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Comparative Sensitivity of Different Tests in the Diagnosis of Infectious Bursal Disease in Broilers

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ABSTRACT

Three serological tests (indirect haemagglutination, serum neutralization and agar gel precipitation) were evaluated for the diagnosis of infectious bursal disease in broilers. Serum neutralization test was found to be the most sensitive (92.11%) method followed by indirect haemagglutination (91.53%) and agar gel precipitation (66.92%) tests. The best relative sensitivity (99.40%) was recorded in serum neutralization test over agar gel precipitation test, whereas the best relative specificity (91.30%) came of agar gel precipitation test to serum neutralization test.

Key Words: Infectious Bursal Disease; Indirect haemagglutination test; Serum neutralization test; Agar gel precipitation test

INTRODUCTION

Infectious bursal disease (IBD) has emerged as havoc for poultry in Pakistan (Siddique *et al.*, 1987). The disease has been sporadic upto 1983 but became quite widespread later in commercial poultry and has inflicted heavy economic losses (Anjum, 1994). The virus attacks the immune system of young birds and causes a severe illness. Symptoms include depression, diarrhoea, vent picking and variable mortality. Lesions occur mostly in the Bursa of Fabricious which becomes swollen and oedematous three to four days-post infection. The morbidity is 10 to 100% in experimental disease, mortality usually ranges from 8-10% and sometimes exceeds 50% (Anjum, 1994). Strict hygienic and proper vaccination measures are adopted for the prophylaxis and control of IBD (Vieltiz, 1993). The IBD is diagnosed in the field conditions based upon the postmortem changes and serological tests. Various serological tests like Agar Gel Precipitation Test (AGPT), Serum Neutralization Test (SNT), Indirect Haemagglutination Test (IHAT) and the Enzyme Linked Immunosorbent Assay (ELISA) are employed for confirmation of IBD (Sah *et al.*, 1988) with variable results. This paper describes the comparative sensitivity and specificity of various serological tests used for the diagnosis of IBD.

MATERIALS AND METHODS

Collection of samples. Bursal samples were collected from 68 affected flocks for the preparation of field antigen and stored at -20°C for further use (Aliev *et al.*, 1989). Five hundred and eight (508) serum samples were collected from the commercial broiler farms with clinical IBD in and around Faisalabad (Pakistan) and kept separately in 2 ml aliquots at -20°C till use.

Procurement of vaccines. Eight live virus IBD vaccines including 1/45 PV, D-78, CUIM, Bursine-2, LZD 228, S-706, VI BURSA-G and LUKERT were procured from local market and were used as vaccinal antigen for the preparation of hyperimmune sera (Amin, 1995) and serological tests after sonification at 600 rapidis per second (rps) for three minutes.

Serodiagnostic response:

I) Indirect haemagglutination test (Saeed, 1991). IHA test was carried out in 508 serum samples collected from IBD field outbreaks to select the vaccinal strains on the basis of highest homologous as well as heterologous titers of IBD virus. The best vaccinal strain was used for the detection of antibodies from the collected serum samples.

ii) Agar gel precipitation test (Rosenberger *et al.*, 1975). The best vaccinal strain that gave the highest homologous and heterologous antibody titer was used to determine the antigen response from 68 sonicated bursal samples. Similarly, all the 508 serum samples were separately processed through AGPT.

iii) Serum neutralization test (Giambrone, 1980). All the serum samples were serologically titrated using the same vaccinal strain of IBD virus used for IHA and AGPT. Flockwise antibody titers (GMT) were also determined through IHA, AGPT and SNT. Chicken embryo fibroblasts were maintained as monolayer in the medium containing Hank's Balance Salt Solution (85%), buffalo colostrum whey (5%), lactalbumin hydrolysate (10%) alongwith kanamycin, penicillin and streptomycin. Cytopathic effect was determined for negative cases of SNT as demonstrated by Amin (1995). The results of various serological tests were statistically analysed through correlation (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

The IHA test was carried out in 508 serum samples and the D-78 vaccinal strain of IBD was selected on the basis of the highest homologous as well as heterologous titers of IBD virus. Homologous antibody titers ranged from 2^9 to 2^{12} where the respective antigens of specific IBD strains were used to sensitize human group 'O' erythrocytes. The combined IBD antibody titer in heterologous system varied from 2^5 to 2^{10} (Table I). Similarly, all the homologous system showed perfect positive band formation through AGPT. These results are in line with the findings of Berg and Meulemans (1991) who reported that the use of virus strain D-78 well qualifies as the best one, whether to be used as test antigen or as vaccinal antigen to raise polyclonal hyperimmune serum. Flockwise IHA antibody titer (GMT) ranged from 4.6 to 512.

Table I. Indirect haemagglutination test antibody titre of homologous and heterologous systems against IBD virus strains in checkered board

	Vaccinal strain antigen of IBD								
Hyper-immune sera	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**GMT**
a	2^{12}	2^8	2^6	2^8	2^9	2^{10}	2^9	2^{10}	512
b	2^8	2^{10}	2^7	2^9	2^{10}	2^8	2^8	2^9	388
c	2^5	2^9	2^{12}	2^7	2^9	2^{10}	2^8	2^7	315
d	2^6	2^8	2^{10}	2^{11}	2^9	2^8	2^7	2^9	362
e	2^6	2^7	2^9	2^{10}	2^9	2^8	2^9	2^{10}	362
f	2^5	2^9	2^{10}	2^7	2^9	2^{10}	2^8	2^9	315
g	2^6	2^7	2^9	2^{10}	2^9	2^8	2^{10}	2^9	362
h	2^{10}	2^6	2^9	2^{10}	2^9	2^8	2^8	2^{11}	512
GMT	147	256	512	548	512	415	362	548	

A= LUKERT; B= 145 P/V; C= VI Bursa G-7; D= LZD 228; E= Barsine-2; F= CU 1M; G= S-706; H= D-78

All 68 pooled bursal samples from 68 affected flocks were subjected to analysis through AGPT and it was found that 43(63.28%) samples were positive with clear cut precipitation band formation. In SNT the same D-78 strain of IBD virus was used to titrate antibody in the test sera. Flock-wise antibody titers (GMT) of SNT test ranged from 8.0 to 415.9. The percentage of positive samples was recorded as 92.11% (n=470/508), 91.53% (n=465/508) and 66.92% (n=340/508) through SNT, IHA and AGPT methods, respectively, whereas a positive correlation (r = 0.959) existed between both the quantitative methods of IHA and SNT.

The comparative efficiency of serodiagnostic tests showed varying levels of sensitivity and specificity. The best relative sensitivity (99.40%) was recorded of SNT to AGPT, whereas the best relative specificity (91.30%) was of AGPT to SNT. These results approved SNT being relatively the most accurate and reliable procedure to quantitate IBD antibody titers as described previously (Giambrone, 1980; Castello *et al.*, 1987; Ismail & Saif, 1990; Lee & Lin, 1992).

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