IN OVO-VACCINATION OF CHICKEN EMBRYOS WITH INFECTIOUS BRONCHITIS VIRUS VACCINE

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Abstract

Study was carried out to evaluate in-ovo vaccination against Infectious Bronchitis (IB) virus. Two hundred forty specific pathogen-free (SPF) fertile chicken eggs of Babcock layer were selected and divided into groups A, B, C, and D (i.e. 60 eggs were kept in each group). The embryonated eggs of group A, B and C were vaccinated with IBV M-41 strain using the manual micro-injection system at 18 days of incubation period while group D were kept as control. After hatching, hatchability percentage, antibody titre in sera of vaccinated chicks was recorded. In-ovo vaccination with different dilution affected the hatchability in embryos as compared to the chicks did not receive vaccine. The chicks of group A received vaccine at the dose of 0.1ml with virus serial dilution 10^{-6} showed 83.33% hatchability while group B received vaccine the dose of 0.1 ml with virus serial dilution 10^{-5} yielded 68.33% hatchability. The chicks of group C were vaccinated with the dose of 0.1 ml dilution 10^{-4} had 63.33% hatchability, Chicks of group D receiving no vaccine showed 96.66% hatchability. The blood of all the vaccinated birds was examined for haemagglutination inhibition test at weekly interval and clear variation in the antibodies titre was evident. The chicks received 0.1 ml dilution 10^{-6} dose of vaccine had the maximum 5.75 antibody titre followed by 10^{-5} (5.5 titre) and 10^{-4} (4.75 titre) at the the end of 5^{th} week. Antibody titre groups A, B and C was gradually decreased to 5.00, 4.62 and 3.15, respectively upto the end of 7^{th} week. No antibody titre was observed in chicks of group D.

Key words: Infectious Bronchitis Virus, hatchability, *in-ovo* vaccination, chicks, antibody titre chicken

INTRODUCTION

In Pakistan, intensive poultry farming is increasing day by day and growing with an average rate of about 10–15% every year. During last five years, it gave more profit to the farmers, however, the poultry industry is facing a variety of problems, particularly the diseases of viral origin. According to an estimate published in various papers in Pakistan, the poultry industry bears yearly loss of 30% due to various infectious diseases (Anonymous, 1994). The annual turnover in the poultry sector estimated to be 7.1 billion rupees or 426 million U.S dollars while the total investment is 65 billion rupees. There are about 300 hatcheries, 140 feed mills and 15 000 commercial poultry farms in the country (Qureshi, 2005). Infectious bronchitis is highly contagious, acute upper respiratory tract disease in chickens. The infectious bronchitis virus (IBV) contains RNA genome belongs to the genus corona virus and family coronaviridae, which consist two genera, Corona virus and Toro virus (Cavanaugh et al., 2002) The clinical signs of the disease are coughing, sneezing, tracheal rales, gasping and nasal discharge. Mortality may occur in very young birds, whereas decrease in weight gain and also feed efficiency are usually observed in older birds. A drop in egg production and egg quality observed when birds in layer flocks got infected (King et al., 1991). Avian infectious bronchitis virus (IBV) that caused tremendous economic losses to the poultry industry worldwide. Different serotypes of this virus showed little cross-protection Lin et al. (2005). Infectious bronchitis virus replicate in tissue of the respiratory and intestinal tracts, kidney, and oviduct. The kidney and other non-respiratory organs are site of persistence of IBV infectious virus being periodically shed in nasal secretion and feces (Dhinaker et al., 1997).

Chicken responds to infectious bronchitis (IBV) field infection or vaccination by producing specific antibodies and cell mediated immunity. Antibodies induced by IBV could be of IgM, IgG, and IgA; although the main antibody found in serum (humoral antibody), is of the IgG class (Cook, 1995). Both live and dead virus vaccines are used in IBV immunization. Live vaccines are initially used to vaccinate broilers, breeder and layer flocks. Mas-

sachusetts serotype vaccines are very commonly used (Cavanagh and Naqi, 2003).

MATERIALS AND METHODS

Two hundred forty specific pathogen-free (SPF) fertile eggs of Babcock Layer were obtained from K&N's, Poultry Diagnostic and Research Institute, Karachi. The eggs were cleaned and disinfected with DS 200 (Divine Pharama Karachi), a quaternary ammonium formulation and were placed in incubator. Before the incubation, incubator was fumigated and sterilized with 10% formalin solution then incubator was kept closed for 24hours After the sterilization, the eggs were kept in incubator at 37°C temperature with 80% humidity. The incubated eggs were candled on 7 days of incubation, the dead embryos and infertile eggs were discarded.

