

INDIRECT HEMAGGLUTINATION TEST FOR THE DETECTION OF ANTIBODIES AGAINST *BOOPHILUS MICROPLUS*

C.S. Hayat, Masood Akhtar, Iftikhar Hussain and Rehan Ahmad
Faculty of Veterinary Science,
University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

Indirect hemagglutination (IHA) test for the detection of antibodies against *Boophilus (B.) microplus* was developed. Midgut, *B. microplus*, antigen was used to sensitize the human group "O" erythrocytes without coupling agent. The optimum temperature for the sensitization of erythrocytes was found to be 37°C. The sensitizing dose of the antigen was calculated as 2.0 mL per 0.01 mL of the packed erythrocytes, diluted in 2 mL phosphate buffered saline, at 37°C for 60 minutes. The IHA results were best seen after 40 minutes of adding the sensitized erythrocytes in the serially diluted samples. IHA was found to be simple, rapid and inexpensive test for the detection of anti-tick antibodies.

INTRODUCTION

Various serological tests have been documented in the literature to detect the anti-tick antibodies from the serum, each having its own merits and demerits (Brossard, 1976; Johnston *et al.*, 1986; Opdebeeck *et al.*, 1988b). Although these are highly sensitive and specific but are very much laborious, expensive and required elaborative equipments. Keeping in view the simplicity, sensitivity, and specificity of the assay, indirect hemagglutination (IHA) test was developed to detect the antibodies against tick, *Boophilus (B.) microplus*.

MATERIALS AND METHODS

Preparation of antigen

Adult semiengorged female ticks, *B. microplus*, were obtained from the tick colony maintained in the Department of Veterinary Parasitology, University of Agriculture, Faisalabad (Akhtar and Hayat, 1993). They were given 3-4 washings with phosphate buffered saline (PBS), pH 7.2. Midgut of the ticks were dissected and midgut antigen was prepared following Opdebeeck *et al.* (1988a). Briefly, the guts were disrupted in an ultrahomogenizer (Ultra-Turrax, Janke and Kunkel) in 0.15 M PBS, containing 1 mM disodium EDTA, sonicated (Rapid 600, Ultra-Sonic, Ltd.) for 10 minutes in 30-60 seconds bursts and centrifuged at 5000 rpm for one hour. The supernatant was used as antigen.

Serum samples

Ten serum samples from buffaloes vaccinated with midgut of *Boophilus microplus*, were used in the present

studies (Akhtar, 1995). Serum samples were collected 15 days post-vaccination.

Indirect Hemagglutination Test Sensitization of erythrocytes

Human blood group "O" erythrocytes were used in the present study. Plasma and buffy coats were removed by centrifugation at 1500 rpm for 5 minutes. The packed erythrocytes were suspended in sterile PBS and were washed thrice by centrifugation for 5 minutes. The packed erythrocytes were then divided into six aliquots in duplicate to calculate the sensitizing dose of the antigen and other optimum conditions for the sensitization of erythrocytes. Each 0.1 mL of packed erythrocytes, diluted in 2 mL of PBS, were suspended in 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of antigen in duplicate in two aliquots. One aliquot was kept at room temperature (24°C) and the other at 37°C in an incubator. The hemagglutination was tested with known positive serum samples on glass slide after every 20 minutes upto 100 minutes with occasional shaking. The suspension was centrifuged at 1500 rpm for 5 minutes and supernatant was discarded. The packed erythrocytes were then washed twice with PBS and centrifuged again at 1500 rpm for 5 minutes. The packed cells were diluted with PBS to make 0.2 per cent suspension.

Test procedure

A two-fold serial dilution of the serum samples were made using 50 µL in microtitration plates. A 50 µL of the sensitized erythrocytes (0.2 %) were added to each well of the plates using four channel pipette. The well contents were mixed with gentle tapping and incubated at 37°C. The hemagglutination pattern was observed after every 10 minutes.

through the bottom where as no hemagglutination was manifested by peculiar central button shaped settling of erythrocytes. The IHA antibody titres of all the samples were recorded in comparison with the positive and negative controls.

RESULTS AND DISCUSSION

Indirect hemagglutination test is frequently used to determine the antibody titres against various parasitic infections with a fair degree of specificity and sensitivity (Ali *et al.*, 1990; Akhtar *et al.*, 1992). But so far the test has not received much attention for the measurement of anti-tick antibodies. Although so many other techniques are extensively used to detect the antibody reactive with tick extracts including indirect immunofluorescence and immunoelectrophoresis (Brossard, 1976), radioimmunoassay and double immunodiffusion (Johnston *et al.*, 1986), complement fixation test (Jackson and Opdebeeck, 1989), and enzyme linked immunosorbant assay (Opdebeeck *et al.*, 1988b). An assay that could detect antibodies using small amount of antigen and require minimum equipment(s) would have great significance. In the present study, IHA test was successfully used to detect the antibodies against midgut of the tick, *Boophilus microplus* antigen.

