Research Article

Effect of Age and Route of Administration on the Efficacy of Live Infectious Bursal Disease Vaccines in Broiler

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Abstract | The present study was conducted to investigate the effect of age and route of administration on the efficacy of live IBDV vaccines on broilers. A total of 120 broilers were randomly divided into six groups (20 birds in each) were coded A, B, C, D, E and F; vaccinated with various combinations of IBD through different routes. Results showed that the birds of different groups received different maternal antibodies did not induce any antibodies as recorded in all groups received vaccines at days 1, 9 or 12 exhibited similar pattern antibody profiles, however, an increase in the mean titer of antibodies was recorded from 17 and onwards. Furthermore, on day 39 of age, the birds of all groups including control were challenged with field strain of IBD virus collected from bursa of clinically infected chicks, No morbidity and mortality were observed. During present study, some milder infection with field strain of IBDV almost a week ago recorded were also found in the control group when challenged with IBDV, IBD vaccines in the presence of specific maternally antibodies of IBD could no induce antibodies titer 1500 with live vaccines were protective against the IBDV. Based on the findings of present study, it can be concluded that all vaccinated group displayed similar antibody response irrespective of the types of vaccines administration to the chicks.

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Introduction

Infectious bursal disease (IBD) or Gumboro disease, an immunosuppressive disease of chickens characterized by destruction of lymphocytes in the bursa of fabricius (BF) and to a lesser extent in other lymphoid organs; the disease is a major problem in concentration poultry production areas throughout the world leads to heavy economical losses to poultry industry. Once the infection is established, the virus persists in the poultry house and continues to infect subsequent flocks (Lukert and Saif, 1991; Azhar Abd El-Aziz, 2000; Lukert and Saif, 2003; Mahmood et al., 2006; Uddin et al., 2010). Infectious bursal disease is caused by a virus of the Birnaviridae family have the potential of immunizing the chicks even in the presence of moderately higher levels of maternally derived antibodies (Wyeth, 2000; Delmas et al., 2011).

Table 1: *Type of IBDV vaccine and rout of administration of vaccines in chickens.*

Age (days)	Groups					
	A	B	C	D	E	F (Control)
1	Bur-706 by spray	Bursine-II by S/C	Vaccine prediction day (VPD)	No vaccine offered	Bursplex by S/C	No vaccine offered
12	Bursine plus in drink water	Bursine plus in drink water	First shot at 10th day	Bursine plus in drink water	Bursine plus in drink water	No vaccine offered.
22	Bursine plus in drink water	Bursine plus in drink water	Second shot at 10th day	Bursine plus in drink water	Bursine plus in drink water	No vaccine offered

Infectious bursal disease spreads horizontally with direct contact of healthy birds to infected ones through contaminated fomites, insects and farm attendants. Infected birds shed virus in their dropping up to two weeks, the incubation period of IBDV ranges from 2-3 days, the clinical signs including sleepiness reduced feed intake, white watery diarrhea, ruffled feathers, depression and reluctance to move, infected bird may become prostrate and dehydrated (Lukert and saif, 1991; Chansiripornchai and Sasipreeyajan, 2009; Sharma et al., 2000). The infection caused by the variant strain of serotype 1 reveals non clinical disease (Elankumaran et al., 2002). Postmortem findings include enlarged, swollen and hemorrhagic cloacal bursa in bird, skeletal muscle darken with hemorrhages (especially thigh and pectoral muscles) while the thymus is opaque thickened gelatinous capsule. The liver and kidney may be swollen and there is also increased mucus in the intestines (Butcher et al., 2003). The disease was first recognized in Karachi, Pakistan during 1971. Since then, it covered whole country. Mortality due to various Gumboro disease outbreaks alone has varied from 25 to 100 percent in young broiler chicks, the prevalence of IBD during 1994 to 1997 in Karachi and its surroundings (based on reported cases) ranged from 10-13 percent (Qureshi, 1999).

Important methods of IBD prevention and control in the poultry industry are disinfection, biosecurity and vaccination at the appropriate time (Chansiripornchai and Sasipreeyajan, 2009).

There are more than a dozen of vaccines and vaccination programs are practices to control the disease but none of them are found entirely effective. Different strains of IBDV vaccines are available based on their virulence and antigenic diversity. The strains with low virulence index are called as mild or intermediate forms, but those with high virulence are designated as intermediate plus or hot forms, in different circumstances, where intermediate vaccines fail to protect the chicks; the need of more virulent strain vaccines is of great importance to control a virulent strain of the disease (Rautenschlein et al., 2003).

