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Some immunogenic and antigenic studies on avian influenza virus infection in Egypt

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بعض الدراسات المناعية والأنتيجينية على عدوى فيروس انفلونزا الطيور فى مصر

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Abstract

Seventy three isolates of AIV were collected from 11 Egyptian governorates during the period from 2010 to 2014, from different poultry flocks, The clinical signs were cyanosis of comb and wattle, echymosis on the shanks and feet, facial edema and greenish diarrhea and drop in egg production .On necropsy, diffuse subcutaneous hemorrhages on the feet and shanks, the lung showed congestion and hemorrhages. Petechiae were noticed throughout on serosal surfaces especially on the peritoneum. The ovaries were hemorrhagic contains yolk from ruptured ova. RT-PCR revealed 43 positive for M gene while 26 positive using H gene primer whereas cleaveage site sequence revealed 17 out 73 positive with percentage 58.9%, 35.6% and 23.2%; respectively.

AI strain (A/chicken/Egypt/M7217B/2013(H5N1) represent the most common clusters of AIV was reassorted at National research center and then adapted on MDCK cell line for preparation of Tissue culture (TC) vaccine in parallel with egg adapted one.

For assessment of protective: Eighty one 7 day old SPF chicks, were divided into four groups (1-4), 20 each in isolators at CLVEB, were challenged at 28 day old with10^6 / ml pathogenic AIV (A /ch / Egy / 1709-6 /2008 Eu 717857) via intranasal route. All birds of group 3 (Unvaccinated challenged) died at 3rd day post- challenge, whereas unvaccinated unchallenged birds (group 4) survived without showing clinical signs. The results revealed that Protection % of vaccinated groups either with prepared tissue culture AI vaccine or egg adapted AI one were 50%.

Key words: avian influenza, vaccine, embryonated chicken egg, tissue cultur

Dedecated to:

My great father and mother

My brother

My sisters

My wife

and My son(samir)(jailan)

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1- Introduction

Avian Influenza (AI) is an important poultry pathogen and a massive menace to the poultry industry. Since the late 1990s, highly pathogenic avian influenza (HPAI) viruses of the H5N1 subtype have devastated the poultry industry of numerous countries. After 2004, H5N1 has spread from Asia to Europe, Africa, and the Middle East, resulting in the killing or culling of hundreds of millions of domestic birds ***(Li et al., 2004)***. Furthermore, highly pathogenic AIVs (HPAIVs) H5N1 infections in poultry constitute a threat to humans and resulted, till the 2nd of October 2014, in 676 human cases with 398 deaths being reported worldwide and in Egypt alone, a total of 185 cases with 68 deaths have been reported ***(WHO, 2014).*** Occasionally, HPAI H5N1 viruses cross the species barrier and infect humans, sometimes with a severe clinical outcome. This direct transmission of HPAI H5N1 virus to humans was first detected in 1997 ***(De Jong et al.1997).***

According to the nucleoprotein (NP) and matrix (M) proteins, influenza viruses are classified into 3 types; A, B and C. AIVs belong to type A which is further diversified based on the surface glycoproteins; hemagglutinin (HA) and neuraminidase (NA), into 18 HA and 11 NA subtypes ***(Tong et al., 2013).***

Based on their virulence, two main AI pathotypes do exist; HPAIVs and low pathogenic ones (LPAIVs). Infection of poultry with HPAIVs usually results in multiorgan systemic disease. Such infections, especially whenever H5 subtype is incriminated, constitute a serious impact on both poultry production, including high rates of morbidity and mortality in susceptible flocks and human health ***(Koopmans et al., 2004; Fauci, 2006).***

In Egypt, in February of 2006, severe outbreaks of HPAI, H5N1; have emerged in several governorates and were associated with drastic mortality up to 100% in infected chickens ***(Aly et al., 2006)***.