Vaccine and vaccination

Infectious bronchitis virus M-41 strain virus vaccine from Salisbury, USA was selected for *in-ovo* vaccination. The dose of 0.1ml was inoculated with 10^{-6}, 10^{-5} and 10^{-4} dilutions. The shell surface of eggs was cleaned and disinfected by iodine solution. After cleaning and disinfecting of the eggs, two holes of 1mm diameter were made in the shell of egg by dental drill machine, then inoculum was inoculated with 0.1ml of IBV (M41) vaccine by using 26 Gauge ×1/2 needle (Socorex Swiss self refilling syringe Cat No.187. 20501). However, two hundred forty-embryonated eggs were divided into 4 groups according to doses inoculated i.e. A, B, C, and D (non-vaccinated considered being control group). The vaccinated eggs were sealed by paraffin wax and then the eggs were transferred to the hatcher. The hatchability percentage of the vaccinated and non-vaccinated embryos was recorded. After the hatching, the newly hatched chicks were counted and percentage was determined.

Haemagglutination test

Two types of haemagglutination tests (i.e. haemagglutination test and spotted haemagglutination test) were conducted for qualitative promotions of the haemagglutination potential of the virus. While, micro-haemagglutination inhibition test was performed as quantitative haemagglutination test.

Spot haemagglutintion test

A drop of 5% red blood cells suspension and a drop of virus containing fluid were mixed on haemagglutination plate. The agglutination of red blood cells to virus within 45 seconds was considered as a positive. (Ammar, 2001)

Micro haemagglutination test –Procedure

A 50 µl of the saline were added to 96 wells of micro titration round bottom plate. In the first well of each series 50 ml virus suspension were added to the wells containing saline and mixed thoroughly with micropipette. A 50 ml of diluted virus suspension were transferred from the first to the second well and mixed properly with micropipette. This process was repeated till the 10^{th} well from which 50 ml of diluted virus suspension was discarded. No virus was added to the 11^{th} and 12^{th} well and kept as controls. Then 50 ml of 1% chicken erythrocyte suspension (prepared from 5% solution of chicken RBCs) were added to wells and incubated for 30 minutes at 4°C. Haemagglutination was recorded the highest dilution and considered to be complete agglutinations.

Haemagglutination inhibition test

Two methods of testing of the antibody titre were tested i.e. constant-serum diluted virus alpha procedure and other constant-virus diluted serum beta procedure. Later beta neutralization test was performed during the study.

Beta neutralization

Procedure

A 50 µl of phosphate buffered saline (PBS pH 7.2) were added to 96 wells of micro-titration plate with rounded bottom. In the first well of each series, 50 ml of serum were added and mixed thoroughly with the micropipette. A 50 ml of diluted serum was transferred from the first well to the second and mixed properly. This process was continued till the 10^{th} well from which 50 ml of diluted serum was discarded. No serum was added to the 11^{th} and 12^{th} well and kept as control wells. A 50 µl of 4 HA units of the virus was added and mixed from 1^{st} to the 11^{th} well. The 11^{th} well contained antigen was considered as virus control. No serum and antigen were added to well 12 and only 50 µl of red blood cells were added and kept as RBC control. The plate was then incubated at 4°C for 30 minutes to record the inter reaction between antigen and antibody. A 50 ml of 1% erythrocyte suspension (prepared from 5% RBCs) were added to each well of the plate and incubated for 30 minutes at 37°C. After 30 minutes, a complete haemagglutination in well No. 11 (antigen control) and no haemagglutination in well No. 12 (diluent control) were recorded. (Rabbaani et al., 2001–2002).

Preparation of vaccine inoculum from M-41 strain of IBV

IBV M-41 vaccine was modified by serial passage in eggs for 27 times to reduce its pathogencity.

Serum samples for antibody titre

After hatching of the chicks, the blood samples were collected with a week interval from 1–5 weeks. The blood was collected from jugular vein and also from wing vein. Blood was collected in disposable syringes (3 ml), which were then placed at room temperature (30°C) in slanting position for two hours and sera were collected in sterile appendrop tubes, which were properly marked with number of the chicks of the group and stored at –20°C till used. The following three doses were prepared in sterile phosphate buffered saline (PBS) and inoculated.

