Study of *Anaplasma* and *Babesia* infection rates of cattle and associated ticks in the South Coast of Guatemala

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Key words

Cattle - Calf - *Boophilus microplus* - Metastigmata - *Anaplasma marginale* - *Babesia bovis* - *Babesia bigemina* - PCR - Immunology - Guatemala.

Summary

Babesia bovis, *B. bigemina* and *Anaplasma marginale* have been suspected to be widespread in the South Coast of Guatemala. The epidemiology of the parasites was studied by monitoring 17 calves at monthly intervals during a period of one year at two farms and by counting and detaching all "standard" female ticks collected on one half of each of the animals. Bovine blood and serum samples were examined by the polymerase chain reaction (PCR) technique and by serological tests, respectively. In addition, the PCR technique was applied on the haemolymph and intestines of the detached ticks. *Boophilus microplus* was the only tick species encountered on the calves. The presence of the three haemoparasites was confirmed in both calves and ticks. High infection rates in the calves were associated with high infection rates in the detached ticks for *B. bigemina*. However, for *B. bovis* and *A. marginale* this association was not found. Furthermore, the PCR and serological results of consecutive samplings of the same animals showed considerable variation in antigen and antibody levels, respectively. No clinical cases of babesiosis and anaplasmosis were observed during the study period, which agrees with an endemically stable situation under current herd management practices.

■ INTRODUCTION

Ticks and tick-borne diseases are considered to be of great importance in cattle in the Central American region. Three haemoparasites, *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*, transmitted by the *Boophilus microplus* tick, are frequently found together under field conditions (7).

Little knowledge on the epidemiology of babesiosis and anaplasmosis is available for the Latin American and Caribbean regions. The majority of studies have reported seroepidemiological results (9).

Recent investigations indicate an endemic situation of bovine babesiosis and anaplasmosis in the region. Pérez *et al.* (15) reported babesiosis and anaplasmosis to be ubiquitous in Costa Rica. In the same country, seroprevalence rates for *B. bovis* ranged from 33 to 100% in the Poás region and from 50 to 100% in the Tilarán region (11). A field study conducted in Yucatan, Mexico, detected an overall prevalence rate of 60.1, 66.7 and 59.6% for *B. bovis*, *B. bigemina* and *A. marginale*, respectively, using the multiplex polymerase chain reaction (PCR) (5).

A countrywide tick survey undertaken in Guatemala by the Interamerican Institute for Agricultural Cooperation (IICA) and the Ministry of Agriculture, demonstrated that *B. microplus* was

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the most important tick species parasitizing cattle in areas lower than 1800 meters above sea level (masl) (12). However, little information is available about the prevalence of bovine babesiosis and anaplasmosis in Guatemala. Serological studies, carried out by the Faculty of Veterinary Medicine and Zootechnics (FMVZ) of the University of San Carlos, showed seroprevalence rates for *A. marginale* ranging from 18.5 to 63.3% in various zones of the country (13, 14, 16, 20). Clinical cases of babesiosis have been reported, however, without laboratory confirmation (12).

The aim of the present study was to elucidate the interrelationship between ticks, parasites and cattle by determining the tick infestation rate and the infection rate of the mammalian host and the tick vector with *B. bovis*, *B. bigemina* and *A. marginale*. Consequently, blood samples were collected from cattle, ticks were detached under field conditions and both were examined using the multiplex PCR assay to detect the three haemoparasites in a relatively fast and highly sensitive manner (3, 5, 6), while the presence of antibodies to both *Babesia* species in the blood was assessed by serological tests.

■ MATERIALS AND METHODS

For this study, two farms were selected, situated in the South Coast of Guatemala. Both were participating in a Herd Health and Production monitoring project (as described for a similar project in Costa Rica by Dwinger *et al.*(2)) of the Faculty of Veterinary Medicine and Zootechnics of the University of San Carlos, Guatemala. Both farms were located in the village of Los Cerritos, municipality of Chiquimulilla, province of Santa Rosa (Northern latitude 13°58'00'', Western longitude 90°18'30'' and 30 masl). The farms had dual purpose cattle (crossbred *Bos indicus* x *Bos taurus*). Cows were hand milked twice daily following calf stimulation for the initiation of milk flow. Following milk extraction, cow and calf were left together until milking was finished (one to three hours).

