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Infectious bursal disease standardization and seroprevalence study in Ranchi in poultry

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ABSTRACT : The work was planned to develop diagnostic assay using hyper immune sera and poultry sera with seroprevalence monitoring in Ranchi (Jharkhand). The best result for dot-ELISA was standardized with 1.2 µl of BursaB2K and Gumboro strain, skimmed milk 1.5 per cent+gelatin 0.5 per cent+BSA 1 per cent as blocking solution, sera dilution of 1:10 and 1:500 conjugate dilution. Statistical analyses were showing there is a non-significant difference in results of dot-ELISA and ELISA test to detect specific anti IBDV antibodies. In the present study, dot-ELISA based seroprevalence study of 92 suspected poultry samples in 18 different places of Ranchi in the year 2014-15, revealed IBD antibodies existed in 38.04 per cent with sensitivity and specificity of 67.57 per cent and 81.82 per cent, respectively. Serodiagnosis helps in monitoring immune status of poultry flock in the area for IBD. There was large variation in IBD positive antibodies in different places of Ranchi, ranging between 14.29 per cent to 100 per cent by dot-ELISA. Similar degree of results were observed with two strains of live attenuated IBD antigen *i.e.* Bursa B2K (invasive intermediate) and Gumboro (intermediate) strain. Statistical analysis revealed non-significant difference between dot-ELISA and ELISA. Dot-ELISA can be taken as promising tool in field.

KEY WORDS : Dot-ELISA, IBD, Seroprevalence, Poultry

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INTRODUCTION

Infectious Bursal Disease (IBD) is an important avian pathogen that inflicts major economic losses to the poultry industry (Burkhardt and Muller, 1987). It is an acute, highly contagious, immunosuppressive and economically important poultry disease caused by Birnaviridae (Okwor *et al*., 2011). The disease damage the humoral immunity producing lymphoid organ Bursa of Fabricius and result in immuno-suppression and increase susceptibility of poultry to opportunistic secondary infection such as Marek's disease and Newcastle disease (Mahgoub, 2012). Broiler birds aged between 21-30 days are most susceptible to IBD infection (Mor *et al*., 2010).

Serological monitoring of IBD is done with an accepted method called automated ELISA (Lasher and Shane, 1994), I-ELISA (Howie and Thorsen, 1981), HI and AGIDT (Rakibul-Hasan *et al*., 2010), Antigen capture enzyme-linked immunosorbent assay (AC-ELISA), Single serum dilution ELISA (SSD-ELISA) (Ramadass *et al*., 2008), immunochromatographic gold-based test (Nurulfiza *et al*., 2011), immunoperoxidase technique (Guvenc *et al*., 2004); Immunocomb based dot-ELISA (Manoharan *et al*., 2004); Monoclonal antibody based dot-ELISA (Swain *et al*., 1999); Passive haemagglutination (Ezeibe *et al*., 2012) and molecular

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techniques like reverse-transcription polymerase chain reaction (Lin *et al*., 1993; de Paula *et al*., 2004 and Makadiya *et al*., 2006).

Isolation and identification of IBD provide the most certain diagnosis but are not usually attempted for routine diagnostic purposes as the virus may prove difficult to isolate (Lukert and Saif, 1991). Many diagnostic assays available in market with high sensitivity and specificity but is least cost effective and highly efficient personnel is also required. Serological diagnosis based on ELISA has been proved to be a rapid and reliable method of screening large number of samples. However, these methods can only be performed under specialized laboratory conditions causing delay in serological screening of large flocks and but disease spread rate is very fast. Comparatively, dot-ELISA is cheaper, reproducible and cost effective. The fine-tuning of breeder flock immunization with serological tests will help in control strategies. Profitable poultry farming depends on early disease diagnosis, effective control measures, treatment, knowledge of seroprevalence of poultry disease with devastating effect can help in chalking out effective control measures for effective poultry farming. As per available literature, dot-Elisa immunodiagnostic tool is more specific, highly sensitive, less time consuming and applicable for field use (Sultana *et al*., 1999; and Subramanyam *et al*., 2010).

