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ANTIBODY RESPONSE OF BROILERS TO CELL CULTURE ADAPTED THERMOSTABLE NEWCASTLE DISEASE I-2 STRAIN VACCINE

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ABSTRACT

The efficacy of cell culture adapted thermostable Newcastle disease virus vaccinal strain I-2 was evaluated at various commercial poultry (broiler) farms. Day-old chicks (n=360/6 farms) were vaccinated (oral route) and blood sample from each bird was collected for serum separation. Antibody response was monitored through haemagglutination inhibition test (HI) and enzyme linked immunosorbant assay (ELISA) on 0, 7, 14, 21, 28 and 35 days post-vaccination (DPV). Maximum geometric mean anti-NDV-HI ($Log_2$7.83) and anti-NDV-ELISA (6017) antibodies titers were observed, respectively on 14th DPV. It was observed that administration of NDV I-2 strain vaccine in broilers birds is feasible and was found to induce more protective antibody response. Thermostable NDV I-2 strain can be a preferred choice against NDV in developing countries.

Key words: Thermostable, Antibody, ELISA, Titer, Serum.

INTRODUCTION

Newcastle disease (ND) is a grave peril and highly transmissible disease in the poultry industry of under developing countries like Pakistan (Mahmood *et al.* 2014). The endemic nature of ND causes huge economic losses to the poultry farmers all over the world (Ananth *et al.* 2008). Avian Paramyxovirus type 1 is the causative agent of this disease (Aldous *et al.* 2003) which belongs to the genus Avula virus of the Sub-family Paramyxovirinae (Xiao *et al.* 2012). The transmission of the disease takes place through contaminated feeding equipments, newly introduced birds, air droplets, sick and carrier birds and other means. ND vaccine has been employed since the last five decades for protection of poultry (OIE 2012). Field records of thermostable I-2 strains application in various countries include Malaysia (Wambura *et al.* 2000) Mozambique (Alders 2004) and Nigeria (Musa *et al.* 2010). Protective levels of antibodies against ND are achieved (Nega *et al.* 2011). The main objectives of development of thermostable vaccine in tropical and sub-tropical countries are the reduction of vaccine wastage, increase in vaccine efficacy, less dependence on cold storage; reduction in cost, ease of application and transportability (Alders and Spradbrow 2001). Thermostable I-2 ND vaccine has successfully provided 100 per cent protection of domestic poultry and 89 per cent to open shed chickens against Newcastle disease (Illango *et al.* 2008). In Pakistan, imported conventional vaccines including LaSota, Hitchnar, B1 etc. have been used for controlling the Newcastle disease. These vaccines are all thermolabile in nature. Therefore the use of cold chain storage system is a pre-requisite for a vaccine to maintain its efficacy and shelf life. However as mentioned before, due to primarily electrical load shedding in Pakistan cold storage of vaccine poses a significant problem all over the country. Additional characteristics of thermostable NDV I-2 vaccines like easy adaptability and applicability which make them more suitable used in rural areas of Pakistan (Mahmood *et al.* 2014). To meet this challenge, our research was under taken to evaluate the antibody level against Newcastle disease by using Vero cell adapted live attenuated thermostable Newcastle disease viral strain I-2 in the local environment of selected region of Pakistan.

MATERIALS AND METHODS

Source of vaccine and reference virus strain: Lyophilized Vero cell line adapted avirulent strain I-2 of NDV for vaccination and an indigenous lantogenic isolate of the virus for haemagglutination inhibition (HI) test were procured from the Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan.

Vaccine preparation: The virus was propagated in Vero cell line. The cells were cultured in 25 cm^2 cell culture flasks (Orange Scientific) and incubated at 37^{o}C in CO_2 incubator with 5% CO_2. The virus (0.25 ml) was inoculated in healthy and confluent monolayer of the Vero cells and incubated at 37^{o}C in CO_2 incubator with 5% CO_2 for 24 hours. The culture supernatant was collected by three freeze-thaw cycles, clarified by centrifugation at @ 5000 g for five minutes. Furthermore, it was passed through a filter with 0.2 um pore size to minimize the risk of contaminations.

Biological titration: The allantoic suspension of NDV was processed for calculation of embryo infective dose 50 (Reed and Muench 1938). Shortly, One ml of the virus was serially diluted as tenfold from 10^2-10^{10}. Beginning from the highest dilution, 0.1 ml of each dilution was inoculated into each of the five 10 day's old chicken embryos. At the end of incubation at 37°C the chicken embryos were chilled at 4°C overnight. After chilling, haemagglutination test was performed on allantoic fluid of each embryos to determine the presence of virus.

Vaccination: The day old broiler chicks (n=60/farm) were vaccinated (dose: 10^6 EID_{50}/bird) through drinking water at six farms surrounding the district of Faisalabad. Tags were used for the identification of the vaccinated birds. Blood samples were collected from each bird. The serum from each sample was separated and stored in properly labelled vials at -80°C for further processing (Olabode *et al.* 2010).

Measuring antibody response: Haemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) were performed to detect anti-NDV-HI and anti-NDV-ELISA antibodies, respectively on 0, 7, 14, 21, 28 and 35 days post-vaccination (DPV).

Statistical analysis: The data regarding anti-NDV antibodies titers (determinately either HI or ELISA tests) was processed using one way analysis of variance (ANOVA) on 0, 7, 14, 21, 28 and 35 DPV. The difference was further studied using Least Significant Difference test. All the analyses were performed using SPSS version 13.0 for Windows (Coakes *et al.* 2006).

