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Studies on the Role of Free Living Birds in the Epidemiology of Prevalent Diseases in Poultry Farms

A Thesis Presented By

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Abstract

A total of 15 broiler chicken flocks belonging to Desouk and Qallin (Kafr el-Sheikh Gov.) as well as to Bassioun (Gharbia Gov.) exhibited rapid increase in mortality rate and respiratory manifestation were thoroughly investigated during the period from December 2013 to January 2015. Rapid field detection of AIV by immunochromatography tests revealed detection of AIV antigen in one (6.67 %) broiler chicken flock. Twenty one tissue pool samples collected from the broiler chicken flocks as well as 244 swabs and one brain tissue pool from 127 free living birds- in the vicinity and inside the affected broiler chicken farms- were subjected for isolation and molecular characterization of NDV and AIV subtypes (H5 and H9). In addition, 75 and 60 samples (pooled; liver, heart and spleen) from broiler chickens and free living birds respectively subjected to isolation and characterization of *E. coli* by using selective media, serotyping, *in vitro* and *in vivo* virulence assay. Nineteen (90.48 %) and 2 (9.52 %) out of 21 samples collected from broiler chicken flocks showed positive isolation for NDV and AIV H5 respectively, whereas 25 (10.2 %) and one (0.41%) out of 245 samples from free living birds showed positive isolation for NDV and AIV H5 respectively and no positive results could be obtained for AIV H9N2 from all samples. Molecular characterization by sequencing of F gen fragment and phylogenetic analysis of the selected 13 NDV isolates, revealed two isolates from broiler chicken flocks and 9 isolates from free living birds had possessed the same velogenic genotype motif of ¹¹²R-R-Q-K-R-F¹¹⁷, in addition to clustering with published class II genotype VII sub genotype d NDVs and closely related to Israel and China isolates, whereas one isolate from broiler chicken flocks had possessed the lentogenic genotype motif of ¹¹²G-R-Q-G-R-L¹¹⁷, in addition to clustering with published class II genotype II NDVs. Molecular characterization of pigeon NDV isolate showed velogenic genotype motif ¹¹²K-R-Q-K-R-F¹¹⁷ and clustered with published class II genotype VI. Molecular characterization of two selected AIV H5 isolates (cattle egrets and broiler chickens) by sequencing of HA gen fragment and phylogenetic analysis revealed clustering the isolates in the same subclade 2.2.1/ C. Isolation and identification of *E. coli* from broiler chicken samples revealed 48/75 (64 %) isolates, whereas free living bird samples revealed 10/60 (16.67%) isolates. *In vitro* pathogenicity test using Congo red (CR) binding assay showed that out of 48 isolates from broiler chickens 22 (45.8 %) showed CR +ve, whereas out of 10 isolate from free living birds 5 (50 %) showed CR positive. Serotyping was carried out on 5 selected CR positive isolates of broiler chickens and all 10 isolates of free living birds. Three *E. coli* serogroups belonged to O44, O55 and O157 from broiler chickens and 6 serogroups belonged to O128, (2) O55, O136, O127 and O164 from free living birds were inoculated in day-old SPF chicks that revealed 100 % mortality with the strain O136 (house sparrow origin), 80 % mortality with the strain O44 (broiler chicken origin), 40 % mortality with strain O55 (dove origin), 20 % with both strains; O157 (chicken origin) and O128 (house sparrow origin), whereas other strains; O55 (chicken origin), O55 (house sparrow origin), O127 (dove origin) and O164 (cattle egret origin) did not caused any mortality in chicks. The study suggested that free living birds can play an important role in prevalence of velogenic NDV genotype VIIId which is responsible for recent outbreaks in Egypt and isolation of genotype VI from pigeons may be proposed to be responsible for next Newcastle disease outbreaks, whereas isolation of AIV H5 from cattle egret focuses on the role of this bird as reservoir for HPAIV H5. In addition, these wild birds can play important role in introduction of pathogenic strains of *E. coli* to broiler chicken farms.

Key words: Newcastle disease- Avian influenza - avian pathogenic *E. coli*- broiler chickens- Free living birds.

Dedication

To my late father God have mercy on him,

To my mother,

My brother and sister,

My wife,

My kids,

My professors,

My sincere friends,

To all who helped

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Praise to Allah, who has guided us to this; and we would never have been guided if Allah had not guided us.”

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1. INTRODUCTION

Egypt is home to an impressive number of bird species which reached until now to more than 481 species of birds that vary from residents to migrants (**Lepage, 2015**). Wild birds are usually regarded as visible indicators of diverse and healthy environments, however from a public health perspective, this positive view is not always valid (**Jones, 2005**) as they can carry a wide range of viral, bacterial, fungal and protozoan pathogens harmful to poultry or other vertebrates including human, these wild birds either being themselves diseased or being seemingly healthy carriers, or the hosts of infected vectors (**Hubálek, 2004**).

Egypt lies at the crossroads of two major spatially-overlapping migration flyways; the Black Sea-Mediterranean flyway and the East African-West Asian flyway linking Africa, Europe and Asia (**FAO, 2005**). There are 34 defined migratory wetlands and stopovers where 7 and 15 regions are located along the coastlines of the Mediterranean and Red seas, respectively (**Abdelwhab and Hafez, 2011**). The Nile Delta is an important wintering stopover for millions of birds between Eurasia and Africa. Therefore, attention has been paid to the possible role of 325 wild birds in the introduction of different pathogens including NDV and AIV (**Ahmed *et al.*, 1980; El-Zoghby *et al.*, 2013 and Sheta *et al.*, 2014**).

