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USE OF DOT-ELISA FOR THE DIAGNOSIS OF INFECTIOUS BURSAL DISEASE IN CHICKENS

Jawaria Sultana, Manzoor Hussain[1], Salman A. Malik and Khalid Naeem[1]
Department of Biological Sciences, Quad-I-Azam University, Islamabad,
[1]Animal Sciences Institute, National Agricultural Research Centre,
Park Road, Islamabad, Pakistan

ABSTRACT

The present study was undertaken to develop dot-blot ELISA (DOT-ELISA), a field oriented diagnostic test to detect infectious bursal disease virus (IBDV). The DOT-ELISA, using nitrocellulose membrane, was standardized and field specimens collected from sick birds, apparently infected with IBDV, were analyzed. Efficacy of DOT-ELISA was compared with agar gel precipitation test (AGPT). The DOT-ELISA was found to be more sensitive diagnostic tool than AGPT, indicating the suitability of DOT-ELISA as rapid field test for the detection of IBDV during early stage of infection.

INTRODUCTION

The need for early and accurate diagnosis of diseases has been a catalyst for developing many diagnostic techniques. This approach is important for developing and planning disease control and eradication programmes. Disease outbreaks and subclinical conditions during infectious bursal disease (IBD) results in significant economic losses by high mortality and lowering productivity of the birds. It is, therefore, important to develop a suitable assay for prompt diagnosis of such infections to minimize these losses.

An outbreak of IBD in a flock may be diagnosed by the rapid onset, clinical signs and acute course of the disease accompanied by characteristic bursal lesions. Current diagnostic assays routinely used for detecting IBDV infection are the immunofluorescence (IF) assay, the agar gel precipitation test (AGPT) and occasionally, electron microscopy (Dash *et al.*, 1991; Nicholas *et al.*, 1985). However, the development of enzyme linked immunosorbent assay (ELISA) has greatly enhanced the capability to diagnose IBD and other infections of poultry and livestock (Carlson *et al.*, 1972). ELISAs are convenient, fast, highly sensitive and reliable for the diagnosis of various diseases.

The DOT-ELISA, to analyze crude viral antigens directly onto nitrocellulose sheet, has been suggested by many researchers. In the present study an adaptation of DOT-ELISA for the detection of IBDV antigen is described and its ability as an early diagnostic tool is demonstrated.

The DOT-ELISA used in this study was developed according to the procedures previously described (Hawkes *et al.*, 1982; Gordon *et al.*, 1988) with some modification.

MATERIALS AND METHODS

Viral Antigen

Fifty bursae from birds, showing clinical signs similar to IBD, were collected from local field outbreaks. A 20% (w/v) bursal homogenate was prepared separately by using tissue homogenizer in sterile Tris-buffer saline (TBS pH 7.5) containing antibiotics (Penicillin 1000 IU/ml, Streptomycin 1000 μg/ml). Each homogenate was centrifuged at 1200 rpm for 10 minutes. The supernatant was passed through 0.45 and 0.2 μm syringe filters (Sartorius AG, Germany) and tested for the presence of IBDV by AGPT. All positive and negative samples were stored at -20°C until used for further experiments.

Antiserum

The anti-IBDV serum was raised in 3-weeks old broiler chicks using D-78 strain of IBD vaccine (Intervet, USA). The vaccinal virus was inactivated by formaldehyde. The preparation was adjuvanted with complete Freund's adjuvant and 0.5 ml was administered subcutaneously in each bird. After one week, birds were again given a similar dose of vaccine prepared in incomplete adjuvant. Blood samples were collected at weekly intervals post-vaccination and the serum was

checked for the presence of viral antibodies by AGPT.

Standardization of DOT-ELISA

A checker board titration was performed to determine the optimum antigen, antibody and conjugate dilutions. For this purpose a 0.45 μm pore size nitrocellulose sheet (Milipore Corporation, Bedford, UK) was cut into strips of 4 x 0.5 cm and marked with lead pencil at 1 cm intervals for the orientation of the antigen dots. Serial two fold dilutions of antigen were made in TBS (10mM Tris, 150 mM NaCl, pH 7.6). Five μl of the known positive antigen diluted from 1:2 to 1:32 were dotted on each strip. Positive and negative controls of IBDV (Dorren Labs., Netherlands) were also applied and allowed to dry at room temperature. Unreacted protein binding sites were blocked by immersing and rocking the strips in 3% fish gelatin in TBS (pH 7.6) for 90 minutes, followed by washing in plain TBS for 5 minutes.

The nitrocellulose strips were placed separately in chicken anti-IBDV positive serum diluted in TBS from 1:20 to 1:160 for 90 minutes at room temperature on a shaker. Thereafter, the strips were washed 3 times in TBS and placed in rabbit anti-chicken IgG conjugated to horseradish peroxidase (Sigma, USA) diluted to 1:500, 1:1000, and 1:2000 in TBS. After 90 minutes at room temperature, the strips were washed again and developed in TBS (50mM Tris, 65 mM NaCl, pH 7.6) containing 0.15% Diaminobenzidine (Sigma, USA), 0.04% $NiCl_2$ and 0.02% H_2O_2. The enzyme-substrate reaction was allowed to proceed for 15 seconds. To stop the reaction, the strips were washed in tap water and air dried before visual interpretation of the results. The highest dilution of the antigen with maximum antibody titer at high conjugate dilution that gave the minimum background colour was considered to be ideal and was used for future analysis of the bursal samples.

