SEROPREVALENCE AND INCIDENCE OF BABESIOSIS AND ANAPLASMOSIS IN A CARORA BREED HERD FROM VENEZUELA.

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SUMMARY: A longitudinal study was conducted in two groups of Carora breed cattle (n =15) which started when experimental calves were one week old and it finished when animals reached 15 months of age. Blood smears were prepared every other week from each calf to monitor microscopically the incidence of *Babesia* spp. and *Anaplasma marginale*, whereas, serum samples were collected with the same frequency to study the seroprevalence of those blood parasites by the indirect immunofluorescence assay (IFA). *Babesia* spp. showed a mean incidence (MI) of 8.5% while *Anaplasma marginale* presented a MI of 22.4% after 15 months of survey, positive calves having no clinical signs. Anaplasmosis showed a higher incidence during the rainy season (May-Nov.) which correlates with larger populations of haematophagous insects for this period. Experimental animals showed an annual mean titer of 1:1,024 to *Babesia* spp. and a mean titer of 1: 950 to *A. marginale* by IFA, with mean antibody levels for the group of calves starting at 1: 40 and increasing to 1: 2,560 after one year of evaluation. The titer of antibodies for those blood parasites increased steadily when calves became older and were transferred from individual boxes to open grazing paddocks. Age, enzootic stability, and the high crossbred of Carora breed animals are discussed as factors that could determine the incidence and seroprevalence rates found against those blood parasites in this cattle dairy breed.

KEY WORDS: Epizootiology, cattle babesiosis, anaplasmosis, Carora breed cattle.

INTRODUCTION

Bovine babesiosis and anaplasmosis are hematic parasitic diseases of cattle caused by *Babesia bigemina*, *Babesia bovis*, and *Anaplasma marginale*, respectively. Both diseases are often found in cattle herds of Venezuela in particular in susceptible high crossbred or pure *Bos taurus* animals. Research on hematic parasitic diseases in Venezuela has been aimed to its prevention and control (SCHROEDER *et alii*, 1971), epizootiological situation (JAMES *et alii*, 1985), immunodiagnostic methods (MONTENEGRO - JAMES *et alii*, 1990; TORO BENITEZ *et alii*, 1980), and "*in vitro*" culture and immunization of cattle against these diseases (MONTENEGRO - JAMES *et alii*, 1992). Previous epizootiological studies (JAMES *et alii*, 1985) have shown that both, bovine babesiosis and anaplasmosis, are enzootic in particular in the West Central Region (WCR) of Venezuela with incidence rates of 7.5% (babesiosis) and 21.2% (anaplasmosis) and with seroprevalence rates of 78.2% and 57.2%, respectively. These seroprevalence figures indicate that in the WCR of Venezuela cattle is mainly raised in areas of enzootic stability; therefore, few cases of clinical disease caused by these blood parasites are occasionally seen. On the other hand, in regions with enzootic instability or areas where imported *Bos taurus* is introduced, babesiosis and anaplasmosis cause high economic losses.

A strategy recommended for raising productive cattle in the tropics is to use animals resistant to ticks and tick-borne diseases, and often these resistant bovines are the product of crossing *Bos taurus* with *Bos indicus* and/or with creole cattle. In Lara State, Venezuela, dairy cattle owners have been developing a tropical breed since 1920 named "Carora Breed", which is the result of empirical genetic crosses among Brown Swiss cattle, indigenous creole bovines, and *Bos indicus* in a

minor degree (HERRERA SILVA, 1960). The Carora breed cattle has showed 2 main characteristics: a) it has an average milk yield/cow/day/ of 11 liters., and b) it shows some resistance to tropical diseases and ticks (HERRERA SILVA, 1960).

The main objectives of this longitudinal study were: 1) To evaluate the *Babesia* and *Anaplasma* infection rates (incidence), and to assess the kinetics of antibodies against these parasites (seroprevalence) in blood samples collected fortnigthly from Carora breed animals. 2) To correlate the results of incidence and seroprevalence with the season of the year (dry or rainy), and with the husbandry system used in ranches raising this tropicalized breed of cattle. The study started when the experimental group of calves was 1-week old, and it finished when these bovines had 15 months of age.

