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A review of research on the diagnosis of babesiosis. In the last three decades some fundamental knowledge concerning the immunoserology of Babesia spp. infections has led to development of serological techniques which provide a means of studying the pathogenesis of babesiosis and the detection of animals with subclinical infections. The antigens used in the serological procedures originated from the parasitized erythrocytes, plasma, and tissues of animals with acute babesiosis. Parasitic and serum soluble antigens were applied in a variety of serological tests, e.g., complement fixation, gel precipitation, agglutination, and fluorescent antibody, for detection of Babesia spp. antibodies. This review summarizes and discusses the recent advances in the serodiagnosis of babesiosis, together with conditions where the use of serological methods may be valuable.
SEROLOGICAL DIAGNOSIS OF BABESIOSIS: A REVIEW

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SUMMARY

In the last three decades some fundamental knowledge concerning the immunoserology of Babesia spp. infections has led to the development of serological techniques which provide a means for studying the pathogenesis of babesiosis and the detection of animals with subclinical infections.

The antigens used in the serological procedures originated from the parasitised erythrocytes, plasma, and tissues of animals with acute babesiosis. Parasitic and serum soluble antigens were applied in a variety of serological tests, e.g., complement fixation, gel precipitation, agglutination, and fluorescent antibody, for detection of Babesia spp. antibodies.

In this review an attempt was made to summarise and discuss the recent advances in the serodiagnosis of babesiosis, together with conditions where the use of serological methods may be valuable.

INTRODUCTION

During the past three decades, many investigators have been concerned with the development of a serological technique that would aid in diagnosing babesiosis. In spite of the great progress achieved in recent years, no satisfactory practical procedure has been devised for the serological diagnosis of babesiosis, particularly in the latent form. A characteristic feature of Babesia spp. infections is that animals which recover from an acute infection become carriers of the respective haemoparasites. These carrier animals cannot be diagnosed by stained blood films using contemporary staining methods. Thus, in order to identify infected animals it was necessary to develop serological methods to detect specific antibodies rather than the Babesia spp. parasites themselves. The antigens used in these techniques originated from parasitised erythrocytes, plasma, serum, and tissues of animals with acute babesiosis and they were applied in several serological tests.

LITERATURE REVIEW

Complement fixation tests

The complement fixation (CF) reaction constituted one of the earliest tests for the diagnosis of babesiosis. A specific CF reaction for Babesia has been found useful in detecting CF antibodies in the serum of animals with chronic babesiosis. However, practical application of the CF test was not conclusively demonstrated.

Hirato et al. (1945), first reported the possibility of the application of the CF test for diagnosing equine babesiosis. The antigen consisted of stromata of erythrocytes from acutely infected horses with Babesia caballi. By means of the CF test it was found that antibodies appeared in the blood 11 to 15 days after the onset of parasitaemia and in one instance persisted for 100 days.

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Mahoney (1962) prepared antigens from the blood of cattle with high parasitaemias of pure infections of either Babesia bigemina or Babesia argentina. In the serum of calves with pure infections of these parasites, homologous CF antibodies developed 7 to 21 days after infection. Antibodies were detected at all stages from the advent of a parasitaemia detectable in thick blood films to a time when the parasites were in numbers too low for detection by microscopic examination. The antigens appeared to possess a degree of species specificity showing only limited reaction with heterologous antibody.

The use of the CF test in epizootiological studies of bovine babesiosis was reported later by Mahoney (1964). Both antigens of B. argentina and B. bigemina were used in this study. During the initial infection, both antigens produced positive CF reactions in 94 per cent to 100 per cent of all cases studied; however, after 4 to 5 months the number of calves positive to the test dropped below 50 per cent. The same pattern was seen in calves born in enzootic areas except that the percentage of reaction dropped considerably. Homologous reactions were consistent and dependable (74 per cent) during the first few months after infection, but positive reactions fell to 8 per cent after several months of infection with B. argentina. With B. bigemina antigen, 95 per cent reacted in the third month of infection but fell to 20 per cent by the seventh month.

Schindler and Dennig (1962), studied the CF reaction in animals infected with Babesia rodhaini and Babesia canis. An antigen for the CF test was prepared from erythrocytes of dogs acutely infected with B. canis by means of immunohaemolysis or by exposing the erythrocytes to 0.3 per cent saponin. Clear supernatant fluid of B. rodhaini lysed in distilled water and centrifuged to remove erythrocyte stromata served as the CF antigens. Positive CF reactions were not detected in mice infected with B. rodhaini as the animals probably succumbed before the antibodies could be demonstrated. Babesia canis antibodies were demonstrated in dogs 11 to 34 days after infection and were also detected in animals that had harboured parasites for years. No serologic CF cross reaction was observed between B. rodhaini and B. canis using the antigens of the respective parasites. Lyophilisation was found to be an efficient means for long-term preservation of the antigen.