Optimum test conditions were determined on the basis of maximum IHA titre and clear button formation in comparison with the control. The optimum temperature for the sensitization of human group "O" erythrocytes without coupling agent was 37°C (Table 1). No sensitization occurred at room temperature (24°C) even after 100 minutes of suspending the washed erythrocytes in midgut antigen. The sensitizing dose of the antigen was calculated as 2.0 mL per 0.01 mL of the packed erythrocytes, diluted in 2 mL PBS, at 37°C. The optimum sensitization took 60 minutes. Although sensitization occurred within 40 minutes, but showed low antibody titre. There was lysis of erythrocytes after 60 minutes. The IHA results were best seen after 40 minutes of adding the sensitized erythrocytes in the serially diluted samples.

The IHA antibody titres in the buffaloes vaccinated with midgut vaccine ranged from 1:2 to 1:32. Among 10 samples processed, two each showed antibody titre 1:2, 1:4, and 1:32 and four each showed 1:8.

The advantage of IHA test for detecting antibody lies in the ease of operation, requirement of minimal quantities of samples and does not require special/elaborate equipment(s). The test is very much cheap as compared with other serological test like

radioimmunoassay, ELISA, complement fixation assay, immunoelectrophoresis, gel precipitation test and fluorescent antibody technique. Although the results of IHA has not been compared with the above mentioned tests in the current study, it has been reported to be more sensitive (Barrett, 1988; Hyde and Patnode, 1989). The test can, therefore, be done even in laboratories where minimal facilities are available for measuring antibodies and in the field to conduct epidemiological surveys.

Table 1: Amount of antigen versus time at 37° for sensitization of erythrocytes.

Time (min.)	Antigen amount (mL)					
	0.5	1.0	1.5	2.0	2.5	3.0
20	-	-	-	-	-	-
40	-	-	-	++	+	+
60	-	-	++	+++	L	L
80	-	-	L	L	L	L
100	-	-	L	L	L	L

- = No reaction, + = Low reaction, ++ = Moderate reaction, +++ = Strong reaction, L = Lysis of red blood cells.

REFERENCES

- Akhtar, M., C.S. Hayat, M. Ashfaq, M. Afaq and B. Hayat, 1992. Preliminary studies on indirect haemagglutination test for assaying colostral antibodies against *Toxocara vitulorum*. Pakistan Vet. J., 12: 140-141.
- Akhtar, M. and C.S. Hayat, 1993. Ecology and laboratory propagation of the tick, *Boophilus microplus*. J. Anim. Plant Sci., 3: 85-89.
- Akhtar, M., 1995. Studies on the immunization of buffalo against *Boophilus microplus*. Ph.D thesis, University of Agriculture, Faisalabad.
- Ali, A., G. Muhammad, I. Hussain, C.S. Hayat, and M. Akhtar, 1990. Hydatidosis in camels. II. Immunodiagnosis of hydatid disease in camels using indirect haemagglutination, latex agglutination, and double diffusion tests. Pakistan Vet. J., 10: 175-179.
- Barrett, J.T., 1988. Text Book of Immunology, 5th edn. The C.V. Mosby Co., St., Loucis, USA.
- Brossard, M., 1976. Relations immunologiques entre Bovines Tiques, plus particulièrement entre Bovines et *Boophilus microplus*. Acta Tropica, 33: 15-18.
- Hyde, R.M. and R.A. Patnode, 1989. Immunology. 2nd ed., Harwal Publ. Company, Media Pennsylvania.

- Jackson, L.A. and J.P. Opdebeeck, 1989. The effect of antigen concentration and vaccine regimen on the immunity induced by the membrane antigens from the midgut of *Boophilus microplus*. Immunol., 68: 27-276.
- Johnston, L.A.Y., D.H. Kemp and R.D. Pearson, 1986. Immunization of cattle against *Boophilus microplus* using extracts derived from adult female ticks: effects of induced immunity on tick populations. Int. J. Parasitol., 16: 27-32.
- Opdebeeck, J.P., J.Y.M. Wong, L.A. Jackson and C. Dobson, 1988a. Vaccine to protect Hereford cattle against the cattle ticks, *Boophilus microplus*. Immunol., 63: 363-368.
- Opdebeeck, J.P., J.Y.M. Wong, L.A. Jackson and C. Dobson, 1988b. Hereford cattle immunized and protected against *Boophilus microplus* with soluble and membrane associated antigens from the midgut of ticks. Parasite Immunol., 10: 405-409.