USE OF RADIOIMMUNOASSAY (RIA) AND ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TECHNIQUES TO DIAGNOSE SOME TICK-BORNE HAEMOPARASITIC DISEASES

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Abstract:

Improved serology is essential for the diagnosis of tick-borne diseases in epidemiological studies. Serodiagnosis of haemoprotozoan diseases is an important adjunt to the use of vaccines for the control of these infections. These test must meet a number of criteria, including sensitivity, specifity and the ability to detect long term infections.

Enzyme linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) techniques were developed for the diagnosis of both Babesia bovis and B.bigemina antibodies. Whlist the RIA technique is more sensitive than the ELISA, the latter has the advantage of stability and relative safety of the detection system. Long term single infections can be diagnosed with these system and they are ideal for detecting low levels of antibody. Further devleopment of the B.bigemina ELISA is necessary, especially as the antigen is quite labile and must be freshly prepared. This is the one disadvantage in an otherwise highly sensitive and specific test system.

Introduction

Throughout Turkey tick-borne haemoparasitic diseases are serious problem with the vector being present in most areas. Babesiosis is a haemoparasitic disease and it appears to be a an economic importance in Turkey. Previous reports on the prevalence of babesiosis were based upon findings on giemsa stained blood examinations (5, 7, 9). In those studies, thin blood smear technique was used in the diagnosis. In babesiosis, a state of premunition is common and diagnose is difficult with blood smears. ELISA and RIA are two of the most sensitive and specific test are being used in the serological studies. With these tests, carrier state of babesia can be diagnosed. During the period of research programme on haemoparasitic diseases at Ankara Nuclear Research Center for Agriculture and Animal Science, Protozoology Laboratory, work has been concentrated on serodiagnostic tests. Mainly, ELISA has been used to carry out epidemiology of bovine and ovine babesiosis in Turkey. Also, RIA and IFAT (Indirect flourescent antibody test) were

developed for the diagnosis of babesiosis. In this review, results of serologic surveys in Turkey were discussed from the previous studies

Materials and Methods

Antigen: Crude antigen was used for the diagnosis of Babesia ovis in the ELISA test. A synthetic highly purified Babesia bovis derived antigen was obtained from CSIRO, Australia and used for the diagnosis of Babesia bovis.

Crude Babesia ovis Antigen Preparation: Dignostic antigen have been prepared by using the method of Mahoney (6). A field strain of B. ovis was isolated from a fatal clinical case in Ankara region of Turkey. This isolate was used to make crude antigen preparation. Six months old sheep known negative was splenectomised and infected with B.ovis strain by blood inoculation. When the parasitemia had reached 8-10 %, 1000 ml. of blood was collected. Antigenic fraction suitable for use in an ELISA was prepared by lysis in hypotonic saline. It was found that a saline concentration of 0.525-0.555 % produced approximately 95% of infected erythrocytes. These infected erythrocytes were washed 3 times with PBS buffer (pH 7.3) and centrifuged at 3000 rpm. for 10 minutes at room temperature. White blood cells were removed by diluting cells after the final centrifugation. For serodiagnosis antigen, 4 volumes of distilled water were added to one volume of infected erythrocyte suspension and supernatant lysate obtained by centrifugation at 10.000 rpm for 30 min. at 4°C. The infected cells were diluted ½ in PBS and stored as 2 mls. Aliquots in the vapour phase of liquid nitrogen. When required, an ailquote was thawed at 37°C in water bath and used in ELISA. Each ml. of infected cell suspension contained approximately $2.5x10^9$ parasites.

ELISA Test Procedure: The ELISA method in this study was similar to that used by Waltisbuhl (10). Flat-bottomed microelisa plates (Dynatech M129b Denkerdark, West Germany) were used in ELISA test. 200µl of diluted antigen was added to each well of a microelisa plate. Antigen diluted in sodium carbonate buffer (0.1 M- pH 9.6). plates were sealed with plate sealers and incubated overnight at 4˚C. After İncubation the antigen solution was removed and 250 µl of 0.5 % gelatine in carbonate buffer added to each well of a microelisa plate. After 1 hour incubation at room temperature the solution was removed. After blocking, the plates washed three times with PBS buffer containing 0.1 % Tween 20 and washed twice with PBS buffer. After five washes with washing buffer, 200 µl of the test sera diluted to 1:500 in PBS Tween 20 buffer were added. The plates were then sealed and incubated overnight at 4 ˚C. After incubation the wells were washed as described above and 200 µl of conjugated second antibody (A goat antibovine H+L chain IgG-Horse Radish Peroxidase Conjugated - Kirkegaard and Perry Lab.Maryland, USA) added to each well of the plate at a dilution of 1:1000 in PBS Tween 20 and the plate incubated for 1 hour at 37˚C. Plates were then washed five times with washing buffer and 200 µl of substrate (5- aminosalicylic acid) solution containing 0.15 % hydrogen peroxidase was added to each well and the plate was

sealed and agitated for 30 min. at room temperature. Then, the plate was immediately read at 492 nm with a Titertek Multiskan spectrophotometer using column as a blank.

RIA Test Procudure: Polyvinyl chloride 'V' well microtiter plates were coated with 200 µl of B.bovis antigen diluted in PBS buffer. The plates were incubated overnight at 4˚C, then washed three times with PBS tween 20. Diluted sera were added to antigen coated well and incubated for 30 min. at 37 ˚C. The plates were incubated for 5 h at 22 ˚C and washed three times with washing solution, then 200 µl of diluted ^{125} I labelled second antibody were added per well. The plates were then incubated for 16 h at 4 ˚C and washed 5 times with washing solution. The wells were cut out and counted in an automatic gamma counter.

Results and Discussion

Serodiagnosis of haemoprotozoon diseases is necessary in order to find out the epidemiological status of these diseases. During the past 15 years, seroepidemiological studies were performed at Lalahan and Sarayköy, Ankara Nuclear Center for Agriculture and Animal Science, Parasitology Laboratories to carry out the incidence of tick-borne diseases (Mainly babesiosis) in Turkey (1, 2, 3, 4, 8, 12). These studies were done in collaboration with Ankara, Konya and Elazığ Veterinary Faculties, Parasitology Sections and A Ministiry of Agriculture. Table 1 and Table 2 show the results of serological surveys for Babesia ovis and B.bovis of some different regions of Turkey respectively.

TABLE 1		
REGION	NUMBER OF SAMPLE	% POSİTİVE
ELAZIG	331	45.0
URFA	607	41.14
ANKARA	509	76.0
KONYA	723	42.14
ÇANAKKALE	360	53.5

Table 1. Number of samples positive for the presence of antibodies to *B. ovis* from various regions of Turkey.

TABLE 2		
REGION	NUMBER OF SAMPLE	% POSİTİVE
MARMARA	416	44.4
CENTRAL	309	61.4
EASTERN	246	30.4
AEGEAN	148	58.7
SOUTHERN	150	58.0
NORTHERN	159	67.9

Table 2. Number of sample positive for the presence of antibodies to *B.bovis* from six geographical regions of Turkey.

From the data obtained in these seroepidemiological surveys indicate that there is high incidence of ovine and bovine babesiosis in Turkey and the disease is common in both temperate sea level zones as well as the colder zones of central Anatolia. In these surveys, ELISA test was used. Also, RIA test was development for the diagnosis of babesiosis. But, this test has not been used in seroepidemiological studies in Turkey. The review by Wright et al (11) ,described that RIA test was somewhat more sensitive than ELISA and it has less application since the radioisotope label ^{125}I has short half-life. It is for this reason that the ELISA is becoming more popular, even if it is somewhat less sensitive.

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