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SEROLOGICAL AND PROTECTIVE COMPARATIVE STUDIES BETWEEN LASOTA AND CHINESE VII D NDV INACTIVATED VACCINES

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ABSTRACT

Newcastle disease (ND) is fatal disease that threatens the poultry production all over the world. ND control strategies based mainly on vaccination. Despite the intensive vaccination programmes that have been implemented for ND control, the outbreaks appear continuously in vaccinated flocks and keep a continuous spread of virus to environment. The field virus isolates obtained from recent outbreaks in Egypt revealed new strain classified as class II velogenic genotype VII sub genotype d. Although the NDV strains from one serotype APMV-1, there is genetic diversity between different genotypes. In this study, the efficacy of two inactivated NDV vaccines that have been prepared from LaSota (genotype II) and VII d NDV (genotype VII) strains evaluated using haemagglutination inhibition test on serum obtained weekly from vaccinated chicks to monitor antibody titer induced by the prepared vaccines and also evaluated for protection % in the vaccines received birds that challenged with 10^6 LD50/0.5 ml I/M at 28 days post vaccination .The protection % was 100 % .this study indicated that, the different Newcastle disease virus strains used for preparation of inactivated vaccines don't affect the protection of such vaccines against disease signs and mortality.

INTRODUCTION

Newcastle disease (ND) is a very serious problem for poultry production in many countries for decades. It is caused by virulent strains of Newcastle disease virus (NDV), an avian paramyxovirus serotype 1 (APMV-1) belonging to the genus Avulavirus, subfamily Paramyxovirinae, family Paramyxoviridae **(Lamb *et al.,* 2005)**. NDV isolates are divided into six lineages (1 to 6). NDV have 5 pathotypes; velogenic viscerotropic, velogenic neurotropic, mesogenic,lentogenic and asymptomatic enteric **(Alexander and senne, 2008)**.There are

antigenic and genetic diversity between the different genotypes despite the fact that all NDV isolates are of one serotype **(Miller *et al.,* 2013)**. Live attenuated and inactivated vaccines are used routinely to control ND in poultry flocks **(Koppad *et al.,* 2010).** In Egypt, NDV is endemic so an intensive vaccination program against NDV is practiced in both large and small-scale poultry production **(Abdel-Glil *et al.,* 2014)**. Despite intensive vaccination programs that being implemented, outbreaks still occur in vaccinated poultry flocks **(Nabila *et al.,* 2014)**. The recent outbreaks between 2011 and 2012 revealed velogenic isolate belong to class II genotype VII sub genotyped that has been isolated from vaccinated broiler farms in Fayoum, Behira and Giza Provinces **(Radwan *et al.,* 2013)**. The main objective of the present work is to evaluate two different formulations of Newcastle disease inactivated vaccine for choice of best vaccination programme against ND in Egypt.

MATERIAL AND METHODS

Vaccines:

Two inactivated NDV vaccines (LaSota and VII d NDV) obtained from VSVRI poultry viral vaccine department.

Challenge strain:

NDV (NDV-B7-RLQP-CH-EG-12) virulent (112RKQKR*F117KM288609) strain was kindly provided by National Laboratory for Veterinary Control on Poultry Production, Animal Health Research Institute used in challenge test with titer of 10^6 EID50/0.1ml

Safety and sterility testing of prepared vaccine formulations:

The obtained vaccines tested for sterility, safety and potency to ensure its efficacy. For sterility testing a Sample from each vaccine was taken and cultured on subaroud dextrose agar, nutrient agar and thioglycolate broth. A double dose of each vaccine administered to 21 day old SPF chicks. Vaccinated birds observed daily for 14 days for any signs, mortalities and local reaction.

Evaluation of vaccine potency:

A total of 120, 22-day-old SPF chickens were reared in isolators and divided into three groups namely the L, V and C with 40 birds in each group. L group (inactivated lasota vaccine treated group), V group (inactivated VII d NDV vaccinated group) and C (control not vaccinated group). Birds received the vaccine at 22-day-old subcutaneously (0.5 mL/dose). Serum samples were collected at 1st, 2nd, 3rd, 4th weeks after vaccination and tested for

Haemagglutination inhibition activity. Birds divided into 4 groups; inactivated VII d NDV vaccinated group, inactivated lasota vaccinated group, and not vaccinated control group divided into 2 groups; not vaccinated not challenged group, not vaccinated challenged group. The challenged groups injected intramuscularly with a dose of 10^6 LD50 per chicken with VII d strain NDV-B7-RLQP-CH-EG-12 after 28 days post vaccination.

RESULTS

Safety and sterility testing of prepared vaccine formulations:

The prepared vaccines were confirmed to be sterile from any bacterial and fungal contamination and safe.

Potency of prepared vaccine:

Serology:

Haemagglutination inhibition test applied on the serum samples for each week to each group shown in Fig. (1).