Farm 1 was visited from September 1994 to July 1995 and farm 2 from September 1994 to April 1995. At the first visit, ten and seven calves, between five and twelve months of age, were selected at farms 1 and 2, respectively, and eartagged. Birth date and breed of the calves were recorded.

During each visit the same calves were monitored. However, two months after the start of the study two calves were replaced by two other calves at farm 2 and the last two months of the study two new calves were included at farm 1.

All animals at both farms, including the calves under examination, were sprayed with acaricides every 22 to 36 days. During the study different acaricides, including organophosphates (coumaphos and trichlorphon), and pyrethroids (deltamethrin) were applied without a specific sequence at intervals of three to five weeks. Sampling visits were planned shortly before each treatment, with a minimum interval of three weeks between spraying and the sampling date.

Average monthly minimum and maximum temperatures and precipitation values (1994-1995) of the village of Montufar, municipality of Moyuta, province of Jutiapa (Northern latitude 13°48'32'', Western longitude 90°09'18'' and 10 masl) were obtained from the National Seismological, Volcanological, Meteorological and Hydrological Institute (figure 1). Relative humidity varied from 60% in December 1994 (dry season) to 91% in August 1995 (rainy season).

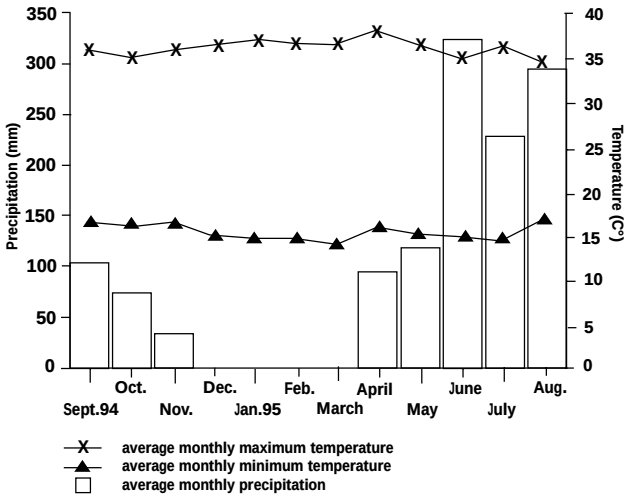

Figure 1*: average monthly maximum and minimum temperatures and precipitation in the village of Montufar, municipality of Moyuta, province of Jutiapa, at an altitude of 10 masl (1994-1995). Source: National Seismological, Volcanological, Meteorological and Hydrological Institute (INSIVUMEH), Guatemala.*

Host infection rate

Two blood samples were collected from each animal aseptically from the jugular vein using disposable needles and vacutainer tubes (Nipro Medical Ind., Japan). One sample was collected in tubes coated with Na-ethylene-diamine-tetra-acetate (EDTA), the other sample in tubes without additive in order to separate serum by centrifugation.

Haematological techniques

The EDTA blood samples were washed three times by centrifugation with phosphate buffered saline (PBS); the supernatant and buffy coat were discarded (7). Packed erythrocytes, stored in sterile microfuge tubes at -20°C (for a maximum duration of 12 months), were analyzed with the multiplex PCR as described by Figueroa *et al.* (5, 7).

As negative control, uninfected bovine blood was collected from a blood donor at the University of Missouri, Columbia. As positive control, infected culture-derived red blood cells were used (17).

Serological techniques

Serum samples were stored at -20°C (up to 14 months) in sterile microfuge tubes. Antibody levels were determined using an indirect enzyme-linked immunosorbent assay (ELISA) for *B. bovis* (4) and an indirect immunofluorescence test (IFT) for *B. bigemina* (1). Results of serological tests were recorded as positive or negative. Serological data for *A. marginale* were not obtained.

