Egytptian Virol. 5 (1): 130-138 (2008)

Preparation and evaluation of inactivated oil emulsion Avian Influenza H5N2 Virus Vaccine for the first time in Egypt

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A Whole virus inactivated oil emulsion vaccine has been prepared from the low pathogenic avian influenza (LPAI) virus A/Turky/CA/209092/02, H5N2 for the first time in Egypt. Virus strain from which the vaccine seed virus has been prepared was derived from the USDA, Ames Iowa, USA. The specific characters of this strain were studied; It was found that the best virus dilution of AI virus for egg inoculation was 10-3 to 10-4, the peak virus titer was obtained 72 hours post inoculation, the predilection sites of the virus multiplication were the allantoic fluid, the chorioallantoic membrane and the amniotic fluid in order, where the allantoic sac route is considered the best site for virus inoculation and the virus was completely inactivated using formalin in a final concentration of 0.1% for 18 hours at 25c°. Evaluation of the prepared vaccine batch based mainly upon vaccination of different groups of specific pathogen free (SPF) chickens using different doses and estimation of the humoral immune response using the hemagglutination inhibition (HI) test. The vaccine provided a faster and more reliable immune response against the H5N2 viral antigen after immunizing 3 weeks old chicken with a dose of 0.3 ml/bird, inoculated subcutaneously (s/c) in the nap of the neck, was fairly enough to evoke the required level of immunity. The approach through which this vaccine could be used as a possible potential vaccine candidate for protection of chickens against the highly pathogenic avian influenza (HPAI) virus has been discussed.

Key Words: Low Pathogenic Avian Influenza (LPAI) Virus – Highly Pathogenic Avian Influenza (HPAI) Virus – SPF Chickens – Inactivated Oil Emulsion Vaccine

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(Received March 2008)
(Accepted May 2008)

.INTRODUCTION

Avian influenza (AI) or better known as the bird flu is an extremely infectious disease caused by a type A strain of the influenza. First identified in Italy a mere one hundred years ago ***Stubbs 1965***.

With the highly pathogenic strains having almost 100% fatality rate. Wild bird populations are often carries of the low pathogenic strains of the virus without the showing any symptoms and contact of domestic flocks with wild migratory birds has been at the origin of many epidemics in poultry. AI can occasionally spread to humans and other animals usually following direct contact with infected birds continuing outbreaks of highly pathogenic avian influenza (HPAI) across Eurasia and Africa caused by a type A influenza of the H5N1 a subtype remains an economic threat to commercial poultry throughout the world by negatively impacting animal health and trade. In recent years the poultry industry world wide has suffered serious damage due to avian flu epidemic, since 2003 the particularly virulent H5N1 strain of the disease has caused more than 125million birds to die or be destroyed in South East Asia (***Leey et al.2007***).

Since February 2006 the highly pathogenic (HP) avian influenza (H5N1) had emerged as the cause of sever disease and high mortality in chickens on production farms and village based production of Egypt, by the time the disease had spread allover the country threatening the poultry industry and causing a great hazard to human; a report by (***information center and decision support center of the council of ministers 2007***) clarified that the total number of birds that have been executed in Egypt as a result of AI illness by about 4 per 34 million birds since the emergence of the disease until march 2007, the report also stated that 80 million (Egyptian pound) deficit in the trade balance during this time due to bird flu.

It is known that biosecurity represents the first line of defense against AI, although in certain circumstances, strict hygienic measures appear to be inapplicable for social and economic conditions and the availability and use of effective vaccine can be valuable tool in controlling out breaks of avian influenza to maximize the outcome of a series of controlling measure in countries that are

currently infected and also a means of reducing the risk of introduction in areas at high risk of infection (*capua and marangon 2007*).
The current study described the preparation of a conventional inactivated whole-virus oil adjuvant vaccine against AI using the low pathogenic subtype H5N2.

MATERIAL AND METHODS

1- Master seed virus

The low pathogenic (LP) A\Turkey\CA\209092\02 (H5N2) avian influenza (AI) virus was used as the vaccinal strain. The virus was provided to the veterinary serum and vaccine research institute by the united state department of agriculture (USDA, Ames) where the virus has previous approval as an H5 AIV seed stock for inactivated vaccine use. The original titer of the virus was $10^{9.5}$ EID50/ml with HA activity 9log2.

