SEROLOGICAL PROFILE OF *BABESIA BOVIS* IN ANIMALS SUBMITTED TO PREMUNITION

P.M. GONÇALVES RUIZ[1]*, J.D. LIMA[1] & L.M.F. PASSOS[1]

(1) Departamento de Medicina Veterinária Preventiva, Escola de Veterinária - UFMG - Caixa Postal 567 CEP: 30 161-970 - Belo Horizonte, MG
*Corresponding author: Fax: + 55 31 4992080, e-mail: pmacedog@dedalus.lcc.ufmg.br

SUMMARY: Thirty-nine Limousin breed cattle, imported from USA, were immunized against *Babesia* spp and *Anaplasma marginale* in Capitólio County, State of Minas Gerais, Brazil. Animals were either inoculated with 5 x 10^8 *Babesia* spp infected erythrocytes and 5 x 10^8 *Anaplasma marginale* or infested with approximately 2,000 larvae of *Boophilus microplus* originated from *Babesia* infected cattle. The immune response to *Babesia bovis* was measured at one-week intervals using the indirect fluorescent antibody test (IFAT). All animals were challenged eight to ten weeks after inoculation and/or infestation. No significant differences were recorded between the groups, regarding antibody titers. Maximum anti-*B. bovis* antibody titers were detected between three and four weeks after the initial inoculation. After this period, antibody titers decreased, increasing again one week after challenge. Antibody titers remained constant during the last three weeks of the experiment.

KEY WORDS: *Babesia bovis*, premunition, indirect fluorescent antibody test, cattle

INTRODUCTION

Bovine babesiosis caused by *Babesia bigemina* (SMITH & KILBORNE, 1893) and *B. bovis* (BABES, 1888) is a widespread disease in tropical and subtropical areas. In Brazil, the two species are transmitted by *Boophilus microplus* ticks (CANESTRINI, 1887).

These diseases have been long incriminated as major obstacles on the importation of improved European cattle breeds, causing important economic losses in the livestock industry (KESSLER *et alli*, 1987).

B. bovis is the agent of major pathological importance under field conditions, resulting in high mortality rates (UILEMBERG, 1995). The occurrence of cerebral babesiose due to *B. bovis* has been reported in some Brazilian states (KESSLER *et alli*, 1983, PATARROYO *et alli*, 1982).

In Brazil, studies related to serology have been addressed to the epidemic risk and to evaluate serological profiles of herds (LINHARES *et alli*; 1992, BARCI *et alli*, 1994; MARTINS *et alli*, 1994).

The indirect fluorescent antibody test (IFAT) has been extensively used in epidemiological studies on bovine babesiosis. Some authors have compared the IFAT with other serological methods (BIDWELL *et alli*, 1978; WEILAND & REITER, 1988), showing that IFAT is highly sensitive and easy to perform.

Due to the lack of commercial vaccines in the Minas Gerais State, premunition is still used as an immunization procedure (LIMA, 1991); however little is known about the immune response induced by this process.

The objective of this work was to determine the dynamic of antibody production against *B. bovis* in the animals submitted to premunition.

MATERIALS AND METHODS

Animals

The experiment was carried out in a farm (Mata Velha) located in Capitólio County, Minas Gerais State, during the period of quarantine of 39 (males and females) Limousin breed cattle. Animals were 24 months old, imported from United States. The serological tests were carried out at the Departamento de Parasitologia do Instituto de Ciências Biológicas and Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais.

The animals were housed in stables, where received standardized feeding, consisting of elephant grass (*Pennisetum purpureum*), balanced ration in limited amount and mineral salt and water "*ad libitum*".

Inoculum preparation

Samples of *Babesia* spp and *A. marginale* used for inoculum were originally isolated from naturally infected animals and multiplied through blood inoculations in calves. All animals were negative for brucelosis, leptospirosis, tuberculosis, blue tongue, bovine leucose, bovine virus diarrhea and infectious bovine rhinotracheitis. Blood samples were collected daily from inoculated animals for determination of parasitemia and packed cell volume (PCV). During the peak of parasitemia, blood was collected, centrifuged and red blood cells were used to inoculate the imported animals. Each inoculum was standardized following the technique recommended by IICA (1987), resulting 5 x 10^8 infected erythrocytes, cryopreserved in liquid nitrogen (-196°C) with dimetilsulfoxide (DMSO) 10%.