Schindler et al (1966) studied the immunological and serological reactions to B. canis infection, applying both CF and the indirect Coons' tests. Animals had been immunised against the infection with live B. canis or soluble antigens of B. canis. Immunity was acquired only after infection with live B. canis. This resistance remained even after B. canis disappeared from the blood and even after disappearance of CF antibodies. Dogs vaccinated with soluble antigen from B. canis produced CF antibodies but not protective immunity.

Mahoney (1967a) found that the most sensitive CF antigens for B. bigemina and B. argentina were crude preparations of the parasites prepared by distilled water lysis of infected erythrocytes followed by centrifugation. In tests on sera obtained from non-exposed cattle, non-specific CF reactions were observed at the 1:5 serum dilution. These non-specific reactions were found more frequently with B. argentina (4-5 per cent) than with B. bigemina (1-6 per cent). The non-specific reaction to B. argentina was prevented by adsorption of the serum with a suspension of normal bovine erythrocyte stromata. Cross adsorption tests indicated that the B. bigemina and B. argentina CF reaction each consisted of major species-specific and a minor group-specific component. These results suggested that substances associated with B. argentina rather than contaminating material of bovine origin in the crude suspensions were responsible for the differences in specificity.
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Mahoney (1967b, c) reported the application of the CF test in the evaluation of resistance against bovine babesiosis produced by vaccination with killed B. argentina, or by passive immunisation of calves against B. argentina. Animals immunised with killed B. argentina reacted to the injection by the formation of CF antibodies. These CF antibody responses during the course of immunisation with killed Babesia parasites were used as a guide that adequate antigenic stimulation was achieved for the production of protective immunity against babesiosis. However, in the experiments with passive immunisation against B. argentina with special reference to the role of CF antibodies, the results were different. The failure of convalescent serum which contained a high level of CF antibodies to give protection suggests that these antibodies were not important in the immune reaction. Therefore, no positive correlation between protecting capacity and CF titre of serum could be demonstrated. The CF antibodies were unlikely to be concerned with immunological resistance to B. argentina.

Frerichs et al. (1969a, b) prepared an antigen from erythrocytes infected with B. caballi. Blood was collected at the height of parasitemia (3 to 7 per cent) and infected erythrocytes were mixed with 10 volumes of cold saline, cold distilled water, or 0.025 M NaCl, and lysed for 1 to 8 hours at 4°C. The lysate was passed through a refrigerated centrifuge at the rate of 2-4 litres per hour at 2.8 kg of air pressure per square cm. The paste, which collected on the wall of the centrifuge cylinder and consisted of parasites and erythrocytic stromata, was mixed with veronal buffered saline, filtered and lyophilised and stored at -20°C. The serological responses of 15 horses were studied by the CF test. The CF titre persisted longer in 14 of the horses than did their capability to transmit the disease and this was a reliable index of exposure to B. caballi. The authors concluded that the greatest difficulty in producing B. caballi antigen was due to insufficient antigenic yield from the low parasitaemia observed with this infection. The use of splenectomised horses, along with the administration of synthetic corticosteroids, greatly alleviated this difficulty. Loss of serological specificity by Babesia equi after it was passaged in horses was the greatest problem in producing this antigen. This problem could be overcome by (1) obtaining fresh isolates under field conditions or (2) storing the parasites in liquid nitrogen before specificity is lost.

Todorovic et al. (1971) isolated CF antigens from B. bigemina- and B. argentina-parasitised erythrocytes and used them in the CF microtitre system for diagnosis of bovine babesiosis in Colombia, South America. The antigens prepared by lysis of infected erythrocytes by ultra sonication had higher reactivity than antigens prepared by the distilled water lysis method. By means of the CF microtitre system it was possible to detect specific Babesia spp. antibodies in serum of cattle 8 days after bloodborne infection. The CF microtitre system was found useful for diagnosing natural and experimental B. bigemina and B. argentina infections in the Colombian cattle.

Gel precipitation tests

Ristic and Sibinovic (1964) obtained an antigen from the lysate of erythrocytes of horses acutely infected with B. caballi by means of precipitation with protamine sulphate. The antigen was used to detect antibodies in horses with babesiosis in the double-gel diffusion test. The reaction of equine sera in the gel precipitation test was correlated with the persistence of the latent infection as determined by transmitting infections from carriers into susceptible horses. The specificity of the test was shown by the absence of reaction with sera of horses with various other infections, including viral infectious anaemia.