2- Embryonated Chicken eggs

Specific pathogen free (SPF) embryonated (9-11 day) old were obtained (Nile-SPF-eggs farm,Koom Oshiem,Fayom,Egypt) the eggs were used for propagation, titration and assurance of complete virus inactivation.

3- Adjuvants:

The vaccine was prepared as an oil adjuvant form using

a. paraffin oil (white X300) white oil quality FDAIAL USP No. 05200 Mobil
b. Aquaous phase emulsifier (Tween 80) polyoxyethlene sorbitan supplied by Sigma.
c. Oil phase emulsifier Arlacil (spam80) sorbitan monooleate, supplied by Micbile.

4- Chemicals:

d. Glycine (NH2-ch2-CooH) produced by El Naser pharmaceutical company was used as 4.8 gm/ Liter
e. Sodium thiomersal 1/10000 adds as 1ml/ Liter

Preparation of working seed:

The master seed was established (A/Turkey/CA/209092/02-H5N2) low pathogenic. From this the working seed was prepared by expansion of an aliquot of

master seed. This working seed was stored in liquid form at below -70 c and other was stored as lyophilized aliquots (1ml) at -70

Virus propagation:

According to ***Garcia et al (1998)*** the working seed was diluted in sterile physiological normal saline PH 7.2 so that about 10^3-10^4 EID50/0.1ml are inoculated into the alantoic cavity of 10 days old embryonated SPF chicken eggs; these are then incubated at 37c for 72 hours. Eggs containing embryos that die within the first 24 hours were discarded. Infected eggs after 72 hours were chilled at 4c□ before being harvested. The harvested allantoaminiotic fluid was stored immediately at 4c□ in container of 1 liter on each one million IU of penicillin and one gram of streptomycin and one million IU of mycostatin were added. The fluid is then tested for bacteriological and fungal contamination before large pools were made for inactivation.

Virus titration:

Obtained virus was titrated in embryonated SPF chicken eggs and the titer was expressed after the calculation according to the method of Read & Munch (1938).obtained titer was 108.5 EID50/ml.

Virus inactivation:

According to OIE publication manual (2004) inactivation of the virus was carried out using formalin in a final concentration of 0.1% of the total volume; the fluid was blended using magnetic stirrer for about 20 hours at 25 c□. samples from the inactivated virus was tested for safety in 10 days old embryonated SPF chicken eggs and two successful blind passage were carried out before it was considered safe.

Preparation of oil adjuvant AI vaccine:

Water in oil emulsion vaccine was prepared as previously described (ston et al. 1978) with both the oil soluble surfactant (span 80) and the aqueous soluble surfactant (tween 80) were added to the oil phase; with the aqueous to the oil ratio of 1:2 was used and the adjusted hydrophil lipophil balance (HLB) according to (Schick 1966) was 7.0

Chickens and housing:

Chicken used in this study were specific pathogen free SPF 1 day old chicks (Nile SPF, eggs, koom oshiem, fayoum, Egypt) the chicks were housed in brooder units within isolation facilities till they became 21 days of age.

Vaccine efficacy studies:

175 SPF chicks were divided into seven groups each of 25 chicks. Groups 1,2,3,4 were received different doses of the prepared vaccine (table 1) Group 5 received the double of the recommended dose (0.6) and considered as safety group. Group 6 (non vaccinated control) was kept with the safety group in the same unite, where as group 7 was kept away from the other vaccinated groups.

Each bird was vaccinated with the recommended dose s/c at the nap of the neck. Ten random blood samples were collected weekly from each group up to 6 weeks post vaccination. Serum was separated and tested for detection of humeral immune response using the heamagglutination inhibition test (HI test) according to method of (Majujabe and Hitchner 1977).

Table (1) vaccine efficacy studies .

Group	Treatment
1	Vaccinated with 0.5 ml
2	Vaccinated with 0.3 ml
3	Vaccinated with 0.15 ml
4	Vaccinated with 0.075 ml
5	Vaccinated with 0.6 ml safety group
6	Non vaccinated kept with safety group
7	Non vaccinated kept away from vaccinated groups

vaccination; moreover the prepared vaccine was accepted after evaluation with an authorized highly specialized committee that hard currency prayed for importation for such vaccine.

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