B. microplus infestation

B. microplus larvae were originated from a *Babesia* spp infected colony. Engorged females were incubated at 27°C, 80% relative humidity for oviposition. Egg masses produced during the first three days were discarded and egg masses produced thereafter were incubated under the same conditions. Larvae (12 and 20 days old) were used to infestate the animals.

Premunition process

Animals to be immunized received 1 ml of inoculum (5 x 10^8 infected erythrocytes) subcutaneously as follows: Group A – 15 animals (12 females and 3 males) were inoculated with blood containing *Babesia* spp and 17 days later, were inoculated with *A. marginale*. Group B - 9 animals (3 females and 6 males) were inoculated with blood containing *A. marginale* and 7 days later, were inoculated with *Babesia* spp. Group C - 15 animals (12 females and 3 males) were infested with 2,000 *B. microplus* larvae and inoculated with *A. marginale* 17 days later. All animals were challenged with infected erythrocytes as follows: 1.5 x 10^6 *B. bovis*, 8.4 x 10^8 *B. bigemina* and 6.7 x 10^6 *A. marginale*. Challenge was given between the 8th and 10th weeks after the initial inoculum.

Laboratorial examination

Blood samples were collected weekly from day 0 until the end of the premunition; these samples were used to determine the packed cell volume (PCV) and to obtain sera to evaluate the immune response. Serum samples were identified and stored at - 4°C until required.

The IFAT method used was essentially as described in IICA (1987). Antigen was prepared from *B. bovis* infected blood. Anti-bovine IgG commercial conjugate (SIGMA) was used at a dilution of 1:1,000. Serum samples were tested in double dilutions, starting from 1:40.

Serological results were analyzed using the Kruskal Wallis test (SAMPAIO, 1998).

RESULTS AND DISCUSSION

The serological profiles observed in groups A, B and C are presented in Fig. 1.

The first detection of anti-*B. bovis* antibodies occurred two weeks after inoculation, with 50% of the animals proved positive, with a progressive increase in titers. Maximum production of antibodies occurred between 21 and 28 days after initial inoculation.

The kinetics of antibodies in *B. bovis* infections reported in the present study differs from that described by TODOROVIC & TELLEZ (1975), who found the first fluorescent antibodies 10 days after initial inoculation. BESSENGER & SCHOELMAN (1983) detected the first antibodies between 14 and 28 days after infection, when a progressive decrease in antibody levels was observed.

The challenge given on the 8th and 10th weeks after the initial inoculation, evoked a antibody response. The highest titers against *B. bovis* were recorded between the 3rd and 4th weeks after initial inoculation and all animals remained positive until the end of the experiment (19 weeks) (Fig. 1).

An overall seroconversion occurred around the 4th week of the experiment, following the same results obtained by LEEFLANG & PERIÉ (1972). PAYNE *et alli* (1990) working with refrigerated vaccines, obtained 100% of seroconversion after vaccinal reaction. On the other hand , SCHENK *et alli* (1993) using attenuated samples of *B. bovis*, *B. bigemina* and *A. centrale*, found seroconversion only after challenge.

The highest titer observed against *B. bovis* was 1:5,120 and it was recorded in a few animals, however the most frequent titer was 1:160. The majority of the animals presented this titer from the 4th to the 11th weeks after initial inoculation.

It was concluded that the type of inoculum used in premunition does not affect the humoral immune response of cattle.