Parasitic serum antigens may be used in a variety of serological tests for the detection of Babesia antibodies, e.g., gel precipitation and latex-agglutination.
Sibinovic et al (1965) observed a precipitation reaction in a double-gel diffusion test when serum from a horse acutely infected with *B. caballi* was allowed to react with serum from a convalescent horse. Antibodies to the serum antigens were detectable at 3 months after infection and persisted at variable levels for an additional 7 months. The antigen disappeared from the serum of infected horses approximately 1 month after infection.

Mahoney (1966) detected a circulating antigen in an acute phase of infection with *B. bigemina* and *B. argentina* by means of gel diffusion technique.

Mahoney and Goodger (1969) reported that two antigenic substances were present in sera collected from acute infection with *B. argentina*; they were absent from serum of non-infected cattle. This was revealed by the gel precipitation technique. Antiserum to infected calf serum prepared in rabbits and then adsorbed with normal calf serum contained precipitating antibodies to components absent from the serum of non-infected calves. Such serum was used in attempts to characterise antigenic substances produced during acute *B. argentina* infection. The first of these antigenic substances was immunologically identical with bovine fibrinogen and the second appeared to be a pigmented macro-molecular complex found in the tissue of normal cattle. Soluble serum antigens of parasitic origin were not detectable; perhaps they either did not induce a strong precipitation response or were in such minute quantity the detection was beyond the sensitivity of the gel diffusion test used in their study.

Ferris et al (1968) discussed the significance and application of *Babesia* serum antigens in sero-diagnosis of babesiosis. A new terminology was suggested for these soluble antigens found in serum or plasma during acute *Babesia* infections. Because they are different from erythrocytic antigens they were termed "ectoantigens". These ectoantigens are complex in nature containing peptides, lipids, phosphatides, and polysaccharides and can be used for stimulation of protective antibodies and also can be used in the diagnosis of acute and chronic infection of *Babesia* species in animals and man.

Todorovic et al (1971) studied the antigenic relationships of *B. bigemina* and *B. argentina* soluble antigens by means of a double-gel diffusion precipitation technique. Antigens were isolated from the infected plasma collected at acute phase of *B. bigemina* and *B. argentina* infections (Todorovic et al, 1968a). Cross reaction was observed in gel between *B. bigemina* and *B. argentina* antigens.

Vizcaino and Todorovic (1973) isolated *B. bigemina* and *B. argentina* soluble antigens from lysate of infected erythrocytes and purified them from host material by Sephadex column chromatography according to the technique described by Goodger (1971). Species specific and genus common antigens were demonstrated by means of gel double diffusion precipitation and immunoelectrophoresis methods.

**Fluorescent antibody tests**

Fluorescent antibody methods have been used for the serological diagnosis of human and animal protozoal diseases.

Ristic et al (1964) used the fluorescent antibody (FA) technique to study the erythrocytic growth and developmental stages of *B. caballi* and *B. equi* isolated from horses in Florida. These workers described the morphological structure of *Babesia* spp. from horses by the FA technique.

Ristic and Sibinovic (1964) used the one-step fluorescein-labelled antibody inhibition test in which an antigen is reacted simultaneously with a mixture of fluorescein-labelled and unlabelled antibody to detect circulating *anti-Babesia* antibody. According to the authors, the one-step fluorescein-labelled antibody inhibition test can be
considered only as a research tool requiring further study and evaluation of its accuracy and specificity.

Garnham and Voller (1965) used the FA technique to study the antibody responses of splenectomised monkeys to Babesia divergens.

Madden and Holbrook (1968) used the indirect fluorescent antibody (IFA) technique to detect antibody in horses experimentally infected with B. caballi. The reactions were determined to be specific. There was no cross reaction with serum from horses experimentally infected with B. equi when used in the IFA test for B. caballi and the test was used to differentiate the two infections. According to the authors, the procedure involved in preparing the reagents and conducting the test was straightforward; however, difficulty arises in reading and interpreting the results. Identification of a strongly positive reaction is relatively simple but differentiation between a weakly positive reaction and a negative reaction requires considerable experience.

