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Detection of Serum Antibody Levels Against Infectious Bursal Disease (IBD) Virus Using Indirect Hemagglutination (IHA) Test in Commercial Broilers

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Abstract: A total of 336 Serum samples were collected from different commercial broiler farms and slaughter shops from in and around Faisalabad. These samples were divided in to three groups according to the age of the birds i.e. 0-3 weeks (86 samples), 3-6 weeks (68 samples) and 6-8 weeks (182 samples). Indirect hemagglutination (IHA) test was used to titrate the serum antibodies against infectious bursal disease virus. Hyper immune serum was raised in laboratory rabbits against IBDV vaccine (D-78). It was used to identify and confirm the IBD virus from bursal tissues isolated from the IBD affected flocks using the qualitative tests i.e. agar gel precipitation and modified counter immuno-electrophoresis. The viral antigen was sonified and used to sensitize the Human "O" erythrocytes without any coupling agent. 1% sensitized erythrocytes suspension was prepared and used to determine the antibody titer. Results indicated that out of 336 test serum samples 221 (65.77%) were positive. The maximum positive samples belonged to 6-8 weeks of age (78.57%) followed by 3-6 weeks (60.29%) and 0-3 weeks (43.02%), respectively. Calculated geometric mean titers for groups 0-3, 3-6 and 6-8 wks of age, were found to be 6.37, 10.84 and 16.14, respectively.

Key words: Indirect hemagglutination test, serum antibodies, infectious bursal disease virus

Introduction

Poultry sector is one of the fundamental and the second biggest industry in Pakistan. Overall mortality rate is 20-30% (Anjum *et al.*, 1993) and most important cause of death is infectious diseases which result in to 60-70% loses (Lukert and Saif, 1997). Infectious bursal disease is an acute; lymphocidal economically important disease of young chickens, which is caused by a dsRNA virus belonging to *Birnaviridae* and is unusually resistant to inactivation by both heat and many disinfectants, which accounts for its persistence in poultry houses (Lukert and Saif, 1997). The disease is of major concern due to its high morbidity and mortality rate during acute phase of infection or immunosuppression during a chronic course. Immunosuppression renders the birds more susceptible to other infections and also interferes with vaccination against other diseases (Nakamura *et al.*, 1992). Morbidity is exhibited through increased processing plant condemnations, higher feed conversions and depressed average daily weight gain (Saif, 1991). Day old progeny chicks are being sold by many hatcheries with unknown status of parental immunity against various prevalent infections particularly against IBD. Vaccination schedules followed at almost all farms thus becomes ineffective and therefore, commercial broilers remain vulnerable to the natural IBD infection due to the neutralization of live virus vaccine by maternal antibodies (Wood *et al.*, 1981).

A number of serodiagnostic tests are available to diagnose the clinical cases including indirect hemagglutination (Aliev *et al.*, 1990), agar gel

precipitation (Castello *et al.*, 1987), enzyme linked immuno-sorbent assay (Cao *et al.*, 1995; Nicholas *et al.*, 1985), counter immuno-electrophoresis (Hussain *et al.*, 2002) and single radial hemolysis (Hussain *et al.*, 2003) tests. IHA test is considered to be inexpensive, quick and easy to perform (Rahman *et al.*, 1994). The present study was conducted to determine the antibody levels against IBD virus using IHA test. The main objective of the work was to establish the prevalence of antibodies against IBD in different age groups of broiler birds.

Materials and Methods

Isolation of the Virus: Infected bursae were collected from IBDV affected birds, weighed and chopped; PBS (pH 7.2) was added to prepare 10% (w/v) suspension and mixed thoroughly by using homogenizer. Homogenized bursae were subjected to ultrasonification in a jacketed vessel using rapids 600 at an intensity of 75 watts/cm² with titanium probe (15cm dia) for 5 minutes to disintegrate the virus particles. Temperature was kept under 20 °C by placing on the ice blocks (Rahman *et al.*, 1994). The sonicated antigen was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected.

Identification of the Virus

Raising of Hyper-immune serum: The hyper-immune serum was raised in rabbits against commercial IBDV vaccine (D-78) (Barnes *et al.*, 1982).

