PREMUNITION IMMUNITY AGAINST *BABESIA BOVIS* AND *BABESIA BIGEMINA* EVALUATED BY INDIRECT FLUORESCENT ANTIBODY TECHNIQUE AND RAPID CONGLUTINATION TEST.

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SUMMARY: Imported Holstein cattle from *Babesia bovis* and *B. bigemina* indenne area was immunized with blood of chronic carrier of these hemoparasites. The inoculum showed high infectivity and virulence, since 95.8% of the animals needed treatment with diamine diaceturate. After the first immunization 19.7% of the cattle remained serologically negative to *B. bovis* and 16.2% and 11.2%. to *B. bigemina* respectively in the Indirect Fluorescent Antibody Technique (IFAT) and Rapid Conglutination Test (RCT). Following the second immunization in the IFAT were detected 88.5% of animals with antibodies against *B. bovis* and 95.1% to *B.bigemina*, while in the RCT was detected 93.4% of positive reactions for both species of *Babesia*. Both serological tests did not show significative statistical difference ($p > 0.05$) in the analysis of immunized animals. Therefore, they are useful tools to evaluate the cattle immunization with blood of bovine *Babesia spp* chronic carrier. Also these serological tests can improve the immunization efficiency because they are indicators of the humoral immune response against *Babesia* genus organisms.

KEY WORDS: *Babesia bovis, B. bigemina*, indirect immunofluorescent antibodies, conglutinin, bovine, serology.

INTRODUCTION

Bovine babesiosis occurs enzootically in almost all Brazilian territory hampering the development of the cattle industry. The disease caused by *B. bovis* and *B. bigemina* is responsible for considerable economic expenses, over 250 million dollars per year (Ministério da Agricultura, 1984). These losses are due to mortality, decrease of herd productivity and costs with treatment (KESSLER *et alii*, 1992). The cattle importation increased in the last years with the scope to improve the genetic background, however, most of them are raised in areas free of *Boophilus* tick, vector of *B. bovis* and *B. bigemina* . These animals need to be immunized before entering in the production system which are normally located in the enzootic areas. In Brazil as well as in most of Latin America countries the blood of a chronic carrier of babesia is widely used (LIMA, 1991), although a more efficient procedure exist with live attenuated vaccines (SCHENK *et alii* , 1993). In the last decades a great deal of serological techniques were developed such as indirect fluorescent antibodies technique (GOFF *et alii*, 1982), enzyme linked immunosorbent assay (BOSE *et alli*, 1990), and the agglutination tests (CHIEVES *et alli*, 1989; MADRUGA *et alii*, 1995. The present paper analyzes the performance of the IFAT and RCT in the monitoring of the cattle humoral response immunized with blood of *B. bovis* and *B. bigemina* carrier bovine.

MATERIALS AND METHODS

Experimental Animals

Sixty one Holstein heifers from Canada with seven to eighteen months old had an adaptation period in the Garanhuns Bovine Clinic of the Federal Rural University of Pernambuco (UFRPE), prior immunization against babesia and foot-mouth