Ross and Lohr (1968) used the IFA test for the detection of B. bigemina antibodies in bovine serum. The test was considered to be highly specific and accurate and sufficiently sensitive to detect a positive response in animals infected 2 years previously. Susceptible and immune clinical responses were obtained when groups of negative and positive reactors to the IFA were challenged with B. bigemina-infected blood. By means of the test it was possible to demonstrate, test reliability of and the relationship between protective antibodies and those detectable by immunofluorescence. Serologic responses to the challenge with B. bigemina infected blood were tested by the IFA test and it was found that titres reaching peaks at 21 days after infection, with maximum titres up to 1:1,280, decreased gradually thereafter but still above minimum levels at the end of a 6 month period. Some evidence was produced indicating a continued downward trend with minimum positive IFA response still detectable 18 to 24 months after a single experimental infection.

Zwart et al (1968) used the IFA test for differentiation of bovine Babesia spp. They found that B. bigemina and B. major have some common and species specific antigens shown by cross immunity experiments. Animals immune to B. bigemina were also immune to B. major; whereas, immune animals to B. major had parasitaemia but not symptoms to B. bigemina challenge. Babesia bigemina but not B. major was susceptible to trypan blue therapy.

Ross and Lohr (1970) found that titres of colostral antibodies to B. bigemina were higher in calves up to 1 month of age than in their dams by means of the IFA test. Colostral antibodies persisted for a mean period of 17 weeks after birth.

Cox and Turner (1970) used specific anti-mouse Ig, IgM, and IgG in the IFA test to detect antibody levels in mice infected with Babesia microti. It was found that the Ig level rises rapidly with B. microti parasitaemia until the twelfth day of infection and continues to rise more slowly until all the parasites disappear. The IgG levels closely parallel those of the Ig and IgM level rise until the twelfth day but do not continue to rise while the B. microti parasitaemia declines; however, the IgG levels rise after the parasites have disappeared from the blood.

Ludford (1969) used the FA stain technique on B. bigemina, B. argentina, B. rodhaini, and B. canis and studied their intraerythrocytic development. The staining showed features of the parasitised erythrocytes not seen in blood films stained by the Giemsa method.

Brocklesby et al (1971) used the IFA test to differentiate Babesia spp. (England) from B. major (Netherland). Serum samples from British cattle infected with Babesia spp. were subjected to the IFA test. The results showed that all samples reacted strongly with an antigen prepared from a strain of B. major, with titres varying from
1 : 320 to 1 : 2,560. The serum reacted mainly with *B. bigemina* antigen with titres ranging from 1 : 40 to 1 : 160. The authors concluded that large *Babesia* spp. of British cattle were a strain of *B. major*.

Ludford et al. (1972) used the IFA test to analyse the antigenic relationships between *B. argentina* and *Plasmodium falciparum* and *Plasmodium vivax*. Cross reaction was observed between *Babesia* and *Plasmodium* parasites.

Leeffang and Perie (1972) described the serological differentiation and antigenic relationship of four *Babesia* spp. by means of the IFA test. The titre of each serum to *B. bigemina*, *B. major*, *B. argentina*, and *B. divergens* was determined against the homologous and heterologous antigens. Titres against the heterologous antigens were constantly lower than those obtained with the homologous antigen.

Donnelly et al. (1972) reported that the IFA test was successfully used to determine infection rate in a herd of cattle following a natural outbreak of *B. divergens*. The level of infection in different herds was correlated with the period at risk. The prevalence of infection was high with 36 per cent of those at risk per month; after exposure for 103 days 80 per cent had antibodies. Morbidity was low; only 15 per cent of those at risk showed any sign of clinical disease.

Goldman et al. (1972) found that the IFA test can be used for diagnosis of *Babesia berbera* and *B. bigemina*, and also as a means of identifying *Babesia* spp. in internal organs. Test antigen was prepared from washed, haemolysed blood in the case of *B. bigemina* and from infected kidney in the case of *B. berbera*. By means of the IFA test, no cross reaction between the two species was observed.

Joyner et al. (1972) studied the antigenic relationship between *B. major* and *B. divergens* by means of the IFA test. High titres of 1 : 640 to 1 : 2,560 were recorded in a homologous system and insignificant titres up to 1 : 160 were shown in the heterologous system. High titres were observed after recovery and it was suggested that the IFA test could be used for survey purposes.

Burridge et al. (1973) found that blood dried on filter paper can be used as a source of antibodies to *B. bigemina* in the IFA test. There was good correlation between the antibody titres of duplicate serum and dried blood samples collected from calves infected with *B. bigemina*. The authors suggested that the use of dried blood samples in the IFA test might facilitate epizootiological studies on *B. bigemina* infections.