It appears possible for free-flying native or feral wild birds to be responsible for direct or indirect spread of infectious agents from contaminated poultry product wastes to susceptible commercial poultry. Direct spread involves infected wild birds gaining access to commercial poultry farms. Indirect spread involves secondary spread to other free-flying birds that may subsequently gain access to commercial poultry farms (**Gilchrist, 2005**).

Respiratory tract infections are the most serious diseases affecting poultry and causes heavy economic losses in the poultry industry worldwide (**Murthy *et al.*, 2008**). Viral or bacterial pathogens are of major importance because they can cause disease independently, or in association with each other (**Roussan *et al.*, 2008**).

Among viral diseases: Avian influenza (AI) and Newcastle disease (ND) which are two of the most devastating viral diseases of domestic and wild birds throughout the world and caused by type A Orthomyxoviruses and type 1 avian Paramyxoviruses, respectively (**Alexander, 1995 and Manvell *et al.*, 2000**). Both diseases are defined as a list A diseases by the office international des Epizootic (**OIE, 2012 and 2014**). Infection of domestic poultry by avian influenza (AI) and Newcastle disease (ND) viruses typically produces syndromes ranging from asymptomatic infection to respiratory disease and drops in egg production to severe systemic disease with near 100% mortality depends on the virus strain as well as the species, age, immune status of the infected poultry, the presence of other microorganisms and adverse environmental conditions. In addition to economic- impact that can occur as an outcome of the restrictions and embargoes placed on the countries of these infections (**Alexander, 1997; Easterday *et al.*, 1997; Swayne and Suarez, 2000**).

Newcastle disease virus (NDV) infections have been established and detected in at least 241 species of birds, representing 27 of the 50 orders of the class *Aves* (**Kaleta and Baldauf, 1988**). It would seem probable that all birds are likely to be susceptible to infection with this virus (**Alexander, 2009**). All NDV isolates are part of a single serotype; however, they display marked genetic diversity (**Miller *et al.*, 2009 and 2010, b**).

Based on genome length and phylogenetic relationships, NDV isolates are classified into two major groups, class I and class II (**Miller *et al.*, 2010, b and Afonso and Miller, 2013**). Class I viruses have worldwide distribution, are isolated mainly from waterfowl and shorebirds, and include nonvirulent strains, with the exception of a single isolate from the Republic of Ireland in 1990 (**Alexander *et al.*, 1992 and Miller *et al.*, 2010, b**). Class II viruses are both virulent and nonvirulent and are recovered from poultry, pet birds, and waterfowl (**Miller *et al.*, 2010, b and Afonso and Miller, 2013**). Based on the mean nucleotide distance, class I viruses are recently shown to be included into a single genotype (genotype I) (**Diel *et al.*, 2012**), whereas class II viruses have been divided into 18 genotypes (I to XVIII) (**Diel *et al.*, 2012 and Snoeck *et al.*, 2013, b**). Genotype VII subgenotype (d) is the most isolated genotype of NDV from recent chicken outbreaks in Egypt with close relationship and nucleotide similarity with Chinese NDV strains (**Radwan *et al.*, 2013 and Hussein *et al.*, 2014**).

Prevalence of NDV in wild birds in Egypt has been investigated by many previous studies: **Ahmed *et al.* (1980); Abozied (1981); Sabban *et al.* (1982) and Mousa *et al.* (1988)**. Recently wild birds were hypothesized to be responsible for the introduction of new Chinese NDV genotype VIIId strain to Egypt and extensive surveillance of wild birds for NDV is a fundamental requirement for understanding the epidemiology of the virus (**Hussein *et al.*, 2014**).

Naturally occurring infections of avian influenza virus (AIV) are reported from free-living birds representing more than 90 species in 13 avian orders in particular, members of the order Anseriformes (ducks, swans, geese) and Charadriiformes (shore birds, gulls, terns) that represent a significant reservoir of influenza A viruses in nature and are often the focus of research and surveillance (**Stallknecht and Shane, 1988; Webster *et al.*, 1992; Olsen *et al.*, 2006 and Munster *et al.*, 2007**).

Avian Influenza is the only species in the genus influenza virus A, which have 18 different haemagglutinin (H1 to H18) subtypes and 11 different neuraminidase (N1 to N11) subtypes (**Tong *et al.*, 2012 and 2013**). Over 118 combinations of AIVs can be isolated from wild except H17N10 and H18N11 which recently isolated only from New World bats (**Tong *et al.*, 2012 and 2013 and Swayne *et al.*, 2013**).

During recent years, the outbreak of the H5N1virus in Asia that has subsequently spread to Russia, Middle East, Europe and Africa, has put increased focus on the role of wild birds as reservoir of HPAIV (**Normile, 2006; Olsen *et al.*, 2006; Capua and Alexander, 2007**). Prevalence of AIV in wild birds has been investigated in Egypt by many studies: **Saad *et al.* (2007); Soliman *et al.* (2012) and El-Zoghby *et al.* (2013)**. Their studies focused on migratory wild birds other than resident wild birds.

Among bacterial diseases: Avian colibacillosis is an infectious disease of birds caused by *Escherichia coli*, which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen (**Barnes *et al.*, 2008**).