Some researchers emphasize that the immunity and virus load in the field often change the first IBDV might interfere in antibody production resulting in low immunity. While others are of the opinion, that before the vaccination, a complete investigation regarding level of maternal antibodies should be properly investigated, different routes of vaccination are being practiced through the spray at the hatchery in day old birds, subcutaneous, or eye drop methods (Sharma et al., 2000; Rautenschlein et al., 2003; Delmas et al., 2011).

Keeping in view the present study was therefore designed to study the efficacy of IBDV live vaccines through different routes in one-day old chicks; with the aims to know the efficacy of various combinations of intermediate and intermediate plus vaccines in chick against IBDV.

Materials and Methods

The present study involving 120 day-old broiler chicks was conducted as a part of M.Sc. (Hons.) research work by the major author to study the effect of age and route of administration on the efficacy of live IBDV vaccines on broilers, at Poultry Farm, Sindh Agriculture University, Tandojam, Pakistan. The day-old broilers chicks were obtained from a well reputed local commercial hatchery and randomly divided into six groups coded as A, B, C, D, E and F; consists 20 birds in each group. All the groups were inoculated with different Gumboro vaccines according to the following schedule (Table 1).

Bur-706 and Bursine–II, (intermediate strains), bursine plus (a hot strain), Bursaplex (antibody= IBDV). VPD= vaccine prediction Day calculated from a formula de-

signed by (Kouwenhoven, 1991) VPD = square root of mean ELISA titer- square root of target titer (2.82) at the termination of the experiment, the blood samples from ten birds of each groups were collected and separated through centrifugation. Before administration of vaccines, the birds of different groups except control were tested by ELISA for maternal antibodies and measured as 583+632 at day one of age. Centrifuged at 1500 rpm, the sera were stored at -20°C and analyzed for IBDV antibodies by ELISA (Table 2).

All the birds were challenge with the virulent field IBD virus at six week of age. The birds were observed for signs and symptoms of the disease after the exposure and morbidity and mortality were recorded, five birds from each group were sacrificed at day five, post challenge and organ body weight ratio for the bursa, spleen and thymus were recorded.

Blood sample collection

One-milliliter blood samples were collected from the wing vein of each bird on different days using 1 ml sterile disposable plastic syringe (Table 2). The blood was the gently poured in 10 ml sterile plastic tubes and allowed to clot in slant position for 30 minutes at room temperature. After 30 minutes the clots were detached from the wall of the tubes with the help of Pasteur's pipette glass and tubes containing clots were incubated at 37°C for two hours. The tubes were then covered with aluminum foil to avoid evaporation of the sera, the sera present in tubes were gently harvested with the help of pasture's pipette and kept in 1.5 tubes and stored at -20°C till used.

Enzyme linked Immunosorbent Assay (ELISA)

An ELISA kit from Bio-check, Holland, was purchased and used to determine IBDV antibody level in the sera of chickens.

Serum samples prepared and used for ELISA

Each serum was diluted in 1:500 through 50th serum 200^{ul} of diluents in a micro titer plate, A 20^{ul} volume of dilution was transferred from initial dilution to another adjacent well containing 180^{ul} dilution. Once again, this was repeated by taking 20^{ul} from the second well to another well containing 180^{ul} of dilution and soon.

Statistical analysis

The data thus collected were statistically analyzed using SPSS. The comparison of antibody titer means were made by using Duncan's Multiple Range (DMR) test (Duncan, 1955).

Table 2: *Blood Sample collected from chicks at different periods and analyzed.*

Blood samples collected (#)	Age of chicks at collection (at day)	Purpose of collection and method used
1	1	To determine the maternal antibody level and to predict vaccination days by ELISA
2	4	ELISA
3	10	ELISA
4	17	ELISA
5	24	ELISA
6	31	ELISA
7	38	ELISA
8	44	ELISA