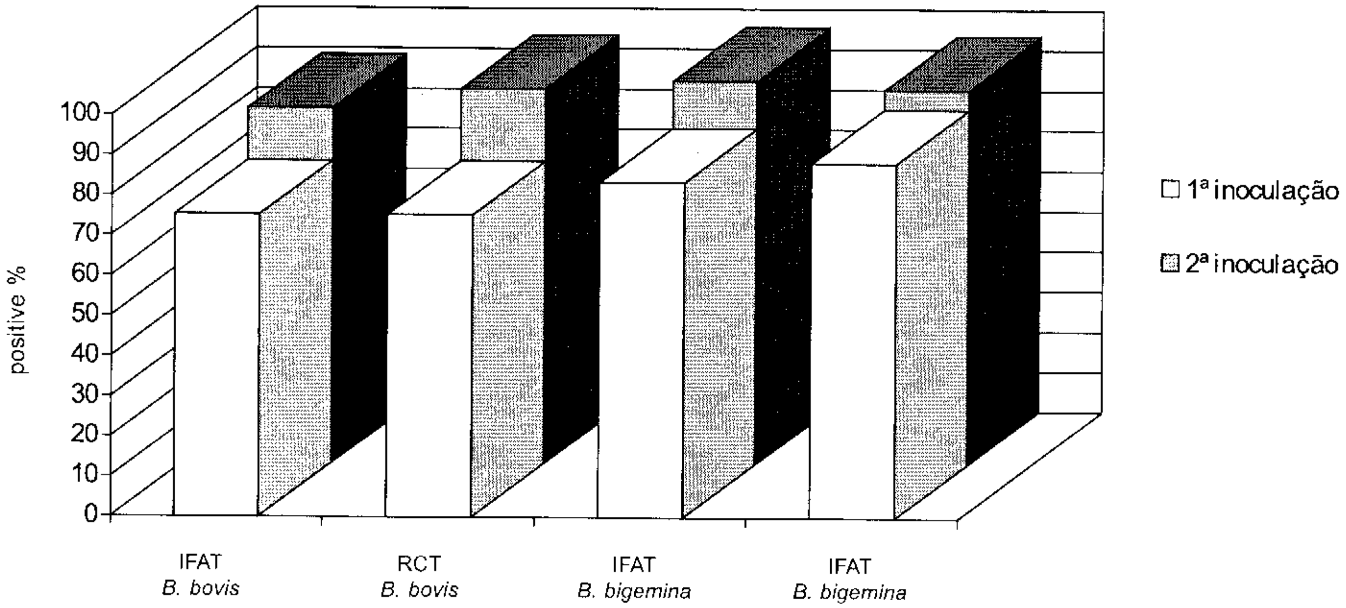

Fig. 1 - Results of the IFAT and RCT after first and second immunization with blood of *Babesia* chronic carrier bovine.

disease. The blood donor was a bull of Chianina breed raised in Garanhuns area, which is considered enzootic for *B. bovis* and *B. bigemina* . This animal was serologically negative for Bovine Leucosis, Blue Tongue, and Bovine Virus Diarrhea.

Immunization

The inoculum was 5 ml of the tick borne disease carrier blood. Forty seven days later a second inoculum was done with equal blood volume from the same donor. Sixty days later the animals were exposed to tick *Boophilus microplus* infestation.

Sera analysis

The immunized animals were blood sampled three times. The first one when the animals arrived at Garanhuns Bovine Clinic, the second 40 days after the first inoculation and the third 27 days after the second inoculation. The blood of each animal after coagulation in Vacutainer tubes was centrifuged at 1030 g for thirty minutes. The sera obtained were stored at -20C.

Serological tests

The IFAT and TCR antigens were produced in the Hemoparasite Laboratory of the Centro Nacional de Pesquisa de Gado de Corte (EMBRAPA-CNPGC) from *B. bovis* and *B. bigemina* stabilates isolated in Rio Grande do Sul and São Paulo States respectively . The methodology employed in the IFAT and RCT were described by MADRUGA *et alii* (1986) and MADRUGA *et alii* (1995), respectively. The IFAT standardization established an initial dilution of controls and test sera at 1:160 and in the RCT was considered positive all sera showing agglutination clumps after 5 minutes of reaction.

Treatment

All animals with severe clinical manifestation of babesiosis, parasitemia in the thin slide smears stained with May-Grunwald-Giemsa and low packed cell volume were treated with half concentration of diamine diaceturate curative dosis.

The statistical analysis was done by chi-square with 5% of significance.