DOT-ELISA application on field test specimens

Homogenate prepared separately from fifty bursal samples were processed both by DOT-ELISA and AGPT for the detection of IBDV. Appropriate IBD-positive and negative controls were applied. The DOT-ELISAs were run, as described in checkerboard titration (above), except that a single dilution each of antigen, antibody and conjugate dilution was used. Results were expressed as either positive or negative based on colour development.

Agar gel precipitation test

AGPT was conducted following the standard protocol (Crowle, 1973). Briefly, Nobel agar was autoclaved and 5 ml aliquots were poured onto the microscopic glass slides. The wells in the agar were cut using the template capable of making seven satellite wells. In the peripheral wells, 30 μl of each test sample (bursal homogenate) along with positive control were added separately while in the central well, 30 μl of anti-IBD positive serum was added. The slides were incubated in a moist chamber at room temperature. The results were recorded after 24-48 hrs and observed with an illuminating light source against a dark background.

RESULTS

Determination of optimum dilutions of the DOT-ELISA reagents

The optimum dilution of an IBD-antigen was found to be 1:8 (the protein concentration was 22μg/5μl). The optimum conjugate dilution was recorded as 1:1000 and of the serum that gave the minimum background colour at the above concentration of antigen and conjugate was 1:80.

DOT-ELISA and AGPT in bursa samples

A total of 50 samples of bursae were collected from suspected birds during local field outbreaks. These samples were analyzed by AGPT and DOT-ELISA. Of these 50 samples, 22 (44%) were positive by AGPT and 31 (62%) were positive by DOT-ELISA (Table 1).

Sensitivity and specificity of the DOT-ELISA relative to AGPT

Sensitivity and specificity of DOT-ELISA relative to AGPT for IBD antigen was also determined. It was observed that out of 50 samples tested, 22 samples found positive by AGPT were also positive by DOT-ELISA. Of the 28 samples recorded negative by AGPT, 9 were found to be positive by DOT-ELISA. None of AGPT positive sample was negative by DOT-ELISA. Thus the sensitivity of DOT-ELISA was 100% relative to the AGPT and the sensitivity of AGPT relative to the DOT-ELISA was 70.9%. Of the 28 samples found negative by AGPT, 19 were found negative by DOT-ELISA, thus giving the specificity of 67.8% relative to AGPT. The overall agreement between DOT-ELISA and AGPT was 82% (Table 2).

Table 1: IBD virus detection by AGPT & DOT-ELISA

Tests employed	Total No. of samples tested	Positive Samples	
		No.	%
AGPT	50	22	44
DOT-ELISA	50	31	62

Table 2: Sensitivity and specificity of DOT-ELISA relative to AGPT for the detection of IBDV-Antigen

Dot-ELISA	AGPT		Total
	Positive	Negative	
Positive	22	09	31
Negative	00	19	19
Total	22	28	50

Sensitivity of DOT-ELISA relative to AGPT = 31/31 x 100 =100%; Sensitivity of AGPT relative to DOT-ELISA = 22/31 x 100 =70.96%; Specificity of DOT-ELISA relative to AGPT = 19/28 x 100 = 67.80%; The overall agreement between DOT-ELISA and AGPT = 22+19/50 x 100 = 82%

DISCUSSION

The results in this study imply that the sensitivity of DOT-ELISA is sufficient for rapid and accurate diagnosis of IBD in chickens. Some other diagnostic assays such as AGPT have also been previously used to detect IBDV antigen in bursal homogenate (Dash *et al.*, 1991). In our study we were able to detect IBDV using AGPT, but the sensitivity with AGPT was lower as compared with DOT-ELISA.

Nitrocellulose (NC) membrane is the most commonly used support in various assays for its ease of handling, good staining characteristics and irreversible binding of proteins. The bursal homogenate used in this study was absorbed quite easily onto the NC membrane. The visual interpretation of positive reactions generally has caused no problem in the routine assay, which adds to the versatility of DOT-ELISA in its availability to laboratories with limited facilities. In this study, the binding properties of NC membrane were utilized for the immobilization of virus antigen from crude bursal preparation, and their subsequent detection by immuno-enzymatic assays. Successful use of this relatively crude preparation indicated the potential use of this test in the field. This assay has been previously used for the detection of viral antigen from clinical specimens with high specificity and sensitivity. (Heberling and Kalter, 1987; Song *et al.*, 1997).

In the present study the superiority of DOT-ELISA over AGPT as an early diagnostic tool has been demonstrated. It clearly indicated that the infection in a flock would be detectable at very early stage of infection by using DOT-ELISA. Thereupon an early vaccination of the flocks in the vicinity of the infected chickens may be employed before an apparent outbreak appears and produces heavy losses. Attempts are being made to isolate IBDV from positive samples and correlate them with the results of DOT-ELISA. Samples found negative by DOT-ELISA may be obtained from birds not actually infected with IBDV.

Extensive replication of IBD virus occurs in bursa of Fabricius during early stage of the infection. It is important to get samples from sick birds at an appropriate time. Investigations are being carried out to determine the stage of the disease where maximum viral antigen could be obtained for detection by DOT-ELISA.

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