SEROEPIDEMIOLOGICAL STUDY OF *BABESIA BOVIS* IN SUPPORT OF THE URUGUAYAN *BOOPHILUS MICROPLUS* CONTROL PROGRAM.

H. CARDOZO[1], M. A. SOLARI[1], J. ETCHEBARNE[1] & J. H. LARRAURI[2]

(1) Dilave M. C. Rubino, Montevideo, Uryguay, C. C. 6577; (2) Sanidade Animal, DGSG-MGAP, Montevideo, Uruguay.

SUMMARY: Bovine blood samples were collected from a region endemic for *Boophilus microplus* (34º South Lat.) and consisted of 125 ranches with a cattle population of 76,918. A total of 1,728 cattle were bled (1,485 adults and 243 calves less from 1 year of age) from 27 ranches. This sample size was analyzed to provide incidence and prevalence values with a precision of ± 10% at a confidence level of 95%. The FAO/IAEA/ELISA kit was used to detect antibody to *Babesia bovis*. The following results were obtained: 1) Dispersion (proportion of ranches with babesia infection) was estimated to be 70.5% ± 8.8(SD). A positive ranch was defined as having one or more test-positive animals. 2) Apparent prevalence (proportion of cattle with a positive test result) within the region was estimated to be 3.5% ± 0.3, with the range from 0 to 18.5. 3) Incidence based on apparent prevalence in calves less than 1 year of age was estimated to be 2.8%. The dispersion, apparent prevalence, and incidence data for this region of Uruguay will be compared with a repeated sampling of cattle in this area in three years to assess the effectiveness of the eradication/control campaign.

KEY WORDS: *Babesia*, tick eradication, ELISA, seroepidemiology.

INTRODUCTION

Since 1941 the Uruguayan government laws have supported the campaign against the tick, *Boophilus microplus*. Over time there have been advances and reverses in this campaign. Recently greater financial support has resulted in an intensified program (MGAP/Sanidad Animal/BID, Uruguay, 1989). Uruguay is situated between the 30^0 and 35^0 parallels South latitude which represents a marginal area for *B. microplus* development. Since the climate influences the tick population, the enzootic status, changes as does the incidence of parasitic diseases transmitted by ticks, *Babesia bovis, Babesia bigemina* in different parts of the country (CARDOZO *et alii,* 1984; NARI & SOLARI, 1990; SOLARI *et alii,* 1991).

Haemoparasites are responsible for significant losses to the cattle industry of Uruguay (SOLARI *et alii*, 1992). Their distribution is similar throughout the country. Prevalence studies performed with the indirect fluorescence antibody (IFA) technique for *Babesia* and the card agglutination test for *Anaplasma*, have shown ranches of enzootic instability for *B. bovis* and *Anaplasma marginale*, and enzootic stability for *B. bigemina* and *A. marginale* (BERDIÉ *et alii*, 1979; CARDOZO *et alii*, 1981; SOLARI, 1987).

The Ministry of Agriculture and Fisheries is seriously considering the eradication of ticks south of the Rio Negro and plan to develop effective control of the disease in the northern region of the country (MGAP/Sanidad Animal/BID,Uruguay, 1989).

The implementation of a program to control *B. microplus* requires a thorough knowledge of the haemoparasite status in the areas where the campaign is going to be enforced. Accordingly, it is necessary to:

- Evaluate the campaign by monitoring disease prevalence at the beginning and at its different stages as it progresses.
- Identify areas of high tick prevalence that are enzootically stable. Acaricide treatment used in stable areas reduces the tick population resulting in cattle not becoming exposed nor immune to the organisms; this makes them susceptible to hemoparasite infection, morbidity, and mortality.
- Identify areas of low tick prevalence that are enzootically stable. As tick populations increase, cattle are at greater risk of hemoparasitic infections and their consequences.

The ELISA technique is an established diagnostic procedure and has been used in Uruguay for detection of antibody to *B. bovis* (CARDOZO *et alii*, 1992). The objective of the present work was to carry out a seroepidemiological study (dispersion, prevalence, and transmission to calves), using the ELISA technique in an area where the campaign against *B. microplus* is going to be introduced.

MATERIALS AND METHODS

Experimental area: The 8th Police Section in the Department of Lavalleja (34° South Lat.) was selected. This region consists of 125 ranches with 76,918 cattle. The geographic characteristics of the zone are rocky fields with mountain brush vegetation which makes tick control difficult. Official data indicate a high percentage of ranches in this area are infested with *B. microplus*.

The study population: The population for study was drawn randomly in two steps. First, the ranches were stratified according to the size of the bovine population as shown in Table 1.

Table 1 - Characteristics of the study population of animals.

