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DETECTION OF INFECTIOUS BURSAL DISEASE VIRUS IN DIFFERENT SMALL SCALE COMMERCIAL BROILERS

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ABSTRACT

Hossain MM, Rahman MM, Akter MR, Begum MD, Rahman MK (2015) Detection of infectious Bursal disease virus in different small scale commercial broilers. *J. Innov. Dev. Strategy.* 9(2), 16-20.

The experiment was conducted for the determination of infectious bursal disease virus from broiler chicken in Dinajpur during July-December 2013. A total number of 8 different small scale commercial broiler farms in Dinajpur were suspected to be infected with IBDV were observed. The size of the affected flocks was 500 to 1500 birds. Identification of IBDV was done by Clinico-Pathological investigation and determination of serum antibody titer of birds by indirect ELISA test. Morbidity of the flocks were about 0 to 100% and mortality ranged from 3% to 40%. The chicks aged between 21 to 32 days were affected. Depression, anorexia, ruffled feathers, diarrhoea, extreme weakness and death were the common clinical manifestation. At necropsy the major gross lesions included oedematous swelling or atrophy of the bursa of Fabricius, occasionally with petechial and ecchymotic haemorrhage were often observed in leg and breast muscle. The antibody titers of the suspected broilers were determined by indirect ELISA test. At the different age birds it shows that within eight flocks, four flocks show positive seroconversion and only one flock shows 100% seropositive and rest of flocks shows seronegative with very low antibody titer resulting they might be infected any time with IBDV due to lack of protective level of serum antibody.

Key words: *identification, IBD, IBDV, gumboro, virus*

INTRODUCTION

Poultry is considered as an important source of animal protein all over the world. The production and consumption of eggs and poultry meat has been increasing worldwide over the last three decades as the consumption of eggs has doubled and that of chicken meat has tripled (Rahman *et al.* 1996).

Infectious Bursal Disease (IBD) is a highly contagious, globally occurring viral poultry disease. The outbreak of IBDV was first noticed at the end of 1992 in Bangladesh (Islam *et al.* 1994; Chowdhury *et al.* 1996; Rahman *et al.* 1996) and has become a major problem in the poultry industry, causing upto 80% mortality in the field outbreaks (Chowdhury *et al.* 1996; Islam *et al.* 1997).

This disease in poultry which causes heavy economic losses due to immunosuppression in case of subclinical cases (Jackwood and Sommer-Wagner, 2010) and in acute, it is associated with mortalities, hemorrhages with bursa damage (Jackwood and Saif, 1987). The primary target organ for IBDV is the Bursa of Fabricius (Lukert and Saif, 1997). IBDV affects the actively dividing B-lymphocytes bearing cell surface IgM (Hirai and Calnek, 1979; Miiller 1986), developing the severe morphological alteration of Bursa of Fabricius (Lukert and Saif, 1997) and producing a profound immunosuppression (Ivan *et al.* 2001). The immunosuppression prevents the birds from optimally responding to vaccine (Sharma *et al.* 1994) and ultimately leads to increase the incidence of numerous concurrent infections (Rosenberger and Gelb, 1978; Chowdhury *et al*. 1996).

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 and single radial hemolysis (Hussain *et al.* 2003) tests. Therefore the present work was conducted to identify the IBD virus by the Clinico-pathological changes of Infectious Bursal Disease (IBD) and serum antibody titer against IBDV infection in small scale commercial broilers in Dinajpur.

MATERIALS AND METHODS

The study was divided into two major steps. First Clinico-Pathological investigation and second, determination of serum antibody titer of birds by ELISA test. A total number of 8 different small scale commercial broiler farms in Dinajpur were suspected to be infected with IBDV were observed.

Clinico-Pathological investigation

The age of the affected birds ranged from three weeks to five weeks. Detailed particular of the outbreaks of IBD including history, age, breed, flock size, mortality and clinical signs of affected birds were recorded. All the dead as well as sick birds were subjected to postmortem examination in the Laboratory of the Department of Microbiology, HSTU, Dinajpur. The disease was tentatively diagnosed as IBD on the basis of clinical history, symptoms and post mortem findings of the affected birds. Materials used for post mortem examination were birds (liver, bursa, hearts), post mortem tray, scissors, simple forceps, gloves, masks and scalpel. Post mortem examination was carried out and the different disease conditions of the birds were examined and tentative diagnosis was made as per description of (Calnek 1997).

Identification of IBDV by serum antibody titer of birds

Blood samples were collected from the chickens of different infected flocks. The samples were collected from the wing vein using 5 ml syringes. Soon after collection of blood the syringes with blood were kept slantly at 4-8°C for overnight, so that blood can clot in one side of the syringe. Then the clotted blood was removed carefully with sterile needle and sera were poured into sterilized graduated centrifuge test tubes. For each syringe separate needle was used. The sera subjected to centrifugation at 1000 rpm for 10 minutes for purification. Then the clear sera were collected and kept in clean sterilized vials and stored at -20°C for further use.