Phosphate buffered saline (PBS pH 7.2)

The ingredients of buffered solution	Weight (g)
Sodium chloride	8.00
Potassium chloride	0.20
Disodium hydrogen Orthophosphate	2.31
Potassium dihydrogen Orthophosphate	0.20
Distilled water	1 000 ml

Group A	60 embryonated eggs were vaccinated with dose of 10^{-6} EID_{50}/ 0.1ml
Group B	60 embryonated eggs were vaccinated with dose of 10^{-5} EID_{50}/ 0.1ml
Group C	60 embryonated eggs were vaccinated with dose of 10^{-4} EID_{50}/ 0.1ml
Group D	60 embryonated eggs were vaccinated with dose of 10^{-4} EID_{50}/ 0.1ml

RESULTS

The present study was conducted to evaluate *in-ovo* vaccination against IBV that would be practical alternative and effective substitute to post-hatch vaccination against IBV infection under field conditions. The other purpose was to determine and select the dose of IBV vaccine and its effects on hatchability and subsequent immunity in the baby chicks.

The effect of different dilutions of IBV M-41 strain vaccine on hatchability of embryos

During present investigation the effects of various dilutions of IBV M-41 strain vaccine on the hatchability of the chicks vaccinated at day 18 during embryonating period was recorded and presented in Table 1.

The results showed that the hatchability of the chicks was affected by different dilutions of vaccine. The dilutions 10^{-6}, 10^{-5} and 10^{-4} were inoculated into the embryos at day 18 of the incubation, gave 83.33%, 68.33% and 63.33% hatchability, respectively. However, a dilution 10^{-4} that yielded 63.33% hatchability, which was lower as compared to other doses significantly, affected the hatchability of the chicks. The reason could be the high antigen concentration that sometimes may cause antigenic burst in immune cells and produces serious damage in immune cells and also other cells that cause embryo death and ultimately it goes for reduced hatchability in chicks. Therefore, the dilution 10^{-6} is considered and recommended, which can induce better immunity and affects least hatchability in chicks.

The antibody titre in the sera of chicks vaccinated by *in-ovo* technique with IBV M-41 strain during embryonic age at day 18 of their incubation.

The present results show the humoral immune response of the chicken vaccinated with IBV M-41 vaccine through *in-ovo* vaccination during embryonic age at day 18 of their age. Different doses (0.1 ml of 3 different virus dilutions) of vaccine were inoculated into the embryos and their antibody levels were recorded from the week 1 to week 5 after hatching (Table 2). An increased variation in the mean antibody titre was observed at different weeks in the sera of the chicks in response to different doses of vaccine inoculated at 18 days of their embryonic age. A slight difference in the mean antibody titre was also observed among the groups of chicks in relation to the dose of vaccine administered. However, after 5 week, i.e., 6^{th} and 7^{th} week post-vaccination, a significant difference in the mean titer was observed among the

Tab. 1: The effect of different dilutions of IBV-M-41 strain vaccine on the Hatchability of chicks

Group of chicks	Dose in log serial virus dilution	Dose of vaccine inoculated (CAS)	No. of eggs set	No. of hatched chicks	Hatchability (%)
A	10^{-6} EID_{50}	0.1 ml	60	50	83.33
B	10^{-5} EID_{50}	0.1 ml	60	41	68.33
C	10^{-4} EID_{50}	0.1 ml	60	38	63.33
D (control)	no vaccine	control	60	58	96.66

Tab. 2: The mean antibody titre of IBV-M41 vaccine in the sera of chickens using a micro-injection determined by haemagglutination inhibition test up to seven weeks post-ovo vaccination

Groups of chicks	Doses in log	Dose of vaccine inoculated	Mean antibody titre – week						
			1st	2nd	3rd	4th	5th	6th	7th
A	10^{-6} EID_{50}	0.1 ml	1.37	2.50	2.87	5.00	5.75	5.25	5.00
B	10^{-5} EID_{50}	0.1 ml	1.25	2.25	2.87	4.00	5.50	5.00	4.62
C	10^{-4} EID_{50}	0.1 ml	1.50	2.00	2.50	3.75	4.75	4.00	3.15
D	no vaccine was offered	–	0	0	0	0	0	–	–

weeks and groups of the chicks in their sera. After that, the mean titre of antibody started declining and reached at such level where it was expected to be not capable to protect the birds from the disease therefore revaccination at this stage should be carried-out to continue protection against the disease.