Limited literature was available on IBD seroprevalence reported by dot-ELISA in poultry population. Therefore, the present work had been planned to develop easy, rapid and reliable diagnostic test for IBD infection in poultry which will help in monitoring the concern infection with evaluation of comparative efficacy of standardize dot-ELISA with ELISA.

MATERIAL AND METHODS

The blood samples were collected from 92 chickens from age group of 4-10 wks from suspected cases from Ranchi during January 2014 to April 2015.

Raising of hyper immune serum (HIS) :

Three healthy cross bred rabbit over 4 months of age with approx. 2.50-4.50 kg body wt. were procured from rabbit unit, RVC, Kanke, India. One rabbit was kept as a control whereas another two rabbit was used for the production of HIS separately with 100µg of Bursa B2K (invasive intermediate) and Gumboro (intermediate) strain of vaccine antigen, PBS and Freund's complete adjuvant and incomplete adjuvant. By 0^{th} day, 14^{th} day, 21^{st} day and 28^{th} day, booster dosing along with blood aspiration for IBD antibody presence was performed by AGID test. On 35^{th} day, presence of IBD viral antibody in respective rabbits confirmed that rabbits was hyper immunized for IBD antigen. Serum was stored at - 20ºC.

Dot-ELISA :

Dot-ELISA is a highly versatile solid phase immunoassay for detection of antibody or antigen in field use. This assay uses a minute amount of reagent dotted onto nitrocellulose membrane (NCM) bound to plastic stick known as dipstick. The degree of development of brown spot on dipstick was considered as evidence of positivity after treatment with antigen, specific antibody, antispecies enzyme conjugate followed by addition of a precipitable chromogenic substrate *i.e.* diaminobenzidine (DAB).

Varying amount of IB antigen was used. The membrane was dried for 5-10 minutes at room temperature; the free binding sites on NCM were blocked for 40 min at 37°C with varying concentration of skimmed milk, BSA, mixture of skimmed milk+ Gelatin, skimmed milk+BSA, Gelatin+BSA, skimmed milk+Gelatin+BSA.

The dipstick was washed thrice for 5 min each with PBS containing 0.05 per cent Tween 20. Then, it was treated with varying diluted solution of poultry sera and incubated at room temperature for 45 min. New born chick sera were used as negative control. Again washed with PBS and treated with varying diluted solution of specific anti species conjugate (Sigma, USA) and incubate at room temperature for 45 min. After washing add diaminobenzidine chromogenic substrate solution (Himedia) (1 DAB tablet in 10 ml distilled water + 13µl of concentrated hydrogen peroxide 30%) until dots were visible. The dipstick was dipped in stop solution (distilled water) to stop the reaction and allowed for air drying. The development of brown spot dot on dipstick was considered evidence of positivity. The development of brown spot dot on dipstick was considered evidence of positivity. The intensity of brown colour on dipstick was judged by naked eye and numbered on arbitrary scale 0, ++, +++, ++++ indicating negative, moderate, intense and highly intense reaction, respectively, in reference to negative control.

Sensitivity and specificity of dot-ELISA were

calculated, considering ELISA as reference test using following formula (Lalkhen and McCluskey, 2008).

Comparative evaluation of dot-ELISA with reference to ELISA separately for the detection of IBD:

Sensitivity and specificity of dot-ELISA were calculated, considering ELISA as reference test using following formula employed by Lalkhen and McCluskey, (2008):

$$\text{Sensitivity \%} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}$$

$$\text{Specificity \%} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}}$$

where, True positive -	number of positive by test dot-ELISA out of total positive shown by reference test (ELISA)
True negative -	number of negative by dot-ELISA out of total negative shown by reference test (ELISA)
False positive -	number of positive by dot-ELISA out of total negative shown by reference test (ELISA)
False negative -	number of negative dot-ELISA out of total positive shown by reference test (ELISA)

RESULTS AND DISCUSSION

Hyperimmune sera were produced in rabbit and were used for dot-ELISA standardization of IBD in poultry (Fig. 1). The best result for IBD were found with 15 µg *i.e.* 1.2 µl IBD antigen of BursaB2K and Gumboro strain, skimmed milk 1.5 per cent+gelatin 0.5 per cent+BSA 1 per cent as blocking solution, sera dilution of 1:10 and 1:500 conjugate dilution (Fig. 2, 3 and 4). The overall prevalence of IBD infection using dot-ELISA was 38.04 per cent with sensitivity and specificity of 67.57 per cent and 81.82 per cent, respectively (Table 1 and 2). In Jharkhand, for the first time, dot-ELISA technique was used for seromonitoring of IBD. Statistical methods (Chi-square) were used to see significance level between dot-ELISA and ELISA as per standard method described by Snedcor and Cochran (2004). Chi-square test revealed that there was non-significant difference among these serological assays (Table 3).