AGPT and MCCIE Tests: IBDV (field isolate) was confirmed using the modified counter immuno

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electrophoresis (MCCIE) (Hussain *et al.*, 2002) and agar gel precipitation (AGPT) (Castello *et al.*, 1987) tests using the above raised hyper immune serum.

Collection of Serum samples: A total of 336 serum samples were collected from different commercial broiler farms and slaughter shops in and around Faisalabad. All the serum samples were heat inactivated at 56 °C for 25-30 minutes in a water bath (Rahman *et al.*, 1994) and then processed for AGPT, MCCIE and IHA. Out of these 336 samples 86 were from 0-3 weeks, 68 from 3-6 weeks and 182 from 6-8 weeks old broilers.

Indirect Hemagglutination (IHA) test

Washing and Coating of Erythrocytes: 5.0 ml of Human blood group "O" was collected aseptically in a disposable syringe and transferred to a glass tube already containing 1 ml of Sodium citrate (4% Solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 minutes. The plasma and buffy coat was pipetted off. After washing thrice with phosphate buffered saline (PBS), packed RBCs were used for sensitization with antigen. For sensitization, 2 ml antigen and 1 ml of packed washed RBCs in 2 ml of PBS were mixed and placed in the incubator, at 37 °C for 45 minutes (Rahman *et al.*, 1994). The treated erythrocytes were washed thrice with PBS to remove excessive antigen. Finally, one percent suspension of sensitized RBCs were used in indirect hemagglutination test.

Test Procedure: The test was performed according to the method of Aliev *et al.* (1990) and Rahman *et al.* (1994). Briefly, after making two fold serial dilution of the test serum, equal quantity of sensitized human "O" RBCs (1%) were added to each well. The plates were gently tapped to ensure even dispersion of erythrocytes and then kept at 37 °C for 30 minutes of incubation in comparison with the negative control which contain physiological saline and sensitized RBCs. Degree of agglutination in each row was recorded. The serum samples caused a distinct erythrocytic agglutination resulting in characteristic reticulum settling of RBCs throughout the bottom of the well were considered positive. The samples showing peculiar central button-shaped settling of RBCs were recorded as negative. The IHA titer of each sample was narrated as the reciprocal of its end point dilution.

Results and Discussion

Serum antibodies against infectious bursal disease virus can be detected using a number of sero diagnostic tests both qualitatively and quantitatively (Cullen and Wyet, 1975). A total of 336 serum samples were collected from different commercial broiler farms and shops, which were subjected to indirect

hemagglutination test. Out of these, 221 were found positive, with the overall positive percentage of 65.77% (Table 1).

In broiler birds ranging from 0-3 weeks of age, out of 86 serum samples 31 were found positive. The positive percentage of this group was 43.02% (Table 1). IHA antibody titer varied from 1:2 to 1:16 with a geometric mean titer of 6.37 as described in Table 2. The results showed that the serum antibody levels were too low to protect the birds from the infectious bursal disease infection. The reason behind this low level of antibodies might be due to low levels of maternally derived antibodies (MDAs) which are transmitted from the dam to the chicks through yolk and protect the chicks from the harmful effects of IBDV in early ages (Lukert and Saif, 1997). Generally a titer of $\log_2 6$ (1:64) is considered to be protective and gave specific immunity (Lukert and Saif, 1997) but in the present study the overall titer of birds of 0-3 weeks of age, were found in between 1:4 and 1:16 which rendered the birds to sub clinical form of IBDV infection which leads to immunosuppression.

Out of total 68 serum samples, collected from 3-6 weeks old birds, 41 were found positive with a positive percentage of 60.29% (Table 1). IHA antibody titer varied from 1:4 to 1:32 with a geometric mean titer of 10.84 as shown in Table 2. The results of this group indicated higher antibody levels than the previous group which might be due to the presence of infectious agent in the environment or the result of vaccination. Antibody levels of this group were still lower from the expected one. Approximately 10-12 days are required after vaccination to develop minimal protective titer during this "lag time" chickens are susceptible to the infection. (Lukert and Saif, 1997). As severity of the infection is directly related to the susceptible cells present in the bursa therefore, this group is more prone to clinical infection as bursa of Fabricius is at its maximum development (Saif, 1991). In broiler birds ranging in age from 6-8 week, out of 183 serum samples, 143 were found positive, with a positive percentage of 78.57% (Table 1). IHA antibody titer varied from 1:4 to 1:128. Geometric mean titer of this group was 16.14 (Table 2). Birds of this group were having higher antibody levels than the previous two groups and showed relatively decreased susceptibility to clinical infection (Saif, 1991).