MATERIALS AND METHODS

Bovines: The experimental group (EXG) was originally formed by 20 female Carora breed calves with ages between one to seven days old, randomly selected from two ranches (10 calves per ranch) dedicated to milk production. Five calves died during the first three months of this study due to coccidiosis by *Eimeria zurnii*, three calves losses occurred in one ranch and two at the other. consequently, the EXG was finally formed by 15 animals which were properly identified. These ranches were located close to Carora town (10° 35'N and 70° 15'W) in the WCR of Venezuela, and into an ecological area classified as a dry tropical forest (REPUBLICA DE VENEZUELA, MINISTERIO DE AGRICULTURA Y CRIA, 1976). In these ranches calves are raised in three phases: 1) calves one to four months old are kept in individual fenced boxes, 2) five to eight months old calves are penned in medium size grazing paddocks, and 3) 9-18 months old heifers are kept in large open grazing areas.

Collection of Samples: The following samples were collected from each EXG animal: 1)Capillary blood: for preparing thin blood smears, which were fixed with methanol and later stained with Giemsa (Sigma Co.St. Louis, MO. USA). Each stained blood smear was microscopically observed under oil immersion for 5-10' to identify blood parasites; at least 100 microscopical fields were checked before declaring a blood smear either positive or negative to *Anaplasma* or *Babesia* spp. 2) Blood was collected from the jugular vein to obtain serum samples which were kept at -20°C before the IFA test was performed. IFA tests were conducted on all serum samples obtained from EXG bovines. The procedure followed for the conduction of the IFA test was previously published by JAMES *et alii* (1981). The starting serum dilution was 1: 80, the conjugate was FITC in rabbit anti-bovine IgG (Cappel Lab. Cochranville, PA. USA). Samples were examined under an immunofluorescence microscope (Leitz, Laiborlux 11).

Blood samples were collected twice a month, consequently, each monthly result represents the mean value of two observations. Rates of infection in the EXG were calculated through two parameters: 1) **Incidence of parasitemia**, defined as the monthly percentage of animals with positive blood smears to *Babesia* spp. and/or *Anaplasma*, and 2) **seroprevalence**, defined as the monthly mean titer of antibodies detected by IFA against the studied blood parasites divided by the total number of calves checked each month. In addition, **the mean incidence (MI)** for each hemoparasitic disease during the survey was calculated as follows: MI = Σ mean monthly incidence of parasitemia ÷ 15 months.

Two weather parameters were also recorded from the geographical area where this study was carried out: precipitation and temperature. These parameters were daily monitored at «La Pastora» Sugarcane Factory, hidrology and meteorology section, located halfway of those ranches. Only the mean monthly precipitation during the last 10 years in the region (1983-1993) is presented.

Statistical analysis: The differences detected in mean incidence of babesiosis and anaplasmosis in the EXG, during the dry and rainy seasons, were analyzed using the Chi-square test (X2).

RESULTS

The monthly rates of incidence for bovine babesiosis and anaplasmosis in Carora breed cattle are presented in Figure 1. Anaplasmosis showed lower incidence during the dry season (December-April) compared with the high levels of incidence detected in the rainy season (May-November). These differences were significant ($p < 0.05$) after statistical analysis (Table 1). The overall mean incidence for anaplasmosis after monitoring the EXG for 15 months was 22.4%. When the EXG reached five months of age, eight out of 10 calves had positive blood smears to *A. marginale*. The first microscopical observation of *A. marginale* was found in samples collected from seven and 14 days old calves. The overall mean incidence for babesiosis in the EXG during the whole survey was 8.5%. In general, the rates of parasitemia for *Babesia* spp. were lower than those for *A. marginale* (Figure 1), and significant differences were not found for the incidence of this protozoan during the dry vs. rainy season (Table 1).

When the EXG reached seven months of age eight out of 10 calves had positive blood smears to *Babesia* spp., nonetheless, these calves showed no clinical signs. Two calves were positive to *B. bigemina* at the early age of 14 days and *B. bigemina* was the predominant specie seen in blood smears. The monthly rates of seroprevalence for babesiosis and anaplasmosis in the EXG are shown in Figure 2.