In present study we found that overall prevalence of IBD in around Ranchi, Jharkhand was 38.04 per cent with sensitivity and specificity of 67.57 per cent and 81.82 per cent, respectively by dot-ELISA. Similar findings were also reported by Choudhary *et al*. (2012). He reported overall lower incidence rate (33.90%) of IBD by AGPT in and around Ranchi. Dot-ELISA also gave lower prevalence rate (8.4%) of IBD (Alam *et al*., 2012). Some workers reported higher prevalence rate (47.98%) of IBD by sandwitch ELISA technique (Chhabra *et al*., 2004); (73.75%) by AGID test (Karunakaran *et al*., 1993); (64.57%) by dot-ELISA test (Subramanyam *et al*., 2010); (93.00%) by ELISA (Botus *et al*., 2010); (58.8%) by ELISA test (Swai *et al*., 2011) and (91.43%) by AGID,

Table 1 : Overall place wise seroprevalence of IBD infection in poultry using dot- ELISA

Sr. No.	Place	Total no. of samples for IBD	Seroprevalence of IBD positive by Dot ELISA
1.	RVC, Kanke	9	3(33.33)
2.	Hochar	7	1(14.29)
3.	Husir	5	2(40)
4.	Patara toli	9	2(22.22)
5.	Chirondi	4	1(25)
6.	Simartoli	8	3(37.50)
7.	Boria	5	1(20)
8.	Sangrampur	8	4(50)
9.	Arsanday	4	3(75)
10.	Milatcolony	6	2(33.33)
11.	Chuditola	2	1(50)
12.	Bhitha	5	2(40)
13.	Mohotoli	2	2(100)
14.	Chandabe	3	2(66.67)
15.	Kumharia	5	2(40)
16.	Bariatu	2	2(100)
17.	Kokar	5	1(20)
18.	Chutia	3	1(33.33)
	Total (92)	92	35(38.04)

Table 2 : Relative performance of dot ELISA to ELISA for IBD

Test	ELISA (reference test)			
	Results	Positive	Negative	Total results
Dot-ELISA	Positive	25	10	35
	Negative	12	45	56
	Total results	37	55	92

Relative sensitivity = 67.57%; Relative specificity = 81.81%

Table 3 : Statistics for ELISA and dot-ELISA for IBD

Result/Technique	ELISA	Dot-ELISA	Total	χ^2
Positive	37	35	72	
Negative	55	57	112	0.09^{NS}
Total	92	92	184	

P-Value
* and ** indicate significance of values at P=0.05 and 0.01, respectively
NS= Non-significant

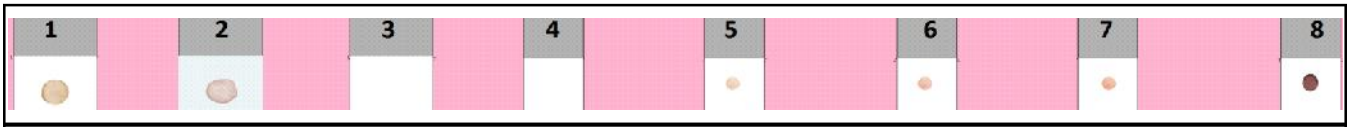

Fig. 1 : Standardization of dot-ELISA for IBD with hyperimmune sera A. 1-Rabbit 1, 2 Rabbit 2, 3- Negative Control Rabbit B . . . . . th day, 6-21st day, 7-28th day, 8-35th day