### Agglutination tests

Curnow and Curnow (1967) reported the indirect haemagglutination test (IHA) for diagnosing *B. argentina* infection in cattle. Formalinised sheep erythrocytes treated with tannic acid were sensitised with an extract of the parasite-erythrocyte stromata mixture used as the antigen in the CF test for *B. argentina*. This antigen was also used to inhibit the reaction in a haemagglutination inhibition test. Bovine erythrocyte stromata antigen prepared from uninfected blood was used to inhibit some non-specific reactions in the IHA test and to adsorb sera before CF testing. By doing this it was possible to remove some of the non-specific reactions. The sensitivity and specificity of the IHA and CF tests were found to be similar.

Todorovic et al. (1967) reported a haemagglutination (HA) test for titrating antibodies in rats infected with *B. rodhaini*. Heparinised blood was collected from uninfected rats to provide erythrocytes for the test. A 2 per cent suspension of trypsinised erythrocytes was used. Serial dilutions of sera were prepared in physiological saline. A positive test was indicated by a single clump which did not break up readily on manual agitation. Haemagglutinins were first detected on 6 days post infection with
the high titres occurring on the twelfth day which coincided with the peak of parasitaemia.

Curnow (1968) reported on the HA test for detection of specific *B. argentina* antibodies by using highly parasitised bovine erythrocytes as an antigen. The reaction appeared to be highly specific for each isolate of *B. argentina*.

Sibinovic et al (1969) used a heat stable polysaccharide antigen extracted from equine erythrocytes infected with *B. caballi* and *B. equi* in the IHA test. The antigen was employed for titration of antibodies in the serum of horses affected with babesiosis by means of a bentonite agglutination test and passive haemagglutination tests. In comparison with the gel precipitation (GP) reaction, bentonite agglutination and passive hemagglutination tests required less antigen and rendered results more rapidly.

The serum antigens isolated from dogs with acute *B. canis* infection were adsorbed to latex particles (0.81 μm in diameter) in accordance with the method described by Todorovic et al (1958) for adsorption of "explasmodial" antigens. These sensitised latex particles were used as agglutinogens in a tube-latex agglutination test (TLA) for titration of antibodies in sera of dogs infected with *B. canis* (Zuckerman and Ristic, 1968). The earliest that antibodies were detected in the TLA test was approximately 15 days following infection; about 2 months later they reached a maximal titre and persisted at low levels during the next 4 months after infection. The persistence of serum agglutinins was generally correlated with the persistence of a carrier stage of infection.

Lohr and Ross (1969) found that an antigen derived from *B. bigemina* can be used in a capillary agglutination (CA) test. The results agreed completely with the parasitic examination of 92 negative and 131 positive serum samples from cattle. There was no cross reaction with other microorganisms, nor with immune serum from cattle infected with *B. divergens*. The CA test was also used to detect antibody response in cattle following single experimental *B. bigemina* infection. Antibodies were first detected soon after the parasites appeared in the erythrocytes. Five to 12 days later, serum titres reached a peak of 1:160 or 1:320. Positive reactions were still clearly recognisable 18 to 26 months after infection.

Ristic et al (1971) also used the CA test for detecting specific babesial antibodies in dogs infected with *B. canis*. An antigen prepared from the blood of splenectomised dogs, acutely infected with *B. canis*, was used to detect an antibody in the serum of dogs, monkeys, and man. The antigen was preserved by lyophilisation. For use in the CA test, the antigen was reconstituted in buffered saline solution, pH 7.2. Reconstituted antigen was serologically active for at least 6 weeks when it was stored at 4°C, the longest period tested.

Goodger (1971) isolated two antigens of *B. argentina* and they were active in the IHA test. One antigen was obtained by gel filtration of soluble material obtained after sonic disruption of parasite suspensions. The other was prepared from the lysate of infected erythrocytes, first by removing haemoglobin on DEAE cellulose and separating the remaining material on Sephadex gel. Both antigens gave high titres of 1:12,000 to 1:25,000 with *B. argentina* homologous antisera. There was some low titre, 1:100 to 1:200, cross reaction with *B. bigemina* heterologous antisera. Babesia *bigemina* antigen prepared with the same technique gave lower titres of 1:1,600 with *B. bigemina* homologous antisera than the *B. argentina* antigen did with *B. argentina* homologous antisera and cross reacted strongly with a titre of 1:1,600 with *B. argentina* antisera. These cross reactions could be adsorbed with *B. argentina* antigen without affecting the specific *B. bigemina* reactions.

