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Evaluation of Specific Antibody Response in Backyard Chickens to Infectious Bursal Disease Live Vaccine

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Abstract

Infectious Bursal Disease (IBD) is one of the oldest and widely known poultry diseases all over the world. It is caused by IBD virus of Avibirnavirus genus of family Birnaviridae family. IBD has great economical impact on backyard poultry world as it causes high weakness and mortality. The objective of present study was to evaluate the antibody response in village chickens in India after vaccinating them with IBD live vaccine. Serum was collected at regular intervals from chickens up to 112 days after vaccination. Antibodies against IBD virus were measured using ELISA method. It was observed that the vaccines with both intermediate and intermediate invasive strain caused good immune response in the birds. Serum antibody level was found significantly high in 28 days blood collection, which decreased gradually up to 112 days. It was also observed that Intermediate invasive strain produced higher amount of antibodies than intermediate strain of the vaccine. Furthermore, it is also suggested that good diet and biosecurity measures in backyard chickens and proper vaccination program can benefit the local farmers of Asian countries.

Keywords: Infectious Bursal Disease, Backyard, Antibody.

INTRODUCTION

Infectious Bursal Disease (IBD) is an important disease of poultry, which occurs due to infection of IBD virus of Avibirnavirus genus of Birnaviridae family. It is a fatal disease and causes high and rapid mortality in young chickens leading to significant economic loss. It is a highly contagious viral disease, which is characterized by destruction of lymphoid organs, particularly bursa of fabricius, where B lymphocytes mature and differentiate. The disease was first reported by Cosgrove (1962). Several outbreaks have been reported in many countries like USA, Europe, Africa, Far East, Asia and Australia (Hailu *et al.,* 2009*,* Khan *et al.,* 2009, Lasher & Davis, 1997; Faragher, 1972; Firth, 1974; Jones, 1986; Lasher & Shane, 1994; Muller *et al.,* 2012, Oluwayelu *et al.* 2014, Provost *et al.,* 1972; Van Den Berg, 2000; Van Der Sluis, 1999). IBD has incubation period of two to three days (Kegne and Chanie, 2014; Dorji *et al.,* 2016; Lawal *et al.,* 2016, Li *et al.,* 2018). It causes mortality up to 50 to 60% in laying hens and 25 to 30% in broilers. The hypervirulent strain of virus causes 100% mortality in specific pathogen free chickens (Nunoya *et al*., 1992, Van Den Berg *et al*., 1991). The symptoms of the disease include exhaustion, prostration, dehydration, diarrhoea and ruffled feathers. The mortality starts within third day of infection, reaches to peak within four days and sickness of birds persist for up to seven days. The severity of disease may vary with age and breed of birds, virulence of the strain and degree of passive immunity (Van Den Berg *et al*., 2000). The principal organ affected is bursa of fabricious, additionally, other lymphoid organs are also affected. The virus targets B lymphocyte cells in active division stage (Kaufer & Weiss, 1980; Sharma *et al.,* 1993; Tanimura *et al.,* 1995). Apparent macroscopic inflammation can be observed in infected bursa (McFerran, 1993).

The disease causes high financial losses as it causes high mortality, secondary infections, growth retardation and carcasses have to be discarded due to haemorrhages. IBD is also found prevalent in village chicken in various countries, where backyard poultry is a significant part of poultry business (Wilcox *et al*., 1983; Swai *et al*., 2011; Sule *et al*., 2013; Yuguda *et al*., 2014, Bettridge *et al.,* 2014). In India, 11% egg production is contributed by village chickens (Kumaresan *et al.,* 2008). Economically, rearing and egg production is a vital part of poultry business in villages of Asian and African countries (Gueye, 2000). Vaccination is the principal method to control IBD (Muller *et al.,* 2012). Generally IBD is controlled by vaccination of the flock and the infected birds are culled to prevent the spread of the disease. For proper vaccination of birds with IBD vaccine, strict schedule of vaccination has to be followed. Proper guidelines of poultry farming, sanitation, cleaning, feeding and water supply need to be observed. The vaccine should be selected based on user-easiness, economy, availability, immunogenicity and transportation facility. In countries like Africa and India, people are having village chickens in small numbers. With this background, this study was designed to estimate seroconversion of IBD live vaccine in improving protection from IBD in backyard chickens.

