outbreaks were reported through passive surveillance, and 27 outbreaks in unvaccinated households’ poultry (chickens, ducks and turkeys) were reported by Community-Based Animal Health Outreach (CAHO). Previous surveillance systems in Egypt have highlighted continuous and wide circulation of the virus in vaccinated and non-vaccinated commercial farms, backyard birds and LBMs [24]. About 71 percent of households in rural Upper Egypt raise poultry; the average flock size in 2010 was 23.7 birds in contrast to 73 birds in Lower Egypt [5] [25].

Table 2 : Total AI H5 outbreaks in relation to bird species 2006-2014.

Year	Chicken	Ducks	Turkey	Mixed	Grand Total
2006	1			4	5
2007	12	5	1	18	36
2008	36	3		40	79
2009	31	10	5	43	89
2010	160	53	4	236	453
2011	178	56	1	129	364
2012	35	28	15	30	108
2013	24	14	26	39	103
2014	22	41		51	114
Grand Total	499	210	52	590	1351
Prevalence	36.90%	15.50%	3.90%	43.70%	100%

Illegal trading of unexamined commercial poultry and backyard birds into LBMs is not uncommon; therefore this might explain the higher incidence of the virus in LBMs (11.4%, n = 108/ 944) [25]. These results are in accordance with our surveillance conducted in cooperation with [26]. In contrast to their results, [24][27], reported 6.8% (n = 192/2827) positivity rate in commercial farms, 3.3% (n = 34/1024) in LBMs and only 0.9% (n = 12/1381) in the backyard flocks.

Total AI H5 outbreaks (Temporal distribution) in relation to bird species 2006-2014.

The highest incidence of HPAI H5N1 outbreaks during the study period were recorded in chicken (36.90%), ducks (15.50%), mixed (43.70%) and only (3.90%) in turkey as shown in Table 4. The species difference for H5, in terms of species and production type, turkey and breeder farms had the lowest risk, followed by layer, broiler, nursery and finally duck farms with the highest risk. In short, about 95 percent

of farms are bio-insecure and vulnerable to exposure and release of infection. Chicken recorded the highest number of positive cases of H9 (n=167, 94% of total positive H9) it is linked to that most of H9 cases were from chicken farm tested before slaughter, while mixed species recorded the highest number of positive H5 (n=46, 46% of total positive H5) this is linked to that most of H5 cases were reported from household. 100% of mixed species in household included ducks.

Table 3: Frequency of +ve cases of H5 and H9 2013.

Sample source (sector)	H5	H9	Total cases
Farm	12	165	177
Household	74	4	78
BM, Poultry Shops	14	9	23
Grand Total	100	178	278

Farm sector recorded the highest number of positive cases of H9 (165, 59% of the total positive AI cases) while household was the highest in H5 (74, 27% of the total positive AI cases). LBM markets positive cases recorded is considerable (17, 6 % of the total positive AI cases) especially that it was recorded in time of no cases have been reported from other poultry sectors (Table 5) Viral circulation in vaccinated and non-vaccinated birds was previously reported; particularly during the winter seasons of 2006 – 2008 [26] [28]. In LBMs, birds of different species with various ages from several locations and different sources (backyards/barnyards and commercial flocks) are usually mixed. Therefore, LBMs are an indicator for A/ H5N1 infections in poultry. Previous surveillance in Egypt has highlighted continuous and wide circulation of the virus in vaccinated and non-vaccinated commercial farms, backyard birds and LBMs.

Results of HI assay of the 2012–2013 viruses conducted against a panel of monoclonal antibodies were used to update a previously published antigenic cartography. Our results indicate that antigenically, subtype H5N1 viruses from Egypt have drifted over time; in 2010, two clusters of viruses (clades 2.2.1 and 2.2.1.1) co-circulated. In 2011–2013, clade 2.2.1 viruses dominated. Recently the WHO has identified 12 new H5N1 clades and the Egyptian subclade 2.2.1 was further split into a new subclade 2.2.1.1, corresponding to genetic subclade B, indicating further divergence of contemporary strains of H5N1 circulating in Egyptian poultry [29].

Meta-analysis Evaluation

Meta-analysis situation and Evaluation of the epidemic studies and their measurements of AIV in Egypt: Meta-analysis carried out on the pro and retrospective epidemic studies in Egypt revealed that; the studies had wide variations according to Cochran's Q statistic and Higgins and Thompson's I^2 . The proportion of total variability explained

by heterogeneity showed a range of low to moderate precision. In spite of the results of meta-analysis carried out on the pro and retrospective epidemic studies concerned with the phylogenic and genetic changes of AIV were précised.

Conclusion

Genetic characterization of Egyptian H5N1 viruses-Analysis of the haemagglutinin (HA) phylogenetic tree identified that the viruses are fall within the clade 2.2.1.1. Genetic characterization of Egyptian H9N2 viruses-Analysis of the haemagglutinin (HA) phylogenetic tree identified that the viruses are fall within the A, B and C groups.

Increased incidence of H5N1 since June 2014. Between 1 December 2014 and 28 February 2015, 333 outbreaks in poultry were observed in Egypt, while between 1 December 2013 and 28 February 2014 there were only 44 reported outbreaks. The most likely reason for the increase in cases is that more poultry in Egypt are infected by H5N1 and so more people are exposed to this virus.

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