Todorovic and Kuttler (1974) isolated an agglutinating antigen of *B. bigemina*
infected erythrocytes and found it highly reactive in a homologous system. The reaction was observed by adding a drop of antigen to two drops of undiluted serum on a card, and mixing for 5 minutes in a rotary motion. *Babesia bigemina* antigen cross reacted with *B. argentina* antisera. The antigen was a fast green dye stained parasitic suspension of *B. bigemina* parasites. It was possible to detect specific *B. bigemina* antibodies in the sera of infected cattle 6 to 8 days after blood-borne infection. The agglutination reaction was observed up to 12 weeks after infections.

Curnow (1973) used a slide agglutination (SA) test to study antigenic differences of *B. bigemina* parasites transmitted by tick- or blood-borne infection. The antigen was a Giemsa-stained and formalinised suspension of *B. bigemina* parasites, prepared by lysing parasitised erythrocytes. It was found that homologous antisera gave strong reactions and heterologous sera had weaker reactions. This demonstrated the test was able to detect antigenic differences between *B. bigemina* parasites. The author concluded that variants of *B. bigemina* in relapsed infections, when transmitted through ticks, caused the variable agglutinins to revert to a common antigenic type, and appears to be strain specific.

**DISCUSSION**

The preceding review has been an attempt to indicate the extent to which *Babesia* serology has developed in the last three decades (Table I). Diagnosis of babesiosis is made by two diagnostic methods: (1) direct identification of the *Babesia* spp. parasites by microscopic examination, or (2) by indirect detection of *Babesia* spp. antibodies by serological tests. It is necessary to emphasise that identification of *Babesia* spp. parasites in the thin or thick blood films is true evidence of infection; however, negative microscopic examination does not exclude it. Actually, in the early phase of infection, in immune cattle populations and in those which have been therapeutically treated, the detection of *Babesia* spp. parasites in the stained blood films is uncommon. Direct identification of the *Babesia* spp. parasites by microscopic examination of blood films is time-consuming and tedious, especially when the vast majority of smears are negative. Indirect identification of *Babesia* spp. antibodies also is not perfect and great danger exists in placing too much confidence in this diagnostic method. A positive serological reaction may simply indicate a previous exposure to *Babesia* spp., current infection, or the presence of antibodies against substances antigenically related to *Babesia* spp. parasites. A false positive reaction observed in serological tests may result from molecular rearrangement of *Babesia* spp. antigens due to harsh treatment during the process of extraction and storage. Because of the complex nature and undefined chemical characteristics of *Babesia* spp. antigens, the results of these tests are frequently difficult to interpret and of doubtful value, and the need for adequate control systems is obvious.

Since immunity to babesiosis is based on so-called “premunition”, or coinfectious immunity (*Immunitas non sterilizans*), it is important to know the immune status of the cattle population in enzootic areas. Furthermore, the need for a serological test is obvious to determine whether eradication programmes have been successful or whether transmission of babesiosis in enzootic areas has been interrupted.

In the United States, bovine babesiosis is eliminated by eradication of *Boophilus* spp. by systematic dipping programmes; however, babesiosis is still prevalent in Mexico and other countries of the world. A proper serologic surveillance needs to be maintained in those areas which are at constant risk of *Boophilus* spp. exposure, in order to prevent re-introduction of babesiosis, which could bring about devastating outbreaks in cattle populations rendered susceptible by the absence of infection, In
achieving these goals, a practical test for use in diagnosing babesiosis needs to be developed and the test must meet the following criteria: (1) the test has to be simple to perform; (2) the interpretation of results must be free of subjectivity; (3) the test must be rapid; (4) the cost must be minimal; (5) the results have to be sufficiently sensitive and specific; and (6) the test must be capable of performing reliable results in various laboratories and under different field conditions.