MATERIALS AND METHODS

Grouping and rearing of birds

The study was conducted in two villages of Gujarat state – Merda Adraj and Jetpura. The birds were reared by local farmers with free access to feed and water. The chickens were monitored daily for any clinical signs and symptoms until the study completed.

Total 90 Kadaknath backyard birds, 14 day old, were divided in three groups with 30 birds in each group:

Group 1: Treated with intermediate strain Gumboro live vaccine (procured from Hester Biosciences Limited, India)

Group 2: Treated with intermediate invasive strain Gumboro live vaccine (procured from Hester Biosciences Limited, India)

Group 3: Control group, untreated

Experimental procedure

Vaccines were administered intraoccular with single dose of Gumboro live vaccine vaccine (Intermediate strain) and Gumboro live vaccine (Intermediate Invasive strain) respectively, with EID_{50} not less than 1×10^3 /dose, procured from Hester Biosciences Limited, India. From 0 to 112 days of vaccination, blood was withdrawn from birds. After 24 hours, serum was separated and stored at -20 °C until testing.

All sera samples were subjected to indirect ELISA test using ELISA kit (IDEXX laboratories, USA) at Hester Biosciences Limited, Anand laboratory. The ELISA test was performed as per the manufacturer's protocol and instructions. Before use, all the samples and reagents were allowed to room temperature and homogenized by gentle mixing. All the samples were diluted at 1:500 with sample diluents provided by the manufacturer. The antibody used in the study was serum and it is specific to chickens. 100 µl of two negative control samples were added. One was added to well A1 and another was added to B1. 100 µl of two positive control samples were added. One was added to well C1 and another was added to well D1. Both positive and negative controls were provided by IDEXX laboratories, USA. Then 100 µl of diluted samples were added into the appropriate wells and incubated at 25 C for 30 minutes by covering the plate with lid. After that, content of the well was emptied and washed 3 to 5 times by the 350 µl of the sterile distilled water using ELISA washer (Nunc International, USA,). Then 100 µl of the conjugate was added to each well and incubated at 18 to 26℃ for 30 minutes. Following washing for 3 to 5 times with sterile

distilled water, 100 µl of Tetramethyl Benzidine (TMB) substrate reagent was added into each appropriate wells and incubated at 25℃ for 15 minutes. Finally, 100 µl of stop solution was added to the each well to stop the reaction. The stop solution contained sulphuric acid. Then the microtitre ELISA plate was placed in the ELISA reader (Biotek ELX 800, USA) and the intensity of the color produced from the ELISA test was measured photometrically at 650 nm wavelength.

Data analysis

Simple t test were used for statistical analysis of the obtained data using IBM SPSS 20 software (please add version number, state and country). P < 0.05 was considered statistically significant.

RESULTS

Samples from all groups were collected and test was performed using ELISA kit. Pre-vaccination titres of all groups were measured i.e., group 1, 2 and 3 were 8.0 ± 0.8, 51.73 ± 22.0 and 8.9 ± 0.9 respectively. The ELISA titre increased after vaccination and at 28 days, it reached to highest value post vaccination (3978.8 ± 251.1 in group 1 and 4637.6 ± 185.4 in group 2). As after 28 days, titre decreased and was found to be 504.8 ± 19.5 and 1042.3 ± 189.0 in group 1 and 2 respectively at 112 days. The data of 28 days and 0 days results were compared and same of 28 days and 112 days were compared and significant difference was found (p< 0.05, Figure 1 and 2). Titre of control group was found to be 7.2 ± 0.6 to 15.7 ± 3.5 throughout the study (Figure 3). The titre greater than 396 is considered as positive, hence values below it are considered negligible statistically.

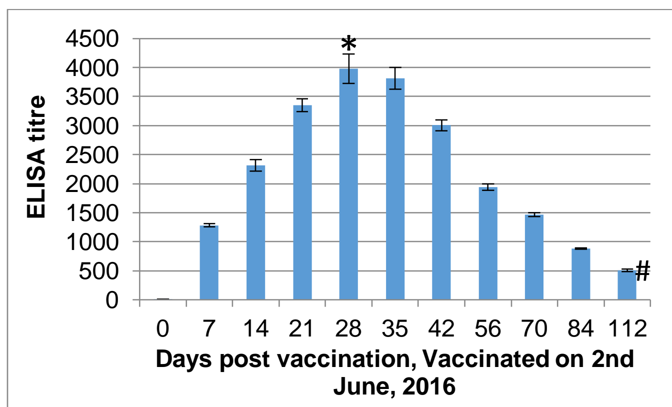

Fig. 1. ELISA titre of group 1 (Vaccinated with IBD live vaccine intermediate strain).
The results are mentioned as mean ± SEM
* data are significantly different from that of 0 days
# data are significantly different from that of 28 days

