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***Corresponding author:**
Surajit Baksi;
Email: drsbaksi_vm@yahoo.com

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Efficacy of Inactivated Fowl Cholera Vaccine in Chickens

Surajit Baksi*, Nirav Rao, Mukandar Khan, Pravinsinh Chauhan, Ashish Chauhan

Hester Biosciences Limited, Ahmedabad, Gujarat, India.

Abstract
Fowl cholera is highly prevalent bacterial disease in poultry population of India. Several areas of India have been reported with outbreaks of fowl cholera. Fowl cholera severely affects the health of flocks, causes morbidity and mortality. Drop in egg production with worsened egg quality also results due to poor health of flock. The disease is also prevalent in village chicken and vaccination is the effective tool to control the prevalence of the disease. In present research, 30 specific pathogen free birds were divided into vaccinated (Group 1; 20 birds) and control (Group 2; 10 birds) groups. Birds from group 1 were vaccinated with inactivated fowl cholera vaccine and 14 days later given booster dose. After 21 days of booster dose, both groups were challenged with virulent strain of fowl cholera disease. At 7 days interval blood was collected and serology was performed for presence of antibodies against the disease with ELISA technique. The vaccine was found effective with respect to serum titre and challenge study as well. From the research, it can be concluded that effective vaccination programs can control the prevalence fowl cholera disease.
Keywords: Fowl cholera, vaccine, efficacy, ELISA technique.

INTRODUCTION

Fowl Cholera (FC) is a serious highly contagious disease, caused by bacteria *Pasteurella multocida*. It is enzootic disease and can spread easily within species. All bird species are affected with fowl cholera globally (Aravinth *et al.,* 2016). FC mainly colonizes the respiratory tract of the normal and birds which can cause stress under respiratory conditions. The clinical sign of disease are fever, anorexia, mucus discharge, ruffled feather and watery diarrhea. Chronic form of disease also seen in birds which leads to depression, dyspnea, lameness, conjunctivitis and torticollis. It causes high morbidity and mortality to the birds and causes significant economic losses to the farmers. Many outbreaks of FC are reported throughout the world (Rimler and Glisson, 1997; Shivachandra et al., 2005). FC is also reported in India with outbreaks in several states (Srinivasan *et al.,* 2011, Hassan *et al.,* 2017, Patil *et al.,* 2015, Shivachandra *et al.,* 2004, Kumar *et al.,* 2012).

The severity and incidence of FC depends of various factors like host species and age, environment, bacterial strain, etc. Generally, *Pasteurella multocida* affects the respiratory system of birds (Maheswaran *et al.,* 1973, Rimler and Glisson, 1997). The physical symptoms appear less before death and the observed symptoms are generally observed in respiratory tract, conjunctiva and surrounding area of head (Christensen and Bisgaard, 2000). The disease is diagnosed by isolation and molecular characterization of strain by polymerase chain reaction (Shivachandra *et al.,* 2005, Sellyei *et al.,* 2010, Furian *et al.,* 2013).

Control of FC depends on vaccination throughout the world. Inactivated vaccines with different strains are available in market for protection of birds against the disease. But, yet 100% protection of the flock remains the challenge. Apart from this, the farming procedure and sanitation are also important factors for prevalence of the disease. In present study, the seroprevalence of the disease was found in different states of India, where poultry population is high. FC is a big challenge in India as far as poultry industry is concerned. In present study, efficacy of inactivated fowl cholera vaccine was studied in Specific Pathogen Free (SPF) layer chickens.

MATERIALS AND METHODS

Efficacy study of inactivated Fowl Cholera vaccine

The efficacy study of inactivated Fowl Cholera vaccine was performed at poultry house at Hester Biosciences Limited, India. A total of 30 specific pathogen free (SPF) layer chicks were selected and reared at poultry house. 20 birds were allocated to Group 1, to which vaccine was inoculated. 10 birds were allocated to Group 2, which was considered as unvaccinated control. The inactivated Fowl Cholera vaccine was procured from Hester Biosciences Limited. Before vaccination, blood was withdrawn from all birds, serum was separated and titre estimation was done through ELISA technique. At the age of 4 weeks, birds from Group 1 were inoculated with 1 dose of vaccine subcutaneously. After 2 weeks of vaccination, inoculate birds from Group 1 were inoculated with booster dose of the same vaccine with single dose subcutaneously. Blood was collected from birds of both groups at the interval of 7,14,21,28,35,42 and 49 days, serum was separated and titre was estimated with ELISA kit.

ELISA procedure

All the reagents were allowed to come to 18-26 °C before testing. 100 µl of negative control was added into duplicate well and the same of positive control was added into duplicate wells. 100 µl of sample was dispensed into appropriate wells. The reagents were incubated at 25 °C for 30 minutes. All solutions were removed and each well was washed with 350 µl wash solution 3 times. After that, 100 µl conjugate was added into each well. Again the solutions were incubated at 25 °C for 30 minutes. Further, the solutions were removed from each well and washed as mentioned before. 100 µl tetramethylbenzidine (TMB) solution was added into each well and incubated at 25 °C for 15 minutes. After that, Stop solution was added into each well and absorbance was measured at 650 nm and recorded and antibody titer was calculated (Perelman *et al.,* 1990; Sander *et al.,* 1998).

Challenge study

After 21 days of booster dose vaccination to birds, challenge all the birds from both the groups with virulent strains of *Pasteurella multocida.* After challenge, observe all the birds daily for any untoward reactions and pathogenic lesions for the period of 14 days.

Data collection and analysis

Serum antibody titre of >396 was considered as positive for presence of antibodies against *Pasteurella multocida*. The results were entered into Microsoft Excel worksheet and analyzed by IBM SPSS 20 software. The data were considered significantly different, if $p<0.05$.