**Table I**

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<th>Author(s) and date</th>
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<td>B. bigemina</td>
<td>RBC</td>
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<td>Ross and Lohr (1968)</td>
<td>I.F.A.</td>
<td>B. major, B. bigemina</td>
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<td>Brocklesby et al (1971)</td>
<td>I.F.A.</td>
<td>B. major, B. bigemina</td>
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<td>Donnelly et al (1972)</td>
<td>I.F.A.</td>
<td>B. divergens</td>
<td>RBC</td>
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<td>Joyner et al (1972)</td>
<td>I.F.A.</td>
<td>B. major, B. divergens</td>
<td>RBC</td>
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<td>Leeflang and Perie (1972)</td>
<td>I.F.A.</td>
<td>B. bigemina, B. argentina</td>
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<td>Ludford et al (1972)</td>
<td>I.F.A.</td>
<td>B. argentina</td>
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<td>Burridge et al (1973)</td>
<td>I.F.A.</td>
<td>B. major, B. divergens</td>
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<td>Curnow (1968)</td>
<td>H.A.</td>
<td>B. argentina</td>
<td>RBC</td>
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<td>Cunor and Curnow (1967)</td>
<td>I.H.A.</td>
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<td>RBC</td>
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<tr>
<td>Goodger (1971)</td>
<td>I.H.A.</td>
<td>B. bigemina, B. argentina</td>
<td>RBC</td>
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<tr>
<td>Lohr and Ross (1969)</td>
<td>C.A.</td>
<td>B. bigemina</td>
<td>RBC</td>
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<tr>
<td>Ristic et al (1971)</td>
<td>C.A.</td>
<td>B. canis</td>
<td>RBC</td>
</tr>
<tr>
<td>Curnow (1973)</td>
<td>S.A.</td>
<td>B. bigemina</td>
<td>RBC</td>
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The CF reaction constituted one of the earliest tests for diagnosis of babesiosis (Hirato et al., 1945). Although complement fixing antibodies are unquestionably produced in the course of Babesia spp. infection, results of the CF test vary according to the antigens used. The CF test described by Mahoney (1962; 1967a) has been used for identification of Babesia spp. infections in Australian cattle. Approximately 0.5 per cent of cattle tested have shown a positive CF reaction but have been negative to a transmission test (Curnow and Curnow, 1967). On the other hand, in the infected cattle after 8-12 months, CF antibody levels declined and the majority of animals showed no CF antibody in the diagnostic range but still carried infection as revealed by subinoculation (Mahoney, 1964). The CF test, which has the lowest sensitivity in comparison with other serological tests, may reliably detect antibodies to B. argentina for 7 months and to B. bigemina for 4 months after a single infection (Mahoney and Ross, 1972). Because of impurities in the CF antigens, the variables and dependability of the CF test are lowered. Mahoney (1962; 1964; 1967a) and Todorovic et al (1971) found limited CF reactions between heterologous Babesia spp. when studying antibody production in pure infections with B. bigemina and B. argentina.

The presence of colostral antibodies that react in the CF test is a source of error. Complement fixing antibodies of colostral origin remain at levels detectable by the CF test for an average of 2 to 3 weeks after birth (Mahoney, 1964). In addition, other technical and mechanical errors which are inherent to other serological tests also apply to the CF technique.

Immunofluorescence techniques which were introduced to study Babesia spp. infections a decade ago have, for the first time, made a test available in which the whole intraerythrocytic Babesia spp. parasites, instead of extracts, constitute antigen. The FA tests are more sensitive than the CF test, and periods in excess of 2 years have been reported (Ross and Lohr, 1968). Cross reaction between Babesia spp. parasites has also been demonstrated. Zwart et al (1968) indicated the possession of both species specific and common genus antigens of B. bigemina and B. argentina by means of the IFA test, while Goldman et al (1972) reported that the serum samples against B. bigemina and B. bovis showed a certain cross reaction against both species. The IFA test has been successfully used for identification of B. major in British cattle, for the incidence rate of B. divergens and for the antigenic relationship among B. bigemina, B. bovis, B. major, and B. divergens (Brocklesby et al, 1971; Donnelly et al, 1972; Ludford, 1969; and Leefflang and Perie, 1972).

The commercial availability of fluorescein-labelled antiglobulin and the observation by Burridge et al (1973) that tests can be made from elute obtained from dried blood samples on filter paper as a source of Babesia spp. antibodies make it possible to carry out tests in any laboratory where a fluorescent microscope and parasitised blood with Babesia spp. are available. Serious limitation in the IFA test for immunodiagnosis of babesiosis results from the subjective nature of the interpretation and from the lack of specificity due to cross reacting antigens when a whole parasite is used. The cross reaction among Babesia spp. and Plasmodium spp. was observed (Ludford et al, 1972). In addition, the presence of colostral antibodies that react in the IFA test for an average of 4 to 5 months after birth is another problem which precludes the use of the IFA test to record the infection rate in calves of this age (Ross and Lohr, 1970).

The gel precipitation test appears to be a very useful serological technique for antigenic characterisation of Babesia spp.; however, the practical application of this technique in diagnosing babesiosis was not demonstrated.