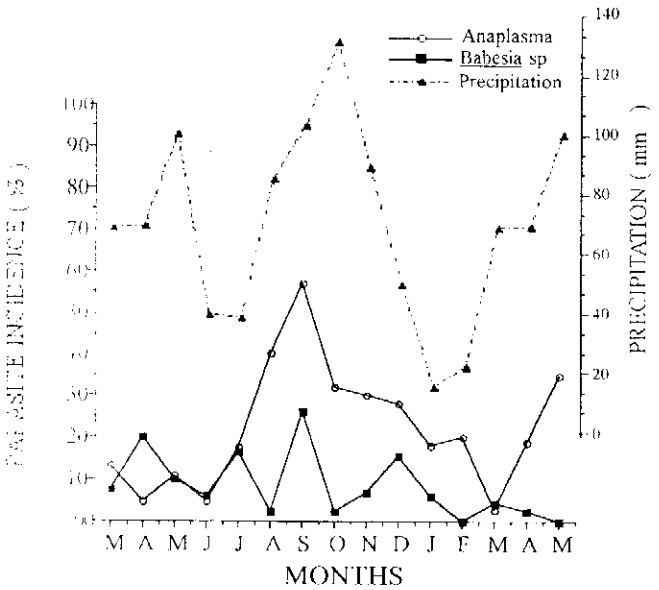

Figure 1 - Mean monthly rates of incidence (%) for bovine babesiosis and anaplasmosis in Carora breed cattle, related to precipitation in the studied region.

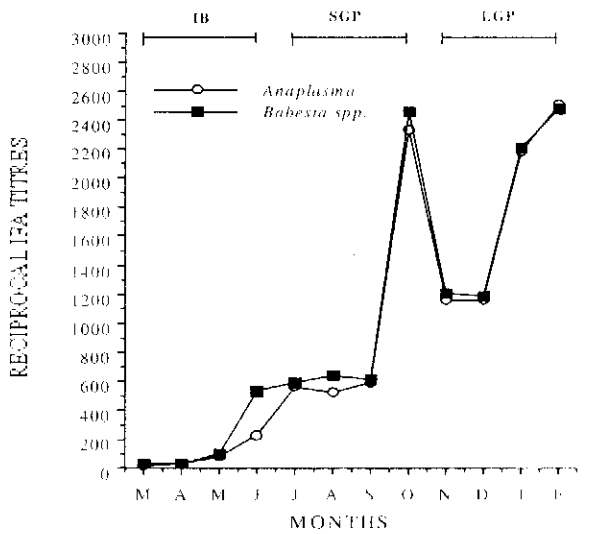

Figure 2 - IF A antibody titres in response to natural infections by *Babesia* spp. and *A. marginale* in Carora breed cattle. IB: in individual boxes. SPG: in small grazing paddocks. LGP: in large grazing paddocks.

Seroprevalence was monitored during one year and it was related to the husbandry system and the group's age. One month old EXG calves had low mean antibody titers for babesiosis (1:30) and anaplasmosis (1:20), and during the first four months of life these EXG calves were kept in individual fenced boxes (IB). When EXG calves were four months old reached mean antibody titers of 1:530 and 1:230, respectively (Figure 2). The rates of seroprevalence for bovine babesiosis and anaplasmosis continued to raise, thus, at eight months of life calves reached mean levels of 1:2,460 and 1:2,335, respectively. From four to eight months the EXG was transferred and kept in small grazing paddocks (SGP). Finally, when the EXG was 12 months old, mean levels of seroprevalence were 1:2,484 for babesiosis and 1:2,510 for anaplasmosis. At this age EXG animals were healthy and grazing in large open paddocks (LGP) (Figure 2).

Table 1 - Mean incidence of bovine babesiosis and anaplasmosis related to season of the year.

	Season	
Parasitosis	Dry (dec.-april)	Rainy (may-nov.)
Babesiosis[a]	8.0	10.0
Anaplasmosis[b]	15.2	28.5

[a] Non significant differences when compared dry vs. rainy
[b] Significant differences ($p<0.05$) dry vs. rainy

DISCUSSION

Rates of incidence and seroprevalence for bovine babesiosis and anaplasmosis were studied in Carora breed cattle during 15 and 12 months respectively. Anaplasmosis showed a high peak of incidence during the rainy season, and lower rates during the dry season. This finding is similar to the annual distribution reported for anaplasmosis in this geographic region and in this tropical dairy breed of cattle by JAMES *et alii* (1985). This distribution for anaplasmosis appears to be determined by the higher population of blood-sucking flies during the rainy season which are important vectors for *A. marginale* in the tropics. The mean incidence of babesiosis was 8.5% during the 15 months survey, and this result could have a two-fold explanation: 1) The high predominance of *B. bigemina* in the region, since this hemoparasite shows greater infectivity for *Boophilus microplus* than *B. bovis* (MAHONEY & MIRRE, 1971) and 2) the high incidence and intensity of *Babesia* spp. sporokinetes in *B. microplus* detected in this region (MELENDEZ & FORLANO, 1996).