Stratum	Number of cattle	Ranches (n)	Number of adult sera per ranch	Number of calf sera per ranch
I	100 - 500	23	55	9
II	501 - 1000	3	55	9
III	1001 - <	1	55	9
Total		27	1485	243

Ranches were selected at random from throughout the area. The number of ranches selected was based on the dispersion of ticks in the area (about 60% of the farms were tick-infested). The statistical model for selecting ranches indicated that 27 ranches would need to be sampled. This represented 21.6% of the total number of herds in the area.

Previous studies in a similar geographic area suggested that a babesial incidence of 2% and prevalence of 5% was expected in the study area. The sample size for each ranch was determined to be 9 calves less than 1 year of age and 55 adults (greater than 2 years of age). This sample size was estimated to provide incidence and prevalence values with a precision of ± 10% at a confidence level of 95%. The 1,728 bovines sampled represented 2.2% of the 76,918 total animals in the area.

After venipuncture, blood samples were placed in a refrigerated box, transported to the laboratory, and serum was harvested within 3 days. The serum tubes were labeled and stored at - 20°C until used. Additional information was obtained from each farm relative to number of bovines, presence of , ticks, and previous experience with hemoparasitic diseases.

Serological testing: An ELISA kit BB09402001 from FAO/IAEA Vienna was used for serological diagnosis as described previously (CARDOZO *et alii*, 1994). The assay was validated for Uruguayan conditions by testing 200 known uninfected and 80 known infected cattle.

Analysis of Results: Dispersion, apparent prevalence and incidence of haemoparasites was calculated with a 95% confidence interval (COCHRAN, 1977; ORGANIZACIÓN PANAMERICANA DE LA SALUD, 1979).

Dispersion was defined as the proportion of ranches with *Babesia* infection. A positive ranch was defined as having one or more test-positive animals. Dispersion was estimated based on stratified sampling. Apparent prevalence was defined as the proportion of cattle with a positive test result and was calculated in accordance with the sampling ratio. Incidence was defined as the apparent prevalence in calves less than 1 year of age and also was calculated in accordance with the sampling ratio.

RESULTS

Assay validation: The calculated sensitivity and specificity of the ELISA test was 99% and 98%, respectively (Fig. 1). The assay performance was essentially the same as previously published (CARDOZO *et alii*, 1994).

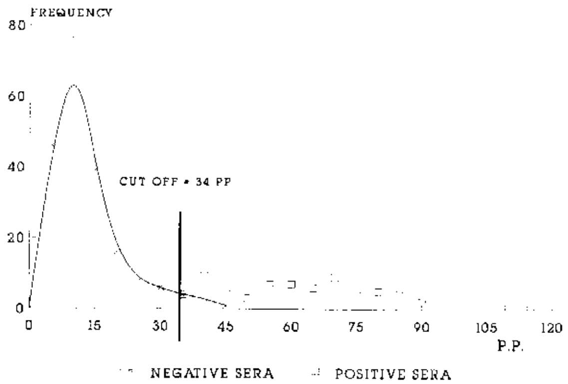

Fig.1. Representation of the cut off in P.P. units from which was established the estimates of sensitivity and specificity of the ELISA assay for antibody to *B. bovis*.

Ranch sampling profile: The 27 ranches included in the survey are profiled in Table 2. The tick infestation status (*Boophilus microplus*) for each ranch was confirmed by producer responses to survey questions. Sixteen of the 27 ranches (59.3%) had tick infestations.

Dispersion of *B. bovis* in the study area was estimated to be 70.5% ± 8.8% (95% confidence interval). Prevalence as a function of ranch size and age is summarized in Table 3. Ranches having 100-500 animals (Stratum I) had 18 times greater prevalence than did ranches having more than 500

Table 3 - Prevalences as a function of stratum and age.

Stratum	Total (%)	Adults (%)	Calves (%)
I	5,4 ± 0,8	5,6 ± 1,3	3,3 ± 1,3
II and III	0,3 ± 0,3	0,3 ± 0,3	0,0 ± 0,0
Police Section	3,5 ± 0,3	3,4 ± 1,1	2,8 ± 1,2

Table 2 - Profile of the ranches sampled.

