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Cairo University

Faculty of Veterinary Medicine
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"Surveillance systems for Avian Influenza viruses in Egypt"

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Abstract

Pro and Retrospective Avian Influenza Situation In Egypt, through: A. Quantitative Observational Studies including: 1 .Officially available collected data all over the years from 2010 till 2014: Surveillance activities in all Egyptian governorates 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) 2. Data collection sheet 3. Sampling. B. Phylogenic and Genetic Changes of H5N1 and H9N2 in Egypt. Meta-analysis Evaluation: To assess heterogeneity in meta-analysis using Coshran's *Q* statistic and Higgins and Thompson's I^2 index.

Total numbers of 70130 samples (cloacal and tracheal swabs) were taken by a team of veterinarians working in the epidemiology unit of GOVS from different poultry sectors (poultry farms, backyard, LBM) all over the years from 2010 till March 2015. The results were revealed that, there has been a real and measurable increase in the 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.

The extensive circulation of HPAI H5N1 in poultry in Egypt since 2006 resulted in the emergence of distinct clades with the recent identification of a further clade: 2.2.1.1. in addition to 2.2.1.. Genetic characterization of Egyptian H9N2 viruses and 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. 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 precised.

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Finally I would like to thank my family and my friends.

Dedication

To my country Egypt…   For better future…

To my Parents and my lovely Sister Rana,

To my soul mate,

And my future son or daughter……..

I hope they all will be happy and I didn't disappoint any of them…

Yara M. M. Al Azab

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LIST of ABBREVIATIONS

AI	Avian influenza
AIV	Avian Influenza viruses
CAHO	Community Animal Health Outreach
CAPMAS	Central Agency for Public Mobilization and Statistics
C&D	Cleaning and disinfection
CFR	Case fatality rate
CLEVB	Central Laboratory for Evaluation of Veterinary Biologics
CLQP	Central laboratory for Veterinary Quality Control on Poultry Production
CRP	Critical risk point
DOB	Day-old bird
DOC	Day Old Chick
ECTAD	Emergency Centre for Transboundary Animal Diseases
EGP	Egyptian pound (EGP 1 = USD 0.167 at the time of study)
EID50	Median Egg Infective Dose
FAO	Food and Agriculture Organization of the United Nations
GMP	Good Manufacturing Practice
GoE	Government of Egypt
GOVS	General Organization for Veterinary Services
HA	Haemagglutinin

HH	Household
HPAI	High pathogenic avian influenza
IDSC	Information and Decision Support Centre
L.E.	Egyptian Pound
LBM	Live bird market
LPAI	Low pathogenic avian influenza
MoALR	Ministry of Agriculture and Land Reclamation
NA	Neuraminidase
NLQP	National Laboratory for Veterinary Quality Control on Poultry Production
ND	Newcastle Disease
RCTs	Randomized controlled trials
RFT	Rapid field test
RPWD	Risk Pathway Diagram
RTPCR	Real-time polymerase chain reaction
SAIDR	Strengthening Avian Influenza Detection and Response Project
SH	Slaughterhouse
SOPs	Standard operating procedures
SPF	Specific Pathogen Free
TCID	Tissue culture infectious dose
USAID	United States Agency for International Development

Introduction

Avian influenza is an infectious disease of poultry caused by influenza "A" viruses of the orthomyxoviridae family. Influenza "A" virus infection in poultry occurs in two forms. The highly pathogenic AI (HPAI; previously known "fowl plague" the most virulent and is associated with viruses of H5 and H7 subtype causes a severe systemic disease with mortality up to 100% , and the low pathogenic AI (LPAI) usually causes minimal clinical signs other than a slight drop in egg production **(Alexander, 2000).**

Avian influenza viruses (AIV) are devastating diseases of poultry firstly observed in Italy in 1878 and was known as “Fowl Plague **(Swayne and Halvorson, 2003**). 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 **(Alexander, 2007; Krauss, 2007).** 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 **(Bouvier, 2009).**

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 **(Fasina et al., 2007).**

Highly pathogenic avian influenza (HPAI) H5N1 represents both an epizootic of enormous scope and a significant pandemic threat to human health. While at present avian influenza remains predominantly an animal disease, with sporadic zoonotic transmission to humans and apparently limited human-to-human transmission, there is ongoing risk for the emergence of strains with increased transmissibility. It is therefore a public health priority to reduce human risk for avian influenza infection by controlling the infection at its source (animal populations), and also taking steps to reduce the possibility of animal-human (zoonotic) transmission. Avian influenza accordingly provides an opportunity to test the utility of integrating human and animal disease surveillance data streams **(Peter et al., 2012).**

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 **(Webster, 1998).** 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 **(Taubenberger et al., 2012).**

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 **(Neumann, 2012)** or H9N2 **(Arafa, 2012a)** or through reassortment with other IAV subtypes, especially H3N2 virus **(Fuller et al., 2013)**

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 of667 (»59%) infected humans **(WHO, 2014)**.

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 **(Meleigy, 2007 ; FAO, 2008).**

In particular, influenza surveillance requires close collaboration both at the various levels of the health system within a country and between countries in order to take immediate global efforts to prevent pandemic. Effective disease control and prevention programs depend primarily on sensitive and representative surveillance systems to detect infectious disease problems early enough to take an immediate action. Surveillance is the most effective public health prevention and control strategy by providing early detection of an epidemic, defining high risk populations in a given year, investigating the extent and distribution of severe cases, identifying the current circulating subtypes of the virus, monitoring for antigenic shift, tracing the origin of the newly emergent/re-emergent virus, and developing vaccines for immunization programs **(Hampson, 1996; Pugliese et al., 1998; Reid et al., 2000).**

AI vaccination in Egypt has been the main tool used over the past three years to control the H5N1 HPAI epidemic. The focus on vaccination appears to have distracted attention away from other essential/critical procedures and disease control measures such as surveillance, biosecurity outbreak investigation, and disease management interventions. Indeed, more than 80% (24 million USD) of the available budget for HPAI control has been devoted to vaccination since the programme was launched **(GOVS, 2009).**

Some countries have succeeded in reducing HPAI incidence and thereby reducing human risk of infection (*e.g.*, Vietnam and China). Vietnam has managed to bring the disease under control within two years by strictly adhering to vaccination guidelines and implementing efficient outbreak management. In contrast, countries which have implemented mass vaccination without extensive outbreak management and biosecurity measures are still fighting to control the infection (*e.g.*, Indonesia and Egypt) **(Domenech et al., 2009).**

The failure of surveillance systems in developing countries is often due to limited available resources, lack of knowledge able staff, disorganization, and poor infrastructure for finding and reporting cases (**Nsubuga, 2006).**

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 **(JAMA, 2012).** 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 (**Thacker, 1988; Petitti, 1994; Bero and Jadad, 1997**).

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 **(Haidich, 2010).**

The aims of the present study are:

1. **To better understanding the epidemiological situation of Avian Influenza in Egypt** including the commercial poultry farms, backyards and live bird markets through collecting and analysis of the officially available data by General Organization of Veterinary Services (GOVS) in cooperation with the Central laboratory for Veterinary Quality Control on Poultry Production (CLQP) and under financial and technical support from USAID and FAO from 2006 till 2015.