

Original Research Article

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Evaluation of Newcastle Disease Antibody Titre in Broiler Poultry

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ABSTRACT

Newcastle disease (ND) also known as Ranikhet disease (RD) is a highly contagious viral disease that attacks many species of domestic and wild bird. In spite of the availability and global employment of NDV vaccines, it always remains a constant threat to poultry producers worldwide. It is prevalent in Indian subcontinent and leads to economic losses. The present study is to design the detection of antibody titre against Newcastle disease in broiler poultry. Total 60 blood samples were collected from broiler on 7th, 14th and 21st day of post vaccination. Haemagglutination inhibition test was performed to quantify the serum antibody titre against NDV. This study revealed that the antibody titre of broiler on 7th, 14th and 21st day was 2⁴ and 2⁵; their respective percentage was 50% & 50%; 25% & 75% and 10% & 90%. All the age group of birds were showing protective titre which was $\geq 2^4$ which indicates the lasota strains vaccine of NDV has significance potency against Newcastle disease of broilers.

Keywords

NDV, Broiler, Haemagglutination test, Haemagglutination inhibition test, Broiler

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Introduction

Newcastle disease (ND) is a highly contagious and fatal disease of chickens. In many developing countries ND is endemic and the disease has the greatest impact on

villages where the livelihood of people depends on poultry farming. The causative agent Newcastle disease virus (NDV) is a member of the genus Avulavirus in the family Paramyxoviridae (Lamb *et al.*, 2005). NDV isolates display a range of virulence in

chickens ranging from inapparent to fatal infection. Based on their pathogenicity in chickens, NDV isolates are categorized into three main pathotypes: lentogenic (low virulence), mesogenic (intermediate virulence) and velogenic (high virulence). The continents having warm climates are known to be reservoirs of virulent NDV strains (Herczeg *et al.*, 1999). It is prevalent in Indian subcontinent and causes losses in millions every year. The disease carries potential to rapidly spread irrespective of national borders and negatively impact international trade of poultry and poultry products. In India according to OIE (2015) a total of 6,93,840 cases with 198 outbreaks were recorded. While, higher seroprevalence of ND in Kuroilers that is 81.4% (Ghosh *et al.*, 2017) was reported in India.

The worth of vaccinations can be estimated best with challenge experiments but they are expensive and time consuming (Czifra *et al.*, 1998). Therefore, serological tests are frequently used to assess protective response (Czifra *et al.*, 1998). Serum antibody titers against Newcastle disease indicate the protective immune response against Newcastle disease of poultry.

For isolation and identification of NDV in embryonated chickens eggs and haemagglutination (HA) and hemagglutination inhibition (HI) test with a NDV mono-specific antiserum is a gold standard for the detection of NDV (OIE, 2012).

Haemagglutination inhibition (HI) test is the method of choice and frequently used to assess immune response. A Lasota vaccine is used to protect the broiler from NDV. Recently the vaccination failure has been seen in field cases. This study is aimed to evaluate the vaccine titre against Newcastle disease virus in broilers.

Materials and Methods

Location of work

The work was conducted in the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh.

Experimental animals

A total of 60 blood samples from commercial broiler farm of Jabalpur were collected. The birds were vaccinated for Newcastle disease virus. The blood samples were collected on 07th, 14th and 21st day post vaccination aseptically.

Antigen

The antigen (Newcastle disease vaccine Lasota strains) used for HI test was collected from a reputed vaccine company.

Preparation of Chickens Red Blood Cell (1 % v/v)

Suspension Chickens blood samples were collected from a live bird market. Blood was collected in a 15ml tube containing 2 ml Alsever's solution as anticoagulant. Collected blood was then centrifuge at 1500 rpm for 5-7 minutes and the supernatant was poured off. PBS (1X) was added into the falcon tube containing blood and centrifuge at 1500 rpm for 5-7 minutes. This step is repeated for 4-5 times for washing chickens blood. 1% v/v suspension of chicken RBC was prepared by adding PBS.