It was further observed from the study that higher dilution of vaccine that produced lesser antibody titre whereas a lower dilution (considered normal dose of vaccine) produced somewhat higher antibody titre but started declining at weeks six and seven suggesting revaccination. From this study, it is also concluded that with the increase in age of chicks, the HI titre in the sera also increased up to 5 weeks. From the investigation, it was further observed that reasonable protective immunity could be obtained by in-ovo vaccination with IBV M-41 vaccine at 18 days of embryonic age.

DISCUSSION

The results regarding the effects of different doses of IBV-M41 strain vaccine on the hatchability percentage of chicks is presented in Table 1. It is concluded from the present study that the higher dose of vaccine has an adverse effect on the hatchability of the embryos indicating that the dose of vaccine can cause embryonic mortality and needs to be investigated thoroughly.

Wakenell et al. (1995) conducted similar kind of study to investigate the effect of *in-ovo* vaccination on the hatchability of chicks. Gagic et al. (1999) also conducted similar kind of investigation; both recorded clear effect of *in-ovo* vaccination on the hatchability of the chicks and noted that a lower dose has lesser effect on the hatchability. Whereas, Stone et al. (1997) in their study indicated that the needle gauge for inoculation and the inoculum volume also affected the hatchability and oil emulsion vaccine exhibited a lower hatchability as compared to other oil vaccine. Chew et al. (1997) investigated that infectious bronchitis virus (IBV), the hatchability of the eggs inoculated with V-IBV at day 18 was significantly reduced (27%) than the eggs, which were not inoculated with IBV or were inoculated with P-IBV (45–58%, $P < 0.01$). The chicks of all treated groups were survived at day 5 of post-hatch.

Therefore, the results of the present study are in agreement to the results of the above authors who observed the effects of *in-ovo* vaccination on the hatchability of chicks, particularly when a higher dose was given to embryos at day 18 of their incubation. The phenomenon is not clear why such mortality of chicks takes place, however, the reason might be due to some problem in proper sealing of hole and also penetrating of the needle deeply which may cause injury to the growing embryo or might be bacterial contamination takes place during *in-ovo* vaccination process, therefore all these required proper examination to come that point to handle the embryos accordingly to reduce the losses. However, Lee et al. (2000) inoculated chicken embryos with eight different strains of infectious bronchitis virus (IBV) representing seven different serotypes at 17 days of embryonation. They investigated that strict epithelio-tropic nature and wide tissue tropism of strains of IBV in the chicken embryos and the universality of ribo-probe. In situ hybridization with this probe will be useful for understanding the tissue tropism and the pathogenesis of IBV *in-vivo*.

The data regarding the antibody titre in the sera of chicks vaccinated by *in-ovo* technique with IBV M-41 during embryonic stage at day 18 of the incubation is presented in Table 2. Gagic et al. (1999) who conducted and observed in their experimental work that *in-ovo* technology could be used to protect chicken against multiple diseases. They recorded both responses through *in-ovo* that developed humoral and cellular immune responses. While Borzemska and Szeleszuzuk (1999) and Sharma (1999) who were the pioneer of postulating the basis of development of technique of *in-ovo* for immunization in birds at the final period of embryogenesis. They agreed that immunization by *in-ovo* vaccination of embryos that enabled the chicks from any disease and also vaccines to be administered before the transfer of maternal antibodies from the yolk sac to the chicken blood stream. Furthermore that Wakenell et al. (2002) who adopted and received the patent for the new *in-ovo* vaccination

technique in USA against Mareks disease, which is now common but vaccination against NDV, ILT and Gumboro disease are in progress and better results are achieved yet. However, the present study is just conformation of the above studies of *in-ovo* vaccination in embryonic stage of various strains of virus conducted by different workers. In this survey, better results regarding the presence of antibody in the sera and its efficacy of protection against the disease are achievements of the *in-ovo* vaccination technique in early age of the chicks. Moreover, Wakenel et al. (1996) were of the same opinion that embryonic vaccination may be an alternative method of vaccination to protect chicken against infectious bronchitis and other diseases which should be brought in practice to protect the chicks immediate after hatch which are at risk.

From the present study, it is concluded that *in-ovo* vaccination at 18 days of embryonated eggs with IB M-41 vaccine is applicable and has commercial potential. However, hatchability was adversely affected by *in-ovo* vaccination, which could be improved with the use automatic injectovac system capable of handling 60 000 eggs at a time.

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