Identify of IBDV by indirect IELISA using a single dilution of serum. Dilution of each test sample was done as 1:500 ratios. Then the reagents were prepared and the test was done by the following procedure:

1. 5 µl of serum sample was directly placed in each well of a dilution plate (polystyrene micro titer plate).
2. 245 µl of Biochek Green sample diluents was added into each well of dilution plate that gave a 1:50 dilution of serum to diluents in the dilution plate.
3. IBD antigen coated plate was removed from sealed bag and recorded location of samples on template.
4. 100 µl of negative control (specific pathogen free serum in phosphate buffer with protein stabilizers and sodium azide preservative 0.1% w/v) was added into wells A1 and B2.
5. 100 µl of positive control (antibodies specific to IBD in phosphate buffer with protein stabilizers and sodium azide preservative 0.1% w/v) was added into wells C1 and D1.
6. 90 µl of sample diluents (Green) was added into each well of a Biochek test plate (except well of negative control and positive control).
7. The 1:50 Serum dilution was mixed with pipette by drawing solution into the pipette and releasing it back into the well. Repeated that 4 times. Then 10ul from the dilution plate containing the 1:50 diluted serum was added it to each corresponding well of the Biochek test kit plate. That gave a final 100ul/well of 1:500 serum dilutions on the Biochek test plate.
8. The plate was covered with lid and incubated at room temperature (22-27°C) for 30 minutes.
9. The contents of wells was aspirated and washed 4 times with wash buffer (350 µl per well). Plate was inverted and tap firmly on absorbent paper.
10. 100 µl of conjugate reagent was added into the appropriate wells. The plate was covered with lid and incubated at room temperature (22-27°C) for 30 minutes.
11. The contents of wells was aspirated and washed 4 times with wash buffer (350 µl per well). The plate was inverted and tap firmly on absorbent paper.
12. 100 µl of substrate reagent was added into the appropriate wells. The plate was covered with lid and incubated at room temperature (22-27°C) for 15 minutes.
13. 100 µl of stop solution was added into the appropriate wells to stop reaction.
14. The reader was blank on air and recorded the absorbance of controls and samples by reading at 405 nm.

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15. Samples with an S/P of 0.2 or greater contain anti-IBD antibodies and are considered positive.

RESULTS AND DISCUSSION

During the visit and selection of farms available in the area, birds were randomly sampled from farms according to eight flocks housing at the time of the visit. Birds of that farms shows some clinical signs that's given below were assumed IBD infected flocks. Randomly some of sick and dead birds are taken to the laboratory for further tests. Clinical findings of the flocks naturally infected with IBDV:

The clinical findings of the affected flocks were-

- Anorexia
- Depression
- Huddle together
- Ruffled feathers
- Diarrhoea and
- Death
- The chickens were affected at the age between 18 to 32 days.

The common clinical manifestations in the chicks suffering from natural IBD include depression, anorexia, ruffled feathers, diarrhoea, extreme weakness and death. Chicks aged between 21 and 42 days were affected mostly. Birds which were vaccinated or not against IBD high morbidity and mortality were recorded. This observation is similar to the reporters of Islam and Samad, 2004; Wang *et al*. 2010 and Majed *et al.* 2013.
Postmortem findings of the chickens naturally infected with IBDV:

After postmortem examination of naturally affected sick and dead birds following lesions were observed.

- Swollen bursa of fabricius.
- Soft and edematous with creamy or yellowish discoloration of the bursal mucosa.
- Congestion and petechial haemorrhage in the serosal or mucosal surface. Gelatinous material was also seen within the lumen of enlarged bursa in few cases.
- Occasionally the bursa became creamy and atrophied and sometimes the lumen contained cheesy mass.
- Swollen kidney with full of uterates.
- Petechial and ecchymotic haemorrhages were found in the leg and breast muscles.
- Haemorrhage in between proventriculus and gizzard.

Gross pathological changes of the affected chickens were studied. At necropsy, the major pathological lesions were oedematous swelling or atrophy of the bursae of fabricious occasionally with hemorrhage and creamy or yellowish discoloration; enlargement or atrophy of the spleen, frequently with white and dark red spots; in some cases haemorrhage in the leg and breast muscles; and occasionally swelling of the kidney. These gross lesions are similar with the findings of other authors (Cheville 1967; Cho and Edgar, 1972; Hirai *et al.* 1973; Dongaonkar *el al.* 1979; Jhala *et al.* 1990; Lone 2007; Khan *et al*. 2009; Hossain *et al*. 2010; Rahman *et al.* 2010 and Alam *et al.* 2011).

Determination of the IBDV specific serum antibody titer of the chickens by indirect ELISA. The results of serological test of the 90 samples from the broilers showed in table 3, only 25 (27.78%) samples showed positive in ELISA for detection of serum antibody titer.