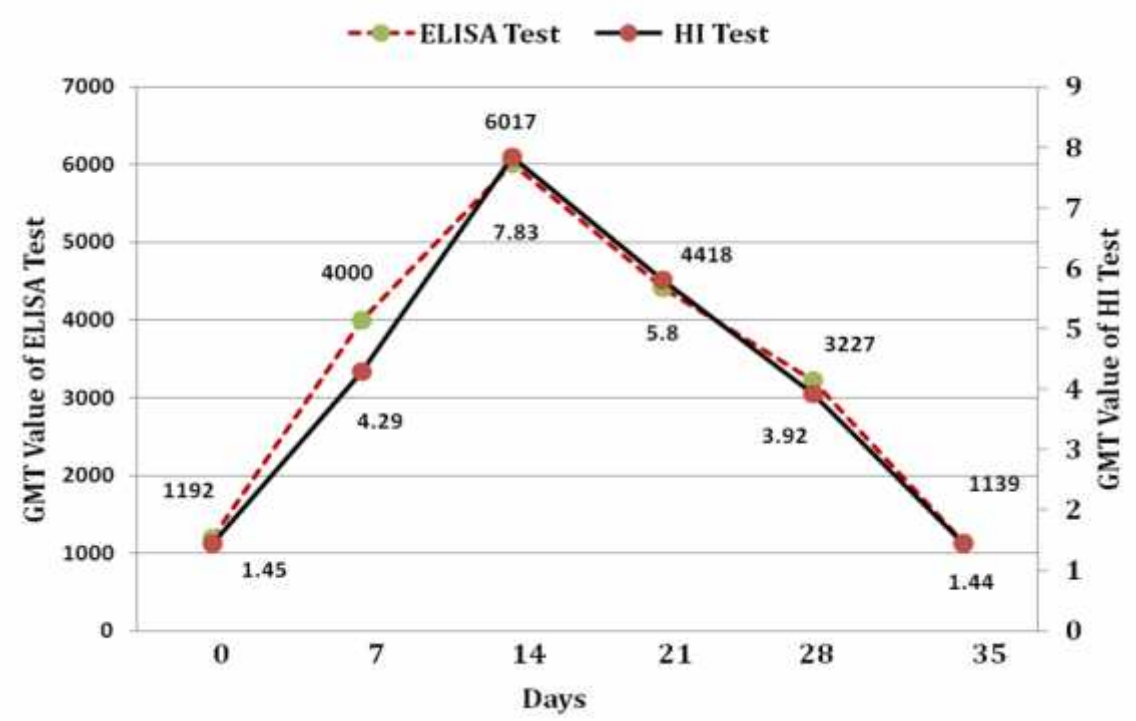
RESULTS AND DISCUSSION

Out of total 360 samples screened 274 (76%) were found positive for anti-NDV-HI and anti-NDV-ELISA antibodies while 86 (24%) were negative. Anti NDV-HI titers of log_2^3 or above is mainly accepted all over the world and it indicate protective level of antibodies against NDV. Serum level of antibodies was detected by using HI test (Uddin *et al.* 2014).

In the current research, single oral cell culture adapted thermostable ND vaccination was capable to produce defensive immunoglobulin response in day old broilers after seven days post vaccination. Thermostable NDV vaccine induced $Log_2$7.83 and 6017 geometric mean anti-NDV-HI and anti-NDV-ELISA antibodies titers, respectively on 14th DPV presented in Graph 1. Similar results had been presented in different studies by Bell *et al.* (1995), Amakye *et al.* (2000), Spradbrow (1992 and 1995), Aldres *et al.* (1994), Wambura *et al.* (2000), Alders (2004), Musa *et al.* (2010) and Wambura and Kataga (2011). Thermostable vaccine dose (10^6 EID_{50}/bird) was sufficient to induce protective titers against challenge viral infection (Bawala *et al.* 2011). Antibody response is directly proportional to immunogen amount in live ND vaccines (Ozan *et al.* 2014).

The protective antibodies response depends upon the vaccination route such as eye drop method, drinking water method etc. (Musa *et al.* 2010). Eye drop method initiates high antibody titer (80%) as compared to in drinking water (60%) as has been investigate by Alders (2005). Our results are also in reported range in the drinking water method (76%). Comparable results were detected in V4 thermostable strain (Guoyuan *et al.* 2013). The antibody titers of thermostable Newcastle disease I-2 vaccines are generally decreased after definite periods of time (Nssien and Adene 2002). In our study, 50 percent antibody titer was reduced on one month post-vaccination. Factors responsible for poor antibody response include season, presence of maternal antibodies, malnutrition, poor breed quality and administration routes (Alexande and Senne 2008). Among these factors, season plays substantial role as dry season affects severely than wet season (Otim *et al.* 2005). This might be due to harsh environmental conditions such as high ambient temperature (Kemboi *et al.* 2013). It had been reported that 100 per cent protection was achieved in wet season as compared to dry season which was 83 percent protection.

The thermostable vaccine induces protective immunity in poultry when correctly applied. It is cheap and thus makes it affordable to all farmers to use. It is not require any strict cold chain facility and easy to administer by farmers. It can make a vital contribution to the improvement of household food security in many developing countries like Pakistan (Mahmood *et al.* 2014). The speed, short time duration and economy of use of this HI assay are higher as compared to the ELISA. The HI test may be more convenient in some developing states like Pakistan that are involved in the production of thermostable NDV vaccine. The control of ND will contribute to improved commercial poultry production by preventing the virus in circulation and act as reservoirs and carriers to themselves and the more susceptible exotic breeds in commercial farms. In some circumstances, it may provide the first contact between small-scale farmers and national veterinary services.



Graph 1: Comparative HI and ELISA Mean antibody titers of cell culture adapted Thermostable ND I-2 vaccine at various selected poultry farms of district Faisalabad, Pakistan

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