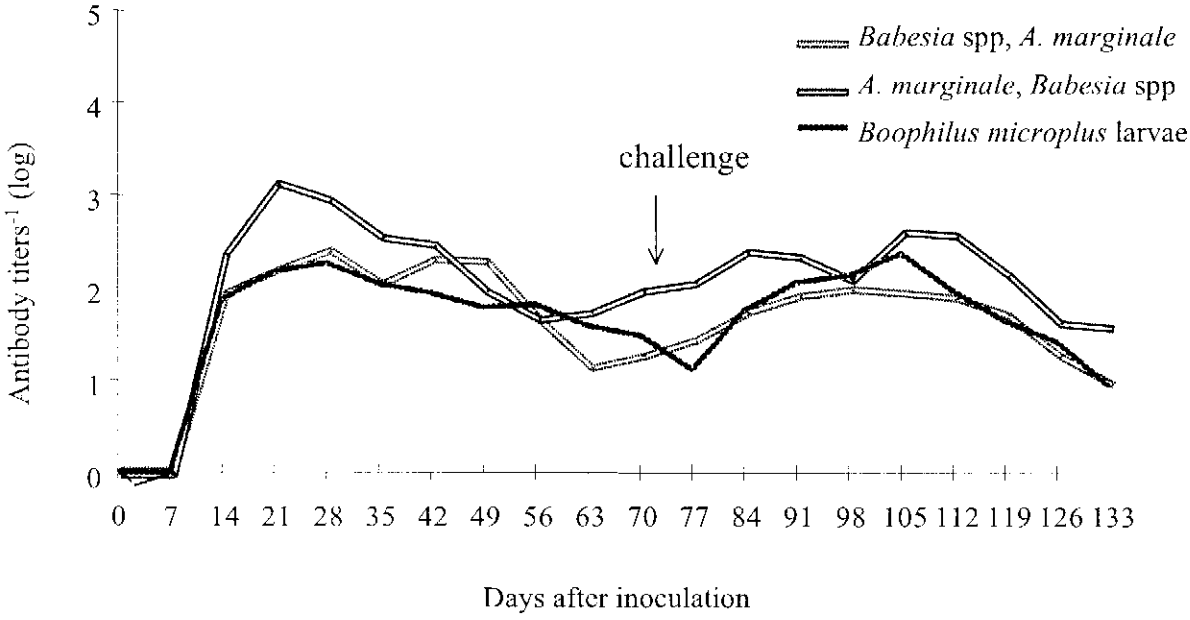

Fig. 1 - Kinetics of antibodies anti-*B. bovis* in cattle after inoculation of blood containing *Babesia* spp, *Anaplasma marginale* or infestation with *B. microplus* larvae.

SUMÁRIO

Trinta e nove animais da raça Limousin, importados dos Estados Unidos, foram submetidos ao processo de premunição, no município de Capitólio (MG). Os animais foram divididos em três grupos e inoculados com sangue contendo 5 x 10^8 *Babesia* spp, 5 x 10^8 *Anaplasma marginale* ou infestados com aproximadamente 2.000 larvas de *Boophilus microplus*, provenientes de animais naturalmente infectados por *Babesia* spp. O perfil sorológico para *B. bovis* foi observado através de exames semanais pela reação de imunofluorescência indireta. Todos os animais foram desafiados entre a 8 e 10 semanas após a inoculação. Todos os animais apresentaram títulos positivos de 1:40 a 1:5.120, durante o processo de premunição. A curva de anticorpos independente da inoculação com *Babesia* spp ou infestação com larvas de *B. microplus*, apresentou o mesmo comportamento nos três grupos. Títulos máximos de anticorpos anti-*B. bovis* foram obtidos entre a 3 e a 4 semanas após a inoculação inicial, havendo em seguida queda progressiva e posterior aumento, uma semana após o desafio. Nas três últimas semanas de experimento a curva manteve-se constante.

PALAVRAS-CHAVE: *Babesia bovis*, premunição, reação de imunofluorescência indireta, bovino

REFERENCES

BARCI, I.A.G.; OLIVEIRA, M.R.; MACHADO, R.Z.; OLIVEIRA, D.A.; ARAÚJO FILHO, R.S. (1994) . Epidemiologia da babesiose bovina em rebanhos produtores de leite tipo B do município de Pindamonhangaba, Vale do Paraíba. *Rev. Bras. Parasitol. Vet., 32*:79-82.