Agglutination reaction provides a sensitive means for detecting Babesia spp.
SEROLOGICAL DIAGNOSIS OF BABESIOSIS

antibodies in various systems, such as haemagglutination, capillary, slide and card agglutination techniques. Antisera which form visible precipitin reactions in gel tests in an undiluted state only may agglutinate Babesia spp. as high as or greater than 1:1000. However, haemagglutination tests require stable cell suspensions and cannot be performed with cells that aggregate spontaneously. The IHA test can be employed only if soluble antigens are available. The IHA technique using tanned or formalised erythrocytes coated with Babesia spp. soluble antigens have been introduced in the serology of Babesia spp. infections by Curnow and Curnow (1967), Sibinovic et al (1969) and Goodger (1971). The reactive antigenic fractions from Babesia spp.-infected erythrocytes were obtained and purified from the host materials with Sephadex and gel chromatography. These antigenic fractions were successfully used in the IHA tests with relatively reproducible results; however, some non-specific reactors were observed as was the case in the IFA and CF tests and the necessity of strict controls in the IHA test cannot be overemphasised.

Agglutination reaction reported in the capillary agglutination (CA) test by Lohr and Ross (1969), slide agglutination (SA) by Curnow (1973) and card agglutination (BCT) test by Todorovic and Kuttler (1974) appears to be of significant importance and the practical application of these reactions in the serology of Babesia spp. needs to be further investigated.

Although several serological tests have been reported (Table I) and noteworthy advances have been made in attempts to purify Babesia spp. antigens from host substances by physical and chemical fractionation, some serological cross reactions have been shown among the various Babesia and Plasmodium spp. (Ldford et al, 1972). The differences in titre of homologous and heterologous sera are significant. The presence of cross reactions does not affect the value of these techniques; when these tests are carefully conducted using an antigen isolated from heavily Babesia spp.-parasitised blood, with a proper control system, a minimum of error in accuracy and specificity should occur.

In the serology of babesiosis, there are still no tests suitable for the detection of early infection. In all tests reported, Babesia spp. parasitaemia appears in the blood before the rise of detectable Babesia spp. antibodies; therefore, the negative results do not indicate that the animal is free from infection. Moreover, none of the available tests will revert to negative within a relatively short time after an infection had terminated by autosterilisation or chemotherapy. Therefore, efforts must continue toward the preparation of more sensitive and specific Babesia spp. antigens which will be able to define the status of infected animals.

In selecting the test of choice it is essential to have precise understanding of the purpose for which serology is to be utilised. Methods devised to date for serological diagnosis of babesiosis are still obviously imperfect. Improvements must still be sought and serological procedures standardised especially for practical use. Serological techniques now available are capable of diagnosing Babesia spp. infections. Nevertheless, until better techniques can be developed, the admittedly imperfect serological techniques now available are capable at diagnosing Babesia spp.

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DIAGNOSTIC SÉROLOGIQUE DE LA BABÉSIOSE: REVUE

Résumé—Durant les trois dernières décades, des découvertes fondamentales sur l'immunoserologie des infections à Babesia spp. ont conduit au développement de techniques sérologiques permettant l'étude de la pathogenèse de la babésiose et de la détection des animaux porteurs d'infections subcliniques. Les antigènes utilisés dans les procédés sérologiques provenaient d'érythrocytes parasitée, de plasma et de tissus d'animaux atteints de babésiose aiguë. Les antigènes parasites et solubles dans le sérum ont été utilisés pour une variété de tests sérologiques (fixation du complément, précipitation en gelose, agglutination et anticorps fluorescents) pour la détection de: anticorps de Babesia spp.

Dans cette revue, un essai est tenté pour résumer et discuter les progrès récents dans le sérodiagnostic de la babésiose, en même temps que les conditions dans lesquelles l'utilisation des méthodes sérologiques peut être valable.
DIAGNÓSTICO SEROLÓGICO DE BABESIOSOS: REVISIÓN

Resumen—En las últimas tres décadas conocimientos fundamentales concernientes a la inmunoserología de las infecciones por Babesia spp. han sido encomendadas al desarrollo de técnicas serológicas que suministren medios para el estudio de la patogenia de la babesiosis y para la identificación de animales con infecciones subclínicas. Los antígenos usados en los procedimientos serológicos fueron obtenidos de eritrocitos parasitados, plasma y tejidos de animales con babesiosis aguda. Antígenos solubles en el suero y parasitarios fueron usados en varias pruebas serológicas; ejemplo: fijación del complemento, precipitación en gelatina, aglutinación y anticuerpos fluorescentes para la identificación de anticuerpos contra Babesia spp.

En esta revisión se ha tratado de resumir y de descubrir los avances recientes en el diagnóstico serológico de la babesiosis junto con las condiciones en las cuales el uso de métodos serológicos pueden ser de considerable valor.