Haemagglutination test and determination of 4HA unit virus for HI test

0.025 ml of PBS was dispensed into each well of a plastic V-bottomed microtitre plate.

0.025 ml of the allantoic fluid/ vaccine (virus suspension) was placed in the first well. Two fold dilutions of 0.025 ml volumes of the virus suspension were made across the plate. A further 0.025 ml of PBS was dispensed to each well. 0.025 ml of 1% (v/v) chicken RBCs was dispensed to each well. The solution was mixed by tapping the plate gently. The RBCs were allowed to settle for about 40 minutes at room temperature. HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs.

The titration was read to the highest dilution giving complete HA (no streaming); represents 1 HA unit (HAU) and calculated accurately from the initial range of dilutions.

Haemagglutination Inhibition (HI) Test (OIE, 2012)

0.025ml of PBS was dispensed into each well of a plastic V-bottomed microtitre plate. 0.025 ml of serum was placed into the first well of the plate. Two fold dilutions of 0.025 ml volumes of the serum were done across the plate. 4 HAU virus/ antigen in 0.025 ml were added to each well and the plate was left for a minimum of 30 minutes at room temperature. 0.025 ml of 1% (v/v) chicken RBCs was added to each well and after gentle mixing, the RBCs was allowed to settle for about 40 minutes at room temperature. The HI was the

highest dilution of serum causing complete inhibition of 4 HAU of antigen.

Statistical analysis

All data were collected and summarized in the computer program MS EXCEL (Microsoft Co.). The statistical data analysis (log based) was performed by IBM-SPSS software. Values were expressed as mean ± SD and geometric mean titer.

Results and Discussion

The Haemagglutination Inhibition (HI) test although conventional but is still the most widely used assay. This test requires cheap reagents, is easy serological method for measuring anti Newcastle disease virus (NDV) antibody levels in poultry sera and is considered the standard laboratory method for diagnosis of NDV. A total of 60 serum samples from broiler poultry were collected and HI titer against NDV was performed to quantify the serum antibody titre from vaccinated flock.

A Newcastle disease HI antibody titer log 2³ (i.e. 1:8) and above is consider as positive for specific immunity (Allan and Gough, 1974; Numan *et al.*, 2005 and Mozaffor Hossain *et al.*, 2010). Again it was reported that the antibody titers ≥ log2⁴ against ND were considered as protective (OIE, 2012).

Table.1 Haemagglutination inhibition titer of broiler against Newcastle Disease Virus

HI titre Age group (Days)	Total number of birds	1:2 (2 ¹)	1:4 (2 ²)	1:8 (2 ³)	1:16 (2 ⁴)	1:32 (2 ⁵)	1:64 (2 ⁶)	1:128 (2 ⁷)	1:256 (2 ⁸)	1:512 (2 ⁹)	Mean ± SE
7 th	20	—	—	—	10	10	—	—	—	—	1.3545±0.0343
14 th	20	—	—	—	5	15	—	—	—	—	1.4298±0.02990
21 th	20	—	—	—	2	18	—	—	—	—	1.4749±0.02070