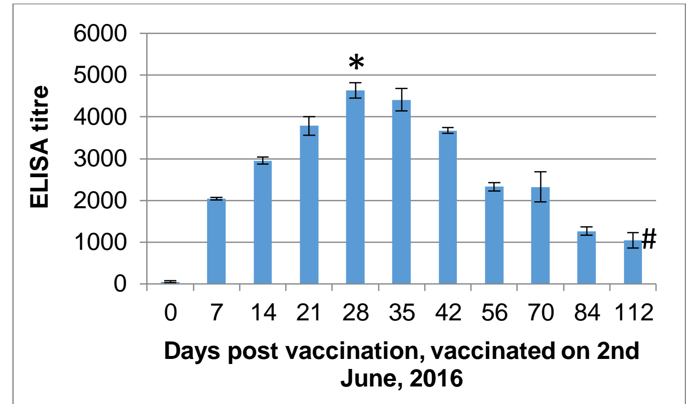

Fig. 2. ELISA titre of group 2 (Vaccinated with IBD live vaccine intermediate Invasive strain).
The results are mentioned as mean ± SEM
* Data are significantly different from that of 0 days
# Data are significantly different from that of 28 days

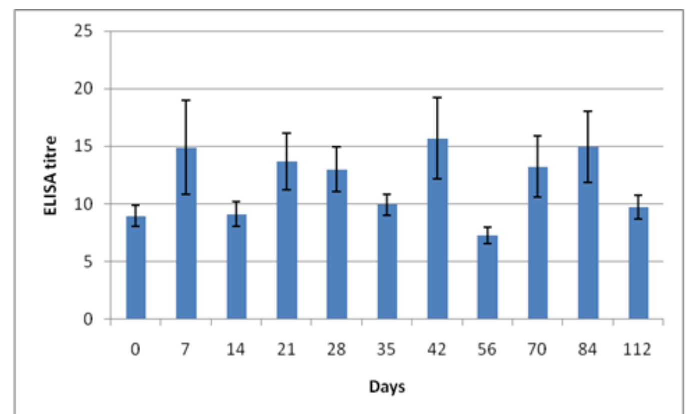

Fig. 3. Post vaccination titre of group 3 (Control)
The results are mentioned as mean ± SEM

DISCUSSION

ELISA has been considered as an accurate and preferable method for detection of specific antibodies against both intermediate and intermediate invasive strains of IBD (Marquardt *et al.,* 1980; Meulemans *et al.,* 1987). The ELISA titre of 396 is considered positive for antibodies against IBD virus as per the guidelines of manufacturer of ELISA kit. Seroprevalence of IBD is commonly found in many parts of world (Sule *et al.,* 2013; Wilcox *et al.,* 1983; Yuguda *et al.,* 2014). It can be found from 0 day titre that the birds were not having infection of Gumboro disease at time of collection. The ELISA titre of vaccinated birds increased to protective amount within 7 days post vaccination as reported by Moraes *et al.,* (2004); Jakka *et al.,* (2014) and Otsyina *et al.,* (2009). The level of titre

attained protective level against the disease significantly ($p<0.05$) after 28 days of vaccination in both the vaccinated group. Jakka *et al.,* (2014) and Moraes *et al.,* (2004) reported that Intermediate invasive strain produces higher level of defence against the disease as compared to Intermediate strain vaccine. Similar results were reported in present study at 28 days of collection, where both vaccines produced maximum level of protection, but statistically there was no significant difference found in the 28 days titre between intermediate and intermediate invasive strain groups. Zaheer *et al.,* 2003 reported satisfactory immune response against commercial IBD vaccine up to 42 days post vaccination. In present study with extended period of serum collection, it was observed that up to 112 days, the titre was protective and can provide protection against the disease up to 16 weeks. The vaccine should be repeated to provide much higher protection after 16 weeks.

CONCLUSION

As IBD is the oldest disease of poultry and great challenge to the poultry farming, it is necessary to protect the flocks from the disease with help of proper biosecurity measures and effective vaccination program against Gumboro disease. It can be concluded from present study that vaccination of flocks at younger age with any of the two strains can protect the chicks from the disease. This will help the farmers to boost the productivity and economy of the industry.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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