RESULTS AND DISCUSSION

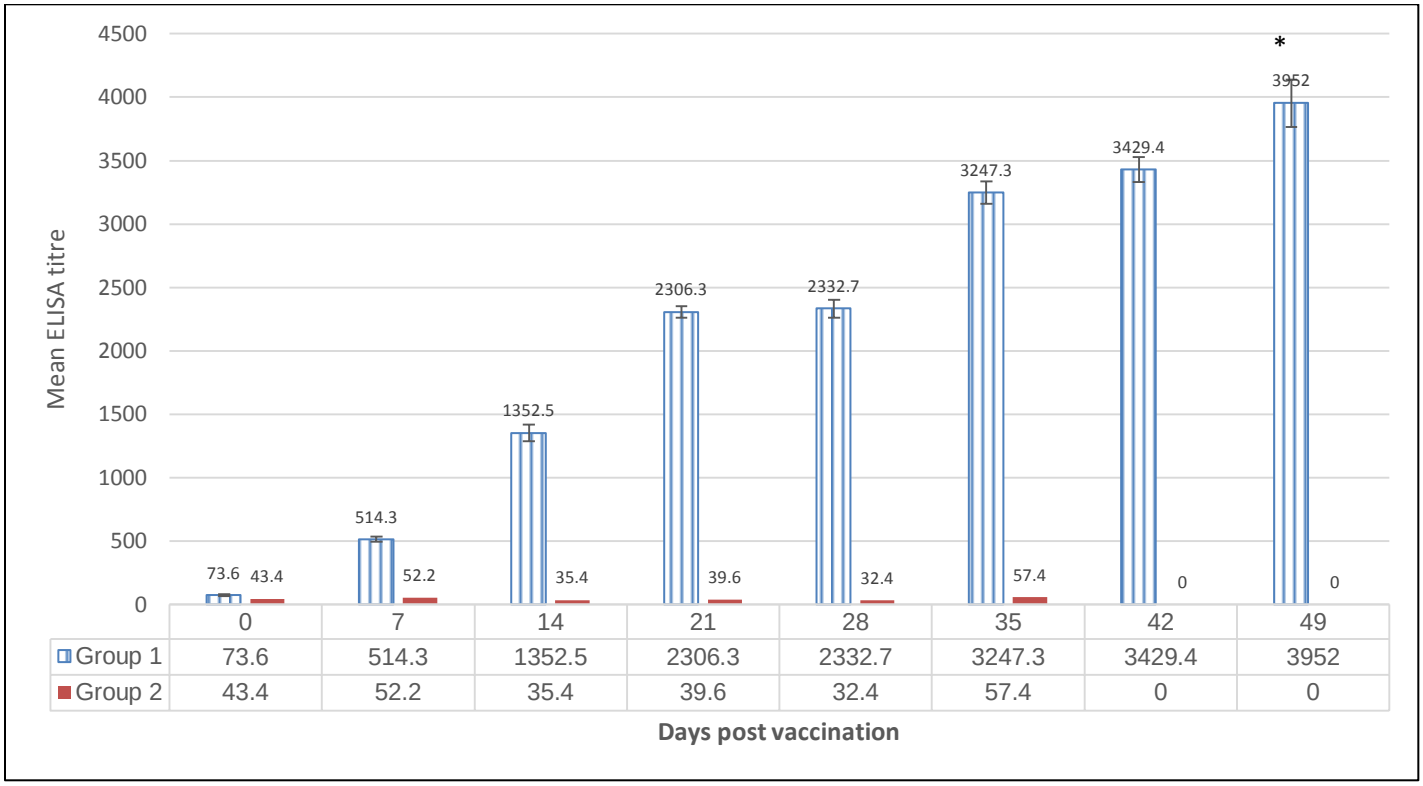
Efficacy study of inactivated Fowl Cholera vaccine

The results of antibody titres were recorded at 0,7,14,21,28,35,42 and 49 days after vaccination (Figure 1). The antibody titre of 396 was considered as positive for infection or immunization. At 0 days of collection, the samples were found negative for presence of antibodies against *P. multocida*. After vaccination, the titre increased and samples were found positive for antibodies at 7,14,21,28,35,42 and 49 days. The antibody level reached to highest level at 49 days collection. In control birds, the

titre was found consistently negative in all 10 birds for 49 days. Similar results were found with other researches, where vaccination was done and serology was performed with ELISA (Belal, 2013; Jaber *et al.,* 2015).

Challenge study results

The birds of both vaccinated and control groups were challenged with virulent strain of *P. multocida* after 21 days of booster vaccination and birds were observed for clinical signs. All the 10 control birds died of Fowl Cholera within 48 hours of challenge. 20 vaccinated birds remained protected from the disease and observed for 14 days. Jabbri and Moazeni, 2005 also performed similar study where vaccinated birds were protected from the virulent strain.



	0	7	14	21	28	35	42	49
Group 1	73.6	514.3	1352.5	2306.3	2332.7	3247.3	3429.4	3952
Group 2	43.4	52.2	35.4	39.6	32.4	57.4	0	0

Fig. 1. Antibody titres against Fowl Cholera vaccine in SPF layer birds
*** p<0.05, significantly different results of 49 days titre of vaccinated birds, compared with 0 days**

CONCLUSION

Fowl Cholera is one of the most important disease of poultry in India. The disease can cause significant economic loss if not controlled in the farms. The results of present study show that the inactivated vaccine of Fowl Cholera protected the birds from the disease after challenge and the antibody titres were also found at protective levels. The disease can be controlled with proper vaccination programs applied at the farms. Effective feeding and drinking water facilities, disinfection, sanitation procedures and controlled diet further can help in prevention of the disease and health of the flocks.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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