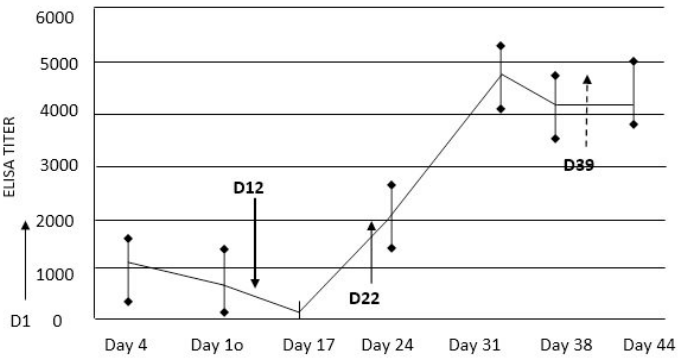

Figure 1: *The mean antibody titer of bursine 706 vaccine in the sera of the chicks of group A determined by ELISA at various ages during experimental period*
Black arrows show the chicks received their vaccine at different days of their age Dashed arrow indicates broilers challenged with IBDV

Results and Discussion

Group-A (1st vaccine of Bursin-706 at day-1 of age through spray; followed by two doses of Bursine plus hot strain at day-12 and 22 in drinking water

The chicks of group A received its first vaccine of Bur 706 at day 1of their age through spray in the presence of maternal antibody measured by ELISA at day 10 as 1583±632. Further decline in the mean antibody titer was recorded (752±411) at day 12 of age (Figure 1). From day 17 and onwards, a sharp increase in mean antibody titer was determined in the sera of the chicks for next two weeks with the mean antibody titer of 4641±791 at day 31 of the chicks for next two weeks with the mean antibody titer in the chicks was their age. However, a slight decrease in the mean antibody titer in the chicks was observed up to 31 and onwards. After that period when these birds were challenged with live virulent field virus at 38- day of their age, no clear difference in the mean

antibody titer was recorded during experimental period up to 44 days. Furthermore all chicks were found normal and no mortality and morbidity were seen.

Group-B (Bursine II at day-1, 12 and 22 of age through sub-cut vaccine at day-9, followed by another dose of the same vaccine at day-19 of age)

The chicks of group B received their first dose of Bursine II vaccine through sub-cutaneous inoculation at day one of their age. The profiles of immune response were observed very similar to that of group A. Briefly, the day old chicks showed the mean maternal antibody titer in their sera as 1583±632, which slowly decreased 1110.8±168 at day four of age and further decline, was recorded as 313.8±146 at day 10. However when those birds were offered booster dose of IBD vaccine (Bursine Plus) at day 12, showed a very low level antibodies (zero) in their sera at day17. The serum samples from vaccinated chicks were collected and tested by ELISA after tow (at day 24 and 31) of inoculation, a sharp rise in the mean antibody titer from zero to 3766.4±920 was determined. After reaching this peak level, a slight decline in the mean antibody titer in the sera of the chicks was noted in the following weeks. At day 38, further decline in the mean antibody titer in the sera of the chicks was recorded and it reached to 2994.1±331 and then persisted for some period although the chicks were challenged with the dose of viral suspension at day 44 of the experimental period (Figure 2).

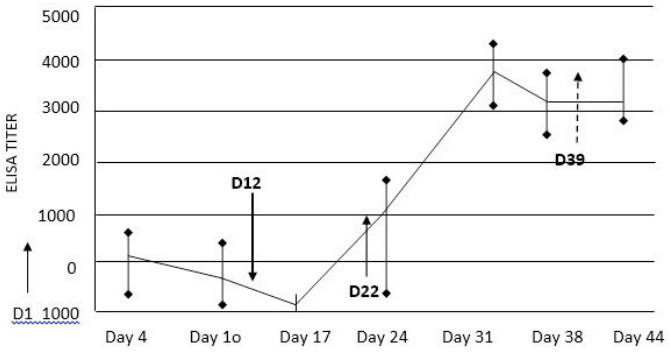

Figure 2: *The mean antibody titer of Bursine 11 (amintermediate strain) vaccine in the sera of the chicks of group administered at different ages through sub cut, determined by ELISA*
Black arrow show the broilers received vaccine at different age. Dashed arrow indicates broilers challenged with IBDV

Group-C (Vaccine prediction day (VPD), first shot at 10th day and second shot at 10th day)