BESSENGER, R. & SCHOEMAN, J.H. (1983). Serological response of cattle to infection with *Babesia bigemina* e *Babesia bovis* in Southern Africa. *Ondest. J. Res., 50*:115-7.

BIDWELL, D.E.; TURP, P.; JOYNER, L.P.; PAYNE, R.C.; PURNELL, R.E. (1978). Comparison of serological tests for babesiosis in British cattle. *Vet. Res., 103*:446-9.

IICA. (1987). *Técnicas para el diagnóstico de babesiosis e anaplasmosis bovinas*. 79 pp., San José, Costa Rica.

KESSLER, R.H.; MADRUGA, C.R.; SCHENCK, M.A.M.; RIBEIRO, O.C. (1983). Babesiose cerebral por *Babesia bovis* (Babes, 1888, Starcovici, 1983) em bezerros do estado do Mato Grosso do Sul. *Pesq. Agrop. Bras., 18*:931-5.

KESSLER, R.H.; MADRUGA, C.R.; JESUS, E.F.; SEMPREBOM, D.V.; VIEIRA-SEMPREBOM, D. (1987). Isolation of pure strains of *Babesia bovis* and *Babesia bigemina*: preliminary test. *Pesq. Agrop. Bras.,22*:1225-30.

LEEFLANG, P. & PERIÉ, N.M. (1972). Comparative imunofluorescent studies on 4 *Babesia* species of cattle. *Res. Vet. Sci., 13*:342-346.

LIMA, J.D. (1991). Premunição: uma alternativa para o controle da tristeza parasitária. *In: Seminário Brasileiro De Parasitologia Veterinária.* São Paulo, 22-26 de setembro, 1991. Anais... p.39-43, 156p., São Paulo.

LINHARES, G.F.C.; MASSARD, C.L.; ARAUJO, J.L.B.; ALVES, L.C. (1992). Levantamento sorológico para *Babesia bigemina* (Smith e Kilborne, 1893) e *Babesia bovis* (Babes, 1888) em bovinos na Região Centro-Oeste do Brasil. *Arq. Univ. Fed. Rural do Rio de Janeiro, 15*:85-9.

MARTINS, J.R.; CORREA, B.L.; CERESER, V.H.; ARTECHE, C.C.P.; GUGLIELMONE, A.A. (1994). Some aspects of the epidemiology of *Babesia bovis* in Santana do Livramento, Southern Brazil. *Rev. Bras. Parasitol. Vet., 32*:75-8.

PAYNE, R.C.; OSORIO, O.; YBAÑEZ, A. (1990). Tick-born diseasis of cattle in Paraguay. II. Immunization against anaplasmisis and babesiosis. *Trop. Anim. Hlth. Prod., 22*:101-8.

PATARROYO, J.H.; VARGAS, M.I.; BICUDO, P.L. (1982). Description of lesions in cattle in a natural outbreak of *Babesia bovis* infection in Brasil. *Vet. Parasitol., 11*:301-8.

SCHENK, M.A.M.; KESSLER, R.M.; MIGUITA, M. (1993). Desenvolvimento de cepas atenuadas de *Babesia bigemina, Babesia bovis* e *Anaplasma centrale*: teste crítico com bovinos Brangus. *Rev. Bras. Parasit. Vet., 22*:75-8.

SAMPAIO, I.B.M. (1998). *Estatística aplicada à experimentação animal.* Belo Horizonte, Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia, 221 pp.

TODOROVIC, R.A. & TELLEZ. (1975). The premunition of adult cattle against babesiosis and anaplasmosis in Colombia, South America. *Trop. Anim. Hlth. Prod., 7*: 125-131.

WEILAND, G. & REITER, I. (1988). *Methods for measurement of the serological response to Babesia. In: RISTIC, M. Babesiosis of domestic animals and man.* CRC Press. Boca Raton, Flórida, pp 143-162.

UILENBERG, G. (1995). International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. *Vet. Parasitol., 57*:19-41.

(Received 27 March 1998, Accepted 7 June 1999)