Flock-A which indicates minimum and maximum antibody titer, is 14 and 696 and there are only 3 positives that mean 27.27% positives (Table 1, Table 2 and Table 3). Than Flock- B, C, and H there is 4 (36.34%), 11 (100%), 7 (58.33%) positives, respectively and Flock - D, E, F, G has no positive results with IBD infection. The result of this study demonstrates serological evidence of Infectious Bursal Disease Virus (IBDV) in chickens. From 90 chicken sera samples, 25 (27.78%) samples were positive, while 65 (72.22%) samples were negative for IBD antibody. Interestingly broiler birds of older age (Flock-C) showed 100% seroconversion of IBDV infection but broiler age of younger birds showed lower percentage of seroconversion (Flock-A, B and H). The presence of IBD antibody in these birds will either be due to maternally derived antibody or infection occurs. However, maternal antibodies to IBD in unvaccinated chickens persist in chicks up to 21 days as determined by ELISA with complete decay by 28 and 35 days (Zaheer and Saeed, 2003).

Table 1. Row O.D. value of serum samples in different flocks against IBDV infection

Flock	1	2	3	4	5	6	7	8	9	10	11	12
A	0.195	0.221	0.223	0.283	0.423	0.471	0.213	0.321	0.188	0.191	0.203	0.520
B	0.160	0.239	0.202	0.384	0.582	0.233	0.280	0.262	0.285	0.471	0.346	0.481
C	1.130	1.719	1.666	1.574	0.550	1.030	0.481	0.393	2.328	0.755	0.723	0.792
D	1.252	0.247	0.286	0.250	0.248	0.205	0.229	0.270	0.286	0.346	0.236	0.197
E		0.222	0.291	0.230	0.297	0.278	0.263	0.322	0.189	0.203	0.183	0.208
F		0.199	0.202	0.300	0.356	0.337	0.288	0.308	0.263	0.293	0.328	0.239
G	0.287	0.364	0.271	0.192	0.241	0.277	0.337	0.373	0.219	0.250	0.280	0.323
H	0.328	0.582	0.474	0.257	0.249	0.421	0.334	0.384	0.423	0.405	0.503	0.370

Table 2. S/P Ratio of serum samples in different flocks against IBDV infection

Flocks	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.043	0.045	0.104	0.242	0.290	0.035	0.142	0.010	0.013	0.025	0.338
B	0.000	0.061	0.024	0.204	0.399	0.055	0.101	0.083	0.106	0.290	0.166	0.299
C	0.000	1.521	1.469	1.378	0.368	0.841	0.299	0.213	2.122	0.570	0.538	0.606
D	0.000	0.069	0.107	0.072	0.070	0.027	0.051	0.091	0.107	0.166	0.058	0.019
E		0.044	0.112	0.052	0.118	0.099	0.084	0.143	0.011	0.025	0.005	0.030
F		0.021	0.024	0.121	0.176	0.157	0.109	0.129	0.084	0.114	0.148	0.061
G	0.108	0.184	0.092	0.014	0.063	0.098	0.157	0.193	0.041	0.072	0.101	0.144
H	0.148	0.399	0.293	0.078	0.071	0.240	0.154	0.204	0.242	0.224	0.321	0.190

Table 3. ELISA antibody titer of the chickens in different flocks against IBDV infection

Flocks	1	2	3	4	5	6	7	8	9	10	11	12
A	-	72	76	190	482	588	57	268	14	19	40	696
B	-	106	38	400	836	94	184	149	194	588	319	608
C	+	3642	3505	3267	765	1898	608	419	5253	1237	1161	1323
D	+	121	196	127	123	43	87	164	196	319	100	29
E		74	207	89	219	180	151	270	16	40	7	49
F		33	38	225	340	300	201	241	151	211	281	106
G	199	357	166	21	110	178	300	376	68	127	184	272
H	281	836	595	139	125	478	293	400	482	443	658	370

Antibody detected in these birds cannot be maternally derived because the age range of birds used for this study was between 21 to 32 days. The presence of IBD antibody in unvaccinated chicken has also been reported by Vui *et al*. (2002) in Vietnam and also in cattle egrets and pigeon in Nigeria by Fagbohun *et al*. (2000). It was not determined which seropositive birds were positive in virus isolation because pooled sample was used. 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).

CONCLUSION

The present study indicates that the chicks aged between 21 to 32 days were affected. Depression, anorexia, ruffled feathers, diarrhoea, extreme weakness and death were the common clinical manifestation. At necropsy the major gross lesions included oedematous swelling or atrophy of the bursa of Fabricius, occasionally with petechial and ecchymotic haemorrhage were often observed in leg and breast muscle.

The antibody titers of the suspected broilers were determined by indirect ELISA technique. At the different age birds it shows that within eight flocks four flocks show positive seroconversion and only one flock shows 100% seropositive and rest of flocks shows seronegative with very low antibody titer resulting they might be infected any time with IBDV due to lack of protective level of serum antibody. So indirect ELISA is an ideal tool for determination of serum antibody titer level of birds for the detection of birds were infected or not and also protective or not against infection.

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