Among the possible reasons for this low level of antibodies in commercial broilers i.e. specific immunity in vaccinated birds, these may be related to the vaccines and vaccination (Sil *et al.*, 2002). The factors of Vaccination failure may be classified as:

- a) Vaccine (type, storage, transportation and handling)
- b) Condition of the bird
- c) Administration of the vaccine

It has been observed that with emergence of new variants, the classical vaccines are no more effective to control the disease (Tariq, 1999). Poor vaccine quality is

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Table 1: Serum Samples Showing Positive or Negative IHA Results

Age (Weeks)	Total Samples	No. of Positive Samples	No. of Negative Samples	Positive percentage
0-3 wks	86	37	39	43.02%
3-6 wks	68	41	27	60.29%
6-8 wks	182	143	52	78.57%
Total	336	221	118	65.77%

Table 2: Distribution of Birds on the basis of IHA titer

Age of the Birds (Weeks)	No. of Positive Samples	Antibody titer using indirect hemagglutination test							GMT
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	
0-3	37	4	13	11	9	-	-	-	6.37
3-6	41	-	14	7	3	16	-	-	10.84
6-8	143	-	22	31	41	26	18	5	16.14

a common problem in developing countries and could be the result of poor manufacturing standards, lack of adequate storage facilities, application of expired vaccine batches and vaccine handling during transportation (Vui *et al.*, 2002). Improper storage might be due to transportation from market to farm or from manufacturer to distributor or from distributor to market or from the electricity failure. Exposure to viricidal agent like phenol or alcohol and improperly disinfected syringes might have detrimental effects on virus viability. Birds receiving continuous treatment with chloramphenicol or furazolidone have been shown to have impaired immune response (Tariq, 1999). Presence of mycotoxins in the feed reduces the host immunity directly by reducing the macrophage engulfing tendency and production of toxic lymphocytes which give poor output in immunity development. Mycotoxin indirectly affects the immunity by producing steroids from the adrenal glands which decrease the lymphocytes leading to immunosuppression (Tariq, 1999). Heat stress and water deprivation also lead to production of steroids and thus resultantly immunosuppression (Sil *et al.*, 2002). Poor nutrition like hypoproteinemia may hurt the immune response (Tariq, 1999). Ammonia present in the farm is a water soluble gas. If it goes above 30 ppm, badly affects the moist mucus membranes of the gut, trachea and nostrils leading to the decreased local immunity i.e. decreased IgA (Tariq, 1999).

Quality of water which is offered to the birds was also found questionable i.e. most areas were supplied with water full of salinity which might hinder the development of specific immunity possibly due the acid base imbalance. Another weak point might be the quality of water to dilute the vaccine before application. Unsuitable vaccination schedule also lead to the neutralization of MDAs and resultantly making the birds more susceptible to the infection.

The half life of the maternal antibodies to IBDV is between 3 and 5 days (Lukert and Saif, 1997) therefore, the exact antibody titer should be known to determine the

age at which the chick will be susceptible to IBD. The antibody titer must not fall below 1:64 before chickens can be vaccinated efficiently (Lukert and Saif, 1997). In the present study, most of the birds had antibody titer below 1:64 and 1:128 which showed the lack of specific immunity and relative susceptibility to the infection. All the facts relating to the low antibody titers in broiler birds showed that the local field IBDV can even break through the MDAs and are antigenically different from vaccinal strains as evident from the lack of specific immunity i.e. IBD field outbreaks even after vaccination.

So it is suggested that strategies must be developed to make the vaccine against the local field IBD virus isolates for the implementation of better disease control program in the country.

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