RESULTS AND DISCUSSION

As expected all animals were negative in the IFAT and RCT for both *Babesia* species in the first sampling prior the immunization. This was expected because they were raised in indenne area for *B. bovis* and *B. bigemina*. After first inoculation of the *Babesia* carrier blood into bovine to be immunized, 75.4% were positive in both serological tests for *B. bovis*. Nine (14.7%) out twelve animals (19.7%) negative in the IFAT and RCT were treated diamidine diaceturate in the acute phase of the infection. The remaining three animals were not treated. Antibodies against *B. bigemina* were detected in 83.6% of the animals in the IFAT, while in the RCT was found 88.5%. Six animals (9.8%) did not show antibodies against this *Babesia* specie in both serological tests and most of them (4) were treated with diamidine diaceturate during acute phase and the remaining did not exhibited any clinical signal or hemoparasites on the thin smears. Following the second immunization only one animals did not display antibodies against *B. bigemina*. Probably the high percentage of animals that not developed antibodies against *B. bovis*, was due to two main factors: low *B. bovis* infectivity of the inoculum or the drug treatment impaired the immune response development. Likely the latter hypothesis is more feasible because most of the serologically negative animals were treated. This is an unfavorable aspect of the immunization with carrier blood because the inoculum is unknown with regards to parasite number as well as organisms virulence. The high infectivity normally requires treatment and in the low infectivity do not induce a properly stimulus for an immunological response. The prior situation seems to be which occurred in this immunization in view of 86.9% were treated after the first inoculation and 29.5% after the second one. These were the same reasons pointed out by LIMA (1991) and GONÇALVES & LIMA (1995) which the cattle did not acquired a satisfactory level of immunity after premunition. After the second inoculum of babesia carrier blood in the animals two (3.7%) and one (1.6%)out sixty one continued not displaying antibodies respectively against *B. bovis* and *B. bigemina* (Fig. 1). However, none of these animals had clinical signals of babesiosis after field challenges through *Boophilus microplus* tick, the only babesia vector in Brazil. Probably these animals were refractory to *Babesia* infection. Among all serological tests developed in last years (Wright, 1990) this work compared the performance of the IFAT and RCT. Although the RCT showed a greater number of serologically positive animals, however, statatiscally the difference was not significative. Similar results also were obtained by IFAT and RCT in the serological evaluation immunized with attenuated *B. bovis* and *B. bigemina* (MADRUGA *et alli*, 1995). Therefore, these serological tests can be applied not only in the epidemiological studies but also in the assessment of the premunition or vaccination with attenuated organisms because the humoral immunity is an indicator of immunity against *Babesia* (MAHONEY *et alii*, 1979). In addition the serological tests are important tools to make the immunization procedure more efficient and economic. As long as the animals which developed antibodies against both *Babesia* species can be released in the field without need of additional time for one second immunization. This procedure will avoid the maintenance in restricted areas. The simplicity, low cost and short time to be performed enable the RCT a serological test useful for field evaluation of preventive measures to control the babesiosis.

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SUMÁRIO

Bovinos da raça Holstein oriundos de áreas indenes a *B. bovis* e *B. bigemina* foram imunizados com sangue de um bovino portador crônico desses hemoparasitos. O inóculo mostrou uma elevada infectividade e virulência, pois 95,8% dos animais necessitaram tratamento com diaceturato de diamidina. Após a primeira imunização 19,7% dos bovinos permaneceram sorológicamente negativos para *B. bovis*, 16,2% e 11,2% para *B. bigemina* respectivamente na técnica de imunofluorescência indireta (TIFI) e teste de conglutinação rápida (TCR). Após a segunda imunização na TIFI foram detectados 88,5% de animais com anticorpos contra *B. bovis* e 95,1% para *B. bigemina* enquanto no TCR foram detectados 93,4% de reações positivas para ambas espécies de *Babesia*.. Os dois testes sorológicos não apresentaram diferenças estatísticamente significativas ($p > 0,05$) na análise dos animais imunizados. Portanto, esses testes sorológicos são úteis para avaliar a premunização de bovinos. Esses testes sorológicos também podem melhorar a eficiência da imunização porque são indicadores da resposta imune humoral contra microrganismos do gênero *Babesia*.

PALAVRAS-CHAVE: *Babesia bovis*, *Babesia bigemina*, imunofluorescência indireta, conglutinina, sorologia.

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