The EXG showed a level of antibody titers against babesiosis and anaplasmosis in constant increase from one to 12 months of age. This finding is different from that ascertained in pure *Bos taurus* calves raised in a temperate country like Argentina (DE RIOS *et alii*, 1988). In this case, calves showed a decreasing antibody titer to *Babesia* spp. during the first five months of life.

The antibody kinetics detected by IFA for *A. marginale* and *Babesia* spp. in the EXG calves from one to seven months old showed a tendency to increase slowly, reaching a mean titer of 1:600 at the 7th month (Figure 2). Afterwards, the titers for both parasites peaked (October) until 1:2,300 and 1:2,400 respectively (Figure 2). This peak in antibody levels seems to correlate with the monthly rates of parasite incidence (Figure 1), whose rates increased on the 7th month (September), one month before the burst of antibody titers for *Anaplasma* and

Babesia spp. It is well known that bovines below one year old are more resistant to clinical babesiosis and anaplasmosis than older ones. This natural resistance has a multifactorial explanation, therefore, in the tropics it is desirable that young animals become infected at this stage of life, since they will remain permanently immunized against these diseases (premunition) (YOUNG, 1988). The antibody kinetics detected by IFA for Anaplasma and Babesia in this study in this cattle, strongly suggests that these animals became premunized during their first year of life, and in general, they will not suffer these diseases as adults, this condition helps to maintain the enzootic stability in the region for these hemoparasitosis.

In the WCR of Venezuela, populations of *Boophilus microplus* are higher during the dry season compared with the population levels during the rainy season, in addition there is a large proportion of *Babesia* spp. infected ticks (MELENDEZ & FORLANO, 1996). This situation brings about a high level of transmission for *Babesia* spp. in the calf population, and it also assures the condition of enzootic stability for babesiosis. Thus, in agreement with GUGLIELMONE (1995) the risk of occurrence of economically relevant outbreaks of babesiosis are minimal in herds maintained in a region with high *Babesia* inoculation rate, and this seems to be the epizootiological situation in the studied area. Another data that supports the state of enzootic stability in the EXG of Carora breed animals is the fact that 80% of the EXG calves carried *Babesia* spp. parasites at seven months of age, and 80% of calves were also positive to *A. marginale* at five months of age. This finding is in agreement with the basic concepts of enzootic stability for these parasites (MAHONEY & ROSS, 1972).

A better understanding of bovine babesiosis and anaplasmosis in a region ought to be based on a comprehensive study of the interactive factors that determine their epizootiology. Therefore, the knowledge of factors like incidence and seroprevalence is fundamental for understanding the epizootiology of babesiosis and anaplasmosis, in particular in a country with herds formed in part by tick resistant bovines like Carora breed cattle, and in part by tick susceptible cattle like Holstein and Brown Swiss.

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SUMÁRIO

Um estudo longitudinal foi feito em dois grupos de bovinos da raça Carora (n=15). Os animais tinham uma semana de idade no começo do experimento findo o qual os bovinos atingiram 15 meses. Esfregaços sanguíneos foram feitos quinzenalmente de cada um dos animais visando a determinação da incidência de *Babesia* spp. e *Anaplasma marginale*, enquanto que amostras de soro foram coletadas na mesma frequência para estudar a soroprevalência desses hemoparasitas pela técnica da imunofluoreseência indireta (IFA). *Babesia* spp. apresentou uma incidência média (IM) de 8,5%, enquanto que *A. marginale* atingiu uma IM de 22,4%. Bezerros positivos não mostraram sintomas clínicos de infecção por esses hemoparasitas. A anaplasmose mostrou uma incidência maior durante a época de chuvas (Mai-Nov) o que está em relação com uma maior população de insetos hematófagos. O grupo experimental apresentou um título médio anual de 1:1024 para *Babesia* spp. e de 1:950 para *A. marginale*. Os níveis de anticorpos no início do experimento foram de 1:40 e atingiram valores de 1:2560 doze meses após. Os títulos de anticorpos contra essas doenças tiveram um incremento sustentado à medida que os bezerros tornaram-se mais velhos e foram levados aos campos de pastagem. A idade dos bezerros,a estabilidade enzootica e a alta mestiçagem da raça Carora são discutidos como os fatores que poderiam determinar os valores de soroprevalência e de incidência achados nesta raça para ambos os hemoparasitos.

PALAVRAS-CHAVE:Epizootiologia, babesiose bovina, anaplasmose, raça Carora.

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