The chicks of group C receive their first dose of IBD vaccine at day 9 of age. The time of vaccination was calculated by using vaccination prediction Day formula in day old chicks through ELISA technique. The mean maternal antibody titer in the sera of bird, record as 1583±632 that declined to 263±156 by day 10 after receiving firs dose of vaccine at day 9, the birds showed further decrease in antibody titer that reached to zero at day 17. Then a steady increase in mean antibody titer was observed with peak level of 382±820 at day 31 of age. A week later, again a sharp decline in the titer was recorded up to day 38 of their age, and antibody mean titer determined as 2460±567. However, the birds received two more doses of vaccines (booster dose), one at day 19 and another at day 28 of age. Whereas; these birds were challenged with the virulent virus obtained from field virus of infected bursa of chicks, at 39th day of their age, no significant (P > 0.05) decrease on the mean titer was observed after birds were challenged with IBDV (Figure 3) furthermore, no mortality and morbidity were recorded during the study period.

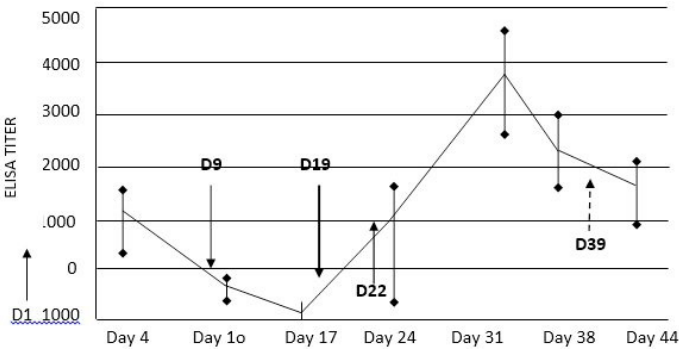

Figure 3: *The means antibody titer of Bursine plus (hot strain) vaccine in the sera of the chicks of group C administered at different ages in drinking water, determined by ELISA*
Black arrow show the broilers received vaccine at different days of their age. Dashed arrow indicates broilers challenged with IBDV

Group D (1st vaccine of Bursine plus at day-12 and another dose at day-22 in drinking water)

A similar trend of immune response was noted as recoded for previous groups. The birds of group D were administered their first dose of IBD vaccine according to the schedule as being practiced for commercial broiler in and around Karachi, the birds obtained their first dose of IBD with Bursine plus vaccine at day 12 of their age, followed by a second dose (booster dose) at day 22. When sera of the birds were assessed by ELISA, similar trend in the antibody formation and response were recorded as found in the sera of the groups studied earlier, during the investigation, it was demonstrated that the birds with maternal antibodies against IBD virus showed some slow response in antibody formation (1560±236) in their sera at day 4 of their age and at day 10 (644±247) of their age. From day 17 and onward, a sharp increase in antibody level was recorded and this continued to day 31 with the mean antibody titer of 3600±995. However, a slight drop in the mean antibody titer was seen after day 31.

Group E (Bursplix by subcutaneous inoculation at day-1, and two more doses of Bursine plus at day-12 and 22 of age in drinking water)

Busaplex vaccine was offered to chickens at the age of day one in the presence of maternal antibodies showed very good response in antibody production without any deteriorating effect on bursa; furthermore, when Bursaplex was inoculated to the chickens at day 1 of their age through subcutaneous in the presence of maternal antibodies responded similarly to those of other groups for different vaccines, during study, a slight decrease in the mean antibody titer in the sera of chick was recorded at day 17 of age their and reached to the level of zero but a steep increase in the mean titer was observed up to day 31 alike to the chickens of other groups and reached to the maximum mean titer of 3671±999. However, a slow decrease in the mean antibody titer was observed when these birds were challenged with virus. This is not meant that the antibodies were in low level that could not provide protection the birds against IBDV. A significant ($P<0.05$) protection was also seen during investigation against infection.

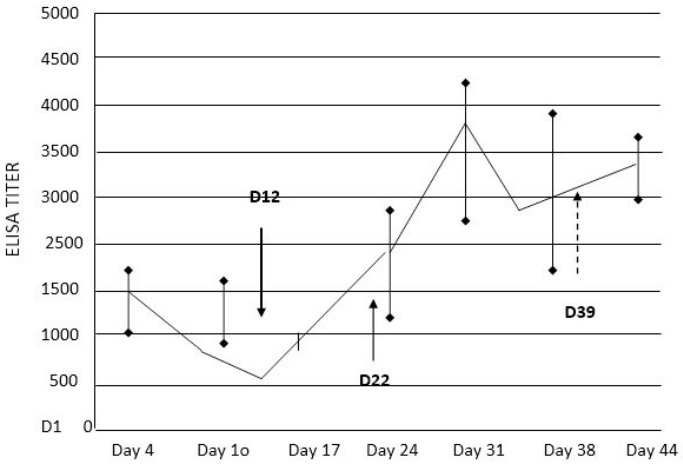

Figure 4: *The mean antibody titer of Bursing plus (Hot strainnn) vaccine in the sera of the chicks of group D administered at different ages by drinking water, determined by ELISA technique*
Black arrow indicate vaccination age of broilers dashed arrow indicates broilers challenged with IBDV