Presumptive diagnosis of HPAI in poultry may include the clinical signs and postmortem lesions but none of these are pathognomonic, and the etiology must be

confirmed by diagnostic tests ***(Swayne and Suarez, 2000).*** These tests include virus isolation, antigen detection, nucleic acid detection and sero-diagnosis. Reverse transcriptase polymerase chain reaction (RT-PCR) for viral nucleic acid detection is considered a valuable and rapid tool for accurate diagnosis followed by sequencing of the full length heamagglutinating gene ***(Spackman et al., 2002; Dybkaer et al., 2004; Chua et al., 2007).***

Control of AI based on biosecurity level, surveillance and vaccination strategy which consider a supportive tool in AIV control strategies was implemented to limit the spread of H5N1 infection and to reduce the losses (***Lee and Suarez, 2005; EFSA, 2008).*** Control policy of H5N1AI in poultry in Egypt was initially based on stamping out and surveillance and, in late 2006; vaccination was implemented as an additional control measure using inactivated whole virion H5N1 or H5N2 vaccines. Mass vaccination using commercial vaccines led to decrease in the incidence of new outbreaks by the end of 2006 and early 2007***(Peyre et al., 2009).*** The government at that time was providing vaccination of household birds free of charge. However, since July 2009, vaccination in household settings has been provisionally suspended until a new vaccination strategy is adopted ***(FAO, 2008).***

There are different types of AI vaccines widely used in Egypt. Reverse genetically H5N1 Chinese strain A/goose/ Guangdong /1/1996 (H5N1) and H5N2 low pathogenic killed Mexican strain vaccine A/chicken/Mexico/232/94 (H5N2) are vaccines 'types widely used in Egypt. These vaccines must decrease shedding of the virus, morbidity, mortality, and transmissibility; reduce field virus replication; and decrease egg production ***(Van den Berg et al., 2008; Swayne, 2009)*** as well as minimize its socioeconomic impacts**.** The poultry industry has received large amount of mass vaccination policy to reduce circulation of the H5N1 virus, but with limited success ***(Hafez, 2010).***

Despite the nationwide vaccination strategy of poultry in Egypt to combat H5N1 AI, continuous circulation of the virus in vaccinated and non-vaccinated commercial and backyard poultry was reported ***(Hafez et al., 2010).***

One of the main causes of vaccination failure as a control measure is the variation in AIV antigenicity which develops gradually by point mutation (antigenic drift) occurring in the major antigenic sites at the globular head of HA ***(Cattoli et al., 2011)*** or drastically by genetic reassortment (antigenic shift) ***(Bouvier and Palese, 2008).*** Antigenic analysis of earlier H5N1 strains in Egypt demonstrated considerable variations ***(Balish et al., 2010)*** with circulation of stable lineages of H5N1 viruses since late 2007. Presence of the virus under immune pressure in vaccinated birds accelerated the mutation rate of the virus to escape from the repertoire of immune response ***(Hafez et al., 2010).***

Increase in incidence of H5N1 outbreaks in vaccinated poultry in Egypt highlighted the significance of continuous monitoring of circulating viruses both genetically and antigenically to define the virus cluster prevalent in the field as well as to select the best vaccine strain which is able to cross-react with circulating viruses from different lineages. The ideal seed virus for vaccine production is, typically, a strain of low pathogenicity that is well matched antigenically with the prevailing virus ***(Chen et al., 2003).*** All H5N1 viruses isolated all over the world since 1996 have multiple basic amino acids in the cleavage site of HA and, therefore, all are highly pathogenic and so they are unsuitable for vaccine production. The main problem is the inability to obtain high yields of virus in embryonated chickens eggs (ECE) ***(Subbarao and Katz, 2004).*** New cell line substrates for the manufacture of inactivated influenza vaccine may alleviate the limitations of egg-based vaccines ***(Mabrouk and Ellis 2002).***

Recent research has focused on the development of cell culture– derived vaccine production technology using either mammalian cell lines for viruses or insect cells for recombinant antigens**.** Production in mammalian cell lines offers significant advantages in the manufacturing process over egg-based production, because it allows more flexibility, faster production, and adequate availability of substrate for virus growth ***(Genzel et al., 2004).***