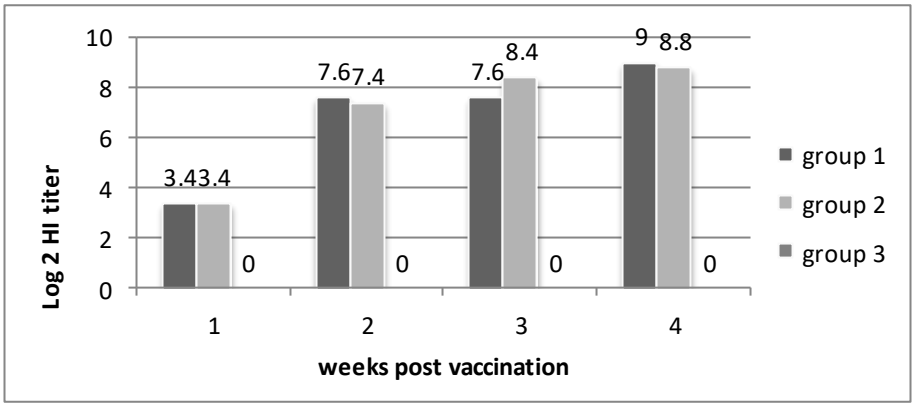

Fig. (1): The mean log2 of HI antibody titer for chickens vaccinated with prepared Inactivated vaccines

Group (1): Chickens vaccinated by inactivated VII d NDV vaccine.

Group (2): Chickens vaccinated by inactivated Lasota vaccine.

Group (3): control non-vaccinated chickens.

Challenge:

The challenge control group birds died within 4-5 days post challenge, the vaccinated challenged birds which kept under observation for 15 days didn't show any clinical signs and mortalities. Protection %=No of survival / total no of challenged birds x100 = 100 %.

DISCUSSION

Newcastle disease (ND) is a contagious disease of poultry that has a negatively effect on poultry production in many countries all over the world. Many efforts have been made to study the methods for eradication of this serious disease. The main method for NDV eradication centres on biosecurity application in poultry flocks and vaccination. Vaccination is the ideal route for control of NDV in endemic areas.Although intensive vaccination programmes have been applied recent outbreaks take place in vaccinated flocks. The isolated strain from recent outbreaks revealed new velogenic genotype VII d NDV. The objective of this study is to evaluate two different formulations of inactivated NDV vaccines by vaccination of 21-day old SPF chicks. Assessment of quality control measures for the obtained inactivated vaccines indicate that they are completely sterile, no bacterial or fungal contaminants and safe for vaccinating chickens which showed no detectable signs of illness as recommendation of **OIE (2004)**.The serological test was carried out on serum samples obtained from vaccinated Chickens revealed that birds have detectable antibodies by the first week post vaccination of a titer ~3.4 log2 in both vaccinated groups and increase to reach its peak in 4th week post vaccination with a titer 8.8 log2 and 9 log2 in lasota vaccinated group and VII d vaccinated group; respectively. Inactivated VII d NDV vaccinated group show slightly higher antibodies by the 2nd week post vaccination than lasota vaccinated group with a titer 7.6 log2 and 7.4 log2 for VII d NDV and Lasota groups; respectively as shown in Fig. (1). By the 3rd week post vaccination lasota vaccinated group showed detectable higher antibodies than VII d NDV vaccinated group with a titer (8.4) and (7.6) for lasota and VII d NDV groups, respectively. HI test results showed slight increase in antibody titer in VII d vaccinated group when compared with HI results of lasota vaccinated group at the time of challenge at 28-day post vaccination.The vaccinated birds showed no mortalities or morbidity in both vaccinated groups after challenge with VII d NDV. Despite of the genetic and antigenic diversity between both strains of the prepared lasota vaccine and the challenge virus,full protection was observed this is reasonable to expect that after NDV vaccination commonly non-specific antibodies elicits and those can neutralize common and similar antigenic sites between the antigenically different NDV strains that can fully protect the vaccinated birds this is in accordance with others **(Alexender *et al.*, 1999 ; Roy *et al.*,2000)** who demonstrated that vaccination of birds by a vaccine strain defended them against the

challenge by a virus strongly differing from the vaccine strain and this is in contrast with data showed by **Panshin *et al.,(* 2002)** that expect that any antigenic modification of antigenic sites (HN and F) proteins can influence the vaccine efficacy and thus overcome the vaccine barrier and cause the disease. The high level of antibodies which has been shown in HI results is sufficient for the protection effect for homologous and heterologous vaccines; this can suggest that protection depend on the level of antibodies at the time of challenge (28 day post vaccination). This in accordance with **(Patti J. Miller *et al.,* 2013; van Boven *et al.,* 2008)** who suggest that heterologous vaccines can prevent transmission if sufficient time is allowed for birds to mount a proper immune response. The previous results expect that, the continuous outbreaks may be due to insufficient immune response induced by applied vaccine or failure in vaccination practice and also may be due to bad storage of vaccine patches and this is similar to **Dortmans *et al.,* (2012)** who supposed that not antigenic variation which responsible for outbreaks but mainly poor flock immunity due to inadequate vaccination practices that can spread the virulent NDV field strains. While non-vaccinated challenged group the typical signs and post mortem findings of NDV appeared clearly after 4th day post challenge with 100 % mortalities. In conclusion, the different Newcastle disease virus strains used for preparation of inactivated vaccines don't affect the protection of such vaccines against disease signs and mortality.

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