Stratum	Ranch Identity	Presence of ticks	Number of cattle	Number of calves	Percent of calves positive	Percent of adults positive	Overall Prevalence
1	820676	+	120	4	-	0	0
1	816529	+	154	32	11	5	6
1	818246	-	156	28	0	9	7
1	820976	-	160	44	11	0	2
1	801033	+	171	58	21	3	7
1	805527	+	180	70	0	6	5
1	805855	-	180	60	0	0	0
1	821204	-	180	11	0	7	6
1	804032	+	200	100	0	0	0
1	802102	-	200	43	0	16	14
1	801948	+	221	63	11	15	14
1	800819	+	250	40	0	0	0
1	805578	+	270	16	11	3	5
1	803346	-	280	50	0	9	8
1	801181	+	310	50	0	2	2
1	S/N[1]	+	320	57	1	4	3
1	806361	+	380	50	21	6	8
1	S/N[2]	+	400	90	0	2	2
1	815506	+	400	200	0	2	2
1	804394	+	420	110	0	22	18
1	803576	-	450	100	11	2	3
1	802552	-	550	140	0	4	4
2	801697	+	582	110	0	7	6
2	S/N[3]	-	660	130	0	2	2
2	815352	-	800	32	0	0	0
2	801972	+	950	100	0	0	0
3	810245	-	1480	4	0	0	0

cattle (Strata II and III combined). Prior estimates of adult and calf prevalence for the strata were 5% and 2%, respectively. These were similar to the observed prevalences in the study (3.4% and 2.8%, respectively for adults and calves).

Seventy-four percent (20/27) of the ranches surveyed had animals that were positive for *B. bovis*. Of the 20 infected ranches, only 1 ranch had a prevalence that exceeded 15%.

The maximum prevalence for any ranch of the area was estimated in 4.1%(mean). Considering that calfhood infections are an indication of the incidence of infection, the maximum incidence was 5.2%. The ranch that had the maximum prevalence did not have any infected calves whereas, the ranch with the maximum incidence had an intermediate prevalence among all ranches (Table 2).

DISCUSSION

Because the sampling strategy employed gave babesial incidence and prevalence data that were consistent with anticipated results, the data obtained in this survey may be extrapolated to the remainder of the population within the 8th police section in the Department of Lavalleja. Among the 70.5% of the ranches that were infected with *B. bovis* within the police section (area), there was no apparent clustering of infected farms (data not shown); rather, infected farms were evenly distributed throughout the region. This data would suggest that the tick is also widely distributed within the area, in agreement with prior tick surveys which indicated that 59.3% of all ranches in the area were tick-infested.

Although 11 ranches indicated that they had not observed ticks on their ranch, 8 of the 11 farms had animals that were positive for *B. bovis*. Because the mean prevalence of seropositive animals on these farms was relatively high (4,2%), it is unlikely that the test was mis-classifying these animals considering that the test sensitivity exceeded 98%. A more likely explanation is that ticks are present on these ranches but are not being observed.

The low prevalence of babesiosis in the region (3.5% ± 0.3%) indicates that the disease is relatively enzootically stable. This is supported by a similar prevalence in adults (3.4%) and incidence in calves (2.8%). On two ranches, the incidence in calves 21% while the prevalence in adult cattle was only 3% and 6%, respectively. This may indicate that the infection is less stable on these farms than for the other farms, which is an observation that has been made previously (FRANCHI, 1992; QUINTANA *et alii*, 1992). Both of these farms were known to be infested with ticks. Only two of the 11 presumably tick-negative farms had sero-positive calves while six of the 11 had adult sero-positive cattle and no sero-positive calves; these ranches tend to confirm the enzootic stability theory.

The relationship between the wide dispersion of vector/haemoparasites and the low overall and local prevalences, depend on several factors: 1) The study area is located in South Latitude 34° where weather conditions allow only 2.5 to 3 tick generations to develop per annum so that high prevalences of infection are more difficult to obtain (CARDOZO *et alii*, 1984); 2) Local variations in prevalence may be due to the interspersed presence of rocky fields covered with brush that protect ticks from cold and heat during certain periods of the year; 3) The low infestation levels may be due to the fact that treatments against ticks are applied to cattle each time that tick infestations are detected by producers. Because these variables may change over time, enzootic stability may also change. Changes in these variables may have resulted in infected herds where they were not expected (PAIVA, 1992). In our experience, this usually happens when tick control is lax and the tick population increases favoring transmission to susceptible cattle.

Four percent of ranches had greater than 15% prevalence. This may have been due to failures in the control or in an improvement of the transmission conditions, placing these ranches at an increased risk of outbreaks. One explanation may be an increased resistance to some acaricides that has occured in Uruguay.

Although other hemoparasitic infections occur in cattle in Uruguay, infections with *B. bovis* are the most common (SOLARI, 1987). A similar situation is present in the Rio Grande region of Brazil (MANIERO O. LEITE, 1988). Although Argentina has carried out a successful campaign and eradicated the tick below the 30^{o} South Latitude, *B. microplus* enzootic areas with high prevalence of haemoparasites still remain above this latitude (GUGLIELMONE, 1991).

Our studies have established a baseline of babesiosis for the 8th Police Department of Uruguay. A similar study after 3 years of the campaign will be used to evaluate the effectiveness of the eradication campaign in Uruguay.

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