Tick infestation rate

During each visit, all ticks between 4.5 and 8 mm in size were detached from one side of the animal and collected in a plastic vial which was closed with a dry cloth. Ticks were counted, measured and the species was determined in the laboratory. The number of female *B. microplus* between 4.5 and 8 mm ("standard" ticks) was considered as an indication of engorged tick production per day per animal (18). The intensity of tick infestation was expressed as the average number of "standard" ticks detached from one side of

the animal (n/2). The infestation rate was expressed as the number of calves infested with ticks per the total number of calves examined.

In April, June and July 1995, ticks were collected from the pasture (20 m^2 at a forested and at an open zone) using the dragging technique as described by Guglielmone *et al.* (10). The ticks were collected in a plastic vial which was closed with moistened absorbent cotton. In the laboratory, ticks were counted and 10% were selected at random for species determination.

Tick infection rate

After determination of the species, 10% of the detached ticks were stored separately in sterile microfuge tubes at -20°C for a maximum of 12 months. To assess infection rates with *Babesia* and *Anaplasma* parasites, analysis with PCR as described by Figueroa *et al.* (5) was performed. Ticks detached during the first month of the study were not investigated by PCR.

Another 10% of the "standard" ticks collected were dissected to separate haemolymph and intestines in order to assess the localization of parasites. Haemolymph was collected by severing a leg with a pincette at the femoral-genual or genual-tibial joint. One drop was placed directly from the leg on a slide, air dried, fixed with methanol and subsequently stained with Giemsa according to standard procedures for microscopic examination. Additionally, of the same tick, haemolymph drops, collected from the severed legs with a sterile toothpick, and intestines, separated after dissecting the tick, were collected and stored in separate vials with two drops of PBS at -20°C for a maximum of 12 months.

Before initiating the PCR technique, ticks and intestines were pretreated according to the following modified scheme of the Puregene kit protocol (Gentra Systems, Inc., Minneapolis MN, USA):

- frozen intestine samples were thawed, subsequently centrifuged at 15,000 *g* for 10 min and the supernatant poured off. The residues of the intestine samples and entire thawed ticks were then homogenized using a microfuge tube pestle;

- 20 µl of the homogenized sample were added to a 1.5 ml microcentrifuge tube containing a 300 µl cell lysis solution, placed on ice and mixed with micropipettor. In a few cases, tick samples, desiccated due to prolonged storage in the freezer, were collected from the tube using two drops of the 300 µl cell lysis solution;

- the lysate was incubated 45 min at 65°C;

- after cooling to room temperature, a 1.5 µl RNase-A solution was added. Samples were mixed by inverting the tube 25 times and incubated at 37°C for 40 min;

- after cooling to room temperature, 100 µl of the protein precipitation solution was added and vortexed vigorously at high speed for 20 sec and centrifuged at 15,000 *g* for 3 min;

- the supernatant was poured into a 1.5 ml centrifuge tube containing 300 µl isopropanol. Samples were mixed by inverting gently 50 times and centrifuged at 15,000 *g* for one minute.

- the supernatant was discarded and tubes drained on clean absorbent paper;

- pellets were resuspended in 300 µl of 70% ethanol by inverting the tubes several times;

- following centrifugation at 15,000 *g* for one minute, ethanol was poured off and the tube allowed to air dry on clean absorbent paper for 15 min;

- a 50 µl DNA hydration solution was added and heated at 65°C for one hour;

- samples were stored at 2-8°C until further analysis with PCR except that no lysis buffer was added and the procedure was started by adding 250 µl of reaction mixture buffer.

To detect haemoparasites in the haemolymph, samples were thawed, centrifuged at 15,000 *g* for 10 min and the supernatant poured off before the PCR was started.

Positive and negative controls, as described previously under host infection rate, were used during the PCR analysis. Furthermore, ticks from a noninfected animal were used as negative tick control.

■ RESULTS

The observation period of nearly one year resulted in 10 visits to farm 1 and 6 visits to farm 2. Clinical cases of babesiosis or anaplasmosis were not reported