Group F (Control)

The birds of group F were kept as a control group without administration of any vaccines. Clearly no maternal antibody titer was recorded in the sera of the chicks against IBD virus. However, this likely seems to be the birds were exposure to the virus sometime ago, therefore a very little immune response was detected in the chicks, briefly, and the group exhibited some decline in the maternal antibody up to day 17 of their age and showed to zero level antibodies in their serum samples as were noted in the vaccinated counterparts. Since the Bursine Plus vaccine was administered to the birds of other groups at day 12 and 22 of their age whereas; no significant difference ($P > 0.05$) in the mean antibody titer was recorded among the vaccinated and non vaccinated birds of various groups. This group (F) also showed similar trend in slight increase antibody titer (723±352) from days 17 to 31 of age. It is concluded that all the birds seemed to be exposed to a field virus during 4th and 5th weeks of their age; this exposure was evident from a sharp decline in titers among vaccinated birds during these weeks (Figure 1, 2, 3, 4 and 5), but a sharp increase in titer of antibody in the sera of control group (Figure 6). This increase in antibody titers continued in group F even after challenged with the virulent field virus recovered from bursal homogenate.

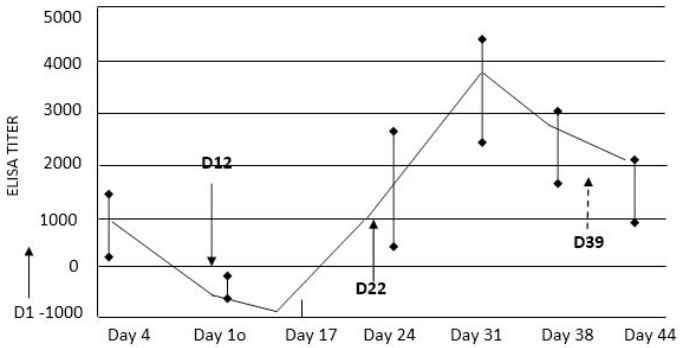

Figure 5: *The mean antibody titer of Bursaplex (IBDV+ IBDV antibody) vaccine in the sera of the chicks of group E administered at different ages in drinking water, determined by ELISA technique*
Black arrow show the broilers received vaccine at different age. Dashed arrow indicates broilers challenged with IBDV

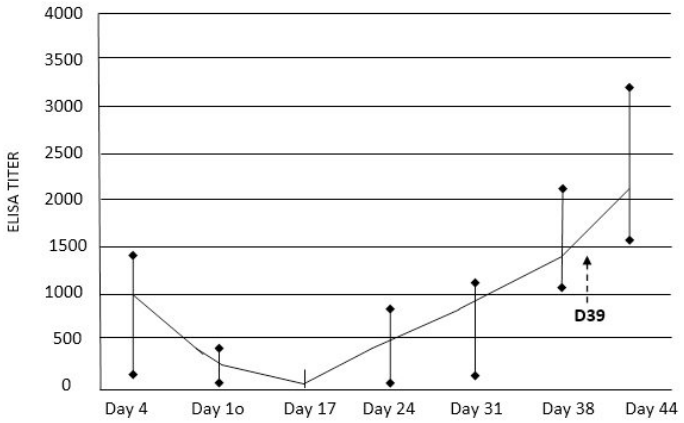

Figure 6: *The mean antibody titer in sera of the chicks of group F without any vaccination, determined by ELISA*
Dashed arrow shows broilers challenged with IBDV