Fig.1 Haemagglutination (HA) Test of broiler

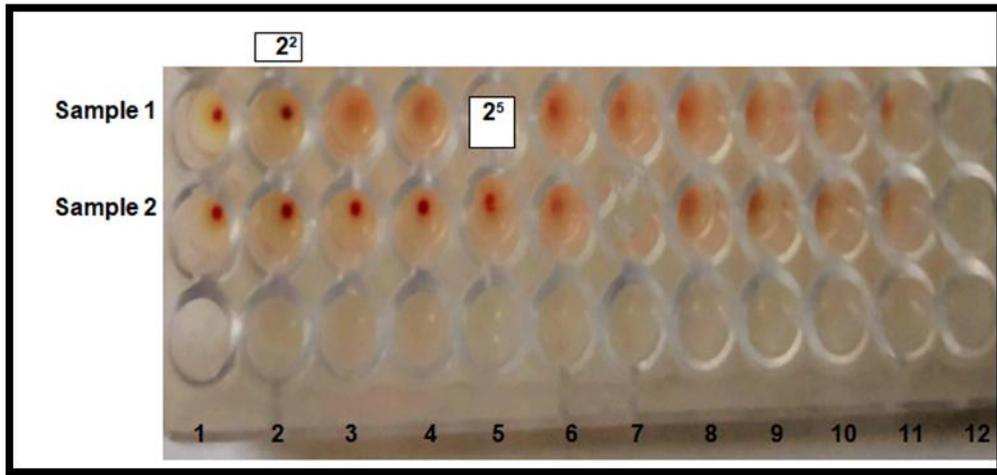
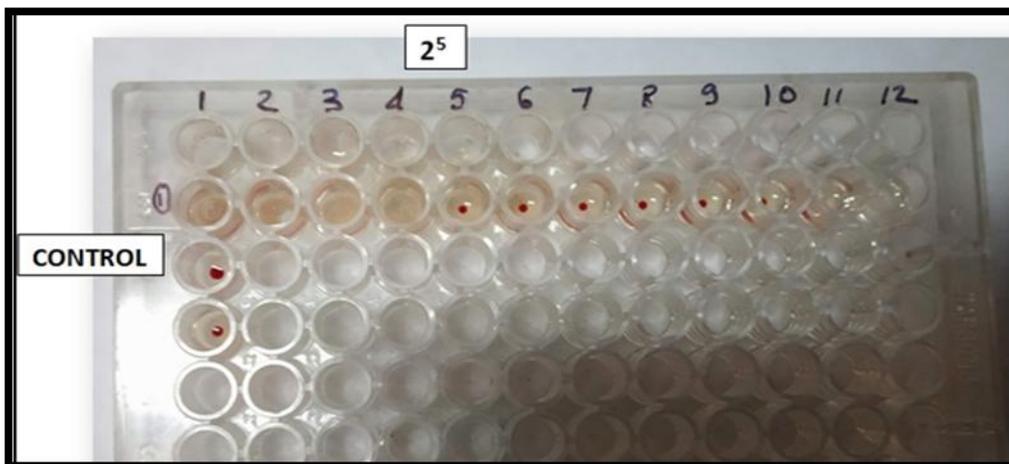


Fig.2 Haemagglutination Inhibition (HI) Test showing HI titre at 2⁵ (1:32)



A total 60 samples were collected at 7th, 14th and 21st day post vaccination. In the present study it was found that the antibody titre of broiler on 7th, 14th and 21st day was 2⁴ and 2⁵; their respective percentage were 50% & 50%; 25% & 75% and 10% & 90%. All the age group of birds was showing protective titre which is $\geq 2^4$ and the values were expressed as mean of log 2 value (Table 01). The maximum titre recorded was 2⁵.

In a study antibody titer levels against NDV in different regions of Morocco by Bell and Moulodi (1988) recorded ranging from 5 to

83% (average 35%) in vaccinated chickens. Rahman *et al.*, 2017 tested a total 92 serum samples and found the Ab $\geq \log 2^4$ titer of 85.9 % (n=79) serum were at protective level; 7.6 % (n=7) were found below the protective level ($\log 2^1$ to $\log 2^2$).

Numan *et al.*, (2005) reported 98.07% of serum samples to be positive for specific immunity against NDV for broilers in Pakistan which is close to finding of the present study. Whereas, Bell and Moulodi (1988) observed varying antibody titer levels ranging from 5 to 83% (average 35%) against

NDV in different regions of Morocco in vaccinated chickens. A study to detect Newcastle disease virus (NDV) antibodies in routinely vaccinated commercial poultry in Nigeria using hemagglutination inhibition assay was conducted. A total of 120 sera tested, 82 (68.33%) had detectable NDV antibodies but only 81.67% had HI protective titre of $\geq 2^4$ and 18.33% showed low seroconversion with titre 2^3 or less (Olufemi *et al.*, 2018).

In the present study the lasota strains vaccine of NDV has significance potency against Newcastle disease of broilers.

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