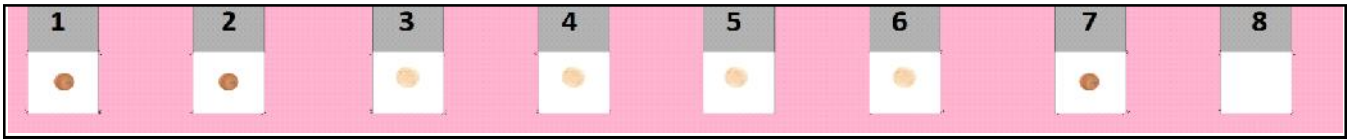

Fig. 2 : Standardization of IBD with different type and amount of antigen, A. Antigen type 1 BursaB2K 2-Gumboro (Similar result) B. Antigen amount 3- 0.5 «l, 4-0.7 «l, 5-0.9 «l, 6-1 «l, 7(best)- 1.2«l, 8- New born chick sera(Negative control)

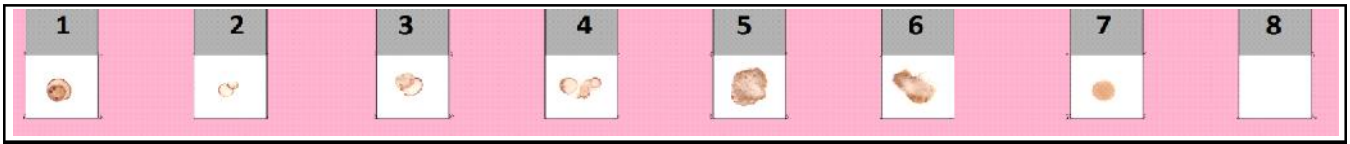

Fig. 3 : Standardization of IBD with different type and amount of blocking solution, A. Skimmed milk: 1-3%, 2-2%; B. Gelatin: 3- 0.5 %, 4-1 %, C. BSA: 5-1.5 %, 6-1 %,7(best)- Skimmed milk (1.5%)+ Gelatin(0.5%)+ BSA (1%), 8- New born chick sera(Negative control)

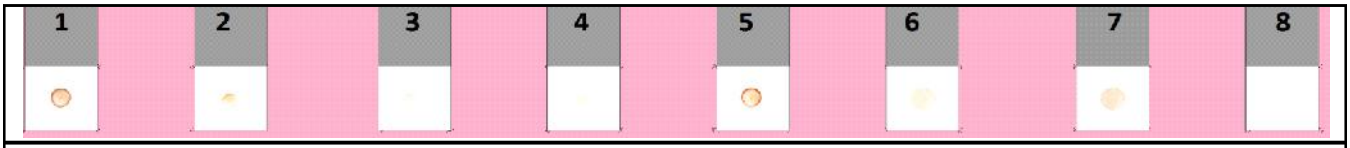

Fig. 4 : Standardization of IBD with different sera and conjugate dilutions, A. Sera dilution 1(best)-1:10 2-1:20 3-1:30 4-1:40 B. Conjugate dilution 5(best)- 1:500, 6-1:800, 7-1:1100, 8- New born chick sera(Negative control)

IHC and RT-PCR with agreement of 87.91 per cent among the three methods (Islam *et al*., 2011).

In our study Freund's adjuvant provided better immune response for HIS production. Iqbal *et al.* (2003) also found similar results for HIS production. In present study, ELISA is more sensitive and specific for detection of IBD antibody than dot-ELISA however, dot-ELISA is more specific and sensitive than AGID and VNT (Sultana *et al*., 1999). But In field condition dot-ELISA is more convenient and cost effective. The cost came out to be approx. less than Rs. 20. This amount is appreciably less compared to Rs. 65.22/sample for IBD (Affinitech ELISA kit). Now-a-day, several workers used molecular techniques like PCR and RT-PCR for specific detection of IBD antibodies. Sensitivity of AGPT, dot-ELISA was compared taking RT-PCR as standard test for IBD at different hours of post- infection (p.i.) and by 14 hr it was possible to detect viral RNA by RT-PCR which was undetectable by AGPT and dot-ELISA. But these techniques are cost effective when used in large sample size and is also not suitable for field condition (Parthiban and Thiagarajan, 2000). Comparatively, dot-ELISA is better and more economical for field use.

Several factors like vaccination status, bio-security measure, management practice and climatic condition may play an important role in the prevalence of IBD. Large number of samples should be tried on future occasion for subsequent work.

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