During present study four different IBD vaccines I, E Bursine 706, Bursine II, Bursaplex and Bursine plus were used and their efficacy in terms of protection in the presence and absence of maternal immunity and also effect of route of administration through spraying, subcutaneous and oral, on the efficacy of the vaccines in broiler

chickens against IBDV were studied. The results of this study showed that statistically no significant (P > 0.05) difference in term of protection against the challenged IBD virus demonstrated in this study. The chicks with maternal antibodies caused some deterioration or elimination effect on antibody formation. This may be due to antigen and antibody complex formation as compare with the groups C, D and F chicks where such kind of effect was not occurred. Generally, antibody production was recorded in firs two weeks of age in all groups of the birds. No significant (P > 0.05) difference in the level of antibody titer was observed in any blood samples of birds of different groups collected at day 4 and 17 of their age, either the birds were administered through injection at day 1 or later. A similar kind of response was observed as reported by many workers that in the presence of maternal antibodies there is a problem of vaccine intake by immune system could be due to interference could be due to interference of maternal antibodies with antigen in the form of antigen-antibody complex (Tsukamoto et al., 1995; Babiker and Tawfeeg, 2008; Rojs et al., 2011; Lone et al., 2012; Angani et al., 2014).

Before using of vaccines particularly IBDV vaccines, one should know the efficacy of each live vaccine and its possible interference with maternal IBDV antibody. Secondly, one should keep in mind to determine maternal antibodies in the sera of the chicks' up to such level that antibodies should not be able to cause any problem against vaccine that the vaccines can work. Without any knowledge about live vaccines, it would be difficult to control highly virulent IBDV (Lukert and Saif, 1991; Qureshi, 1999; Butcher et al., 2003; Hsieh et al., 2010; Rojs et al., 2011). In the study, antibody titer of this group was not much differ from the other groups of the present study; those had received intermediate vaccines at day one of their age. They also found for the use of IBD vaccines in the chicks during the first week of their age in the presence of maternal immunity. Those could cause interference in the production of antibodies. It was also noted that maternal antibodies up to 500 levels could allow good vaccine intake in chicks. But on the contrary, the increase in antibody titer became an evident only after day 17 of their age. The reason for this delay in the antibody production is not clear.

The birds of group D were given their vaccine at day 12 of their age. This is a routine practice among commercial poultry producers. Considering the degree of maternal immunity is being transferred from the yolk of vaccinated dams to the progeny, one could easily understand that at day 12 of age. Maternal antibodies should decline to very low level that it could not interfere in the intake of the vaccine. Surprisingly, no any difference in the behavior of humoral immune response was observed in the group D chicks as found in the rest of the chicks or otter groups received vaccine at day 1 of age. The results of this study are in concurrence with those of Otsyina et al. (2009), El-Mahdy et al. (2013) and Xuemei et al. (2010).

Bursplex is a combination of an intermediate strain of IBD virus and IBD specific antibody the property of the vaccine was the presence of antibody molecules that result in the formation of antigen-antibody complex that allow slow release is an encouraging mechanism particular in the presence of maternal antibodies that cause interference in antibody production the fact is that antigen from antigen-antibody complex is not released until the maternal immunity may decline to very low. Many workers had tried and got good results in birds and even embryos through ovo-vaccination (Sah et al., 1995; Jeurissen and Janse, 1998; Jackwood et al., 1999; Knoblich et al., 2000; Van Den Berg, 2000; Abdel-Alim and Saif, 2001; Corley et al., 2001; Chansiripornchai and Sasipreeyajan, 2009; Uddin et al., 2010). The birds of group E were given Bursaplex on day one of age after 12 and 22 days interval, two more doses were administered to the birds. The slow decline in antibody level was observed in group E chick as compared to other groups in which very quite change titer was recorded.

Conclusions

Based on the findings of present study, it can be concluded that all vaccinated group displayed similar antibody response irrespective of the types of vaccines administration to the chicks. It may also be concluded from the study that the titer 1500 of live IBD vaccines are protective against IBDV infection. It is further observed that two doses one at day from 10-12 of their age either with an intermediate or hot strain and other at day from 22-24 of their age with a hot strain induced protective antibodies against IBDV.

Authors' Contribution

Erum Bughio was the principal author who planned and conducted this research for fulfilling her M.Sc. (Hons.) degree. Dr. Ahmed Sultan Jatoi is corresponding author and assisted in preparation of the draft of this manuscript and also facilitated in the statistical analysis of the data. Whereas; Muzamil

Memon and Reema Bughio helped in data collection and samples were collected by Zafar Ali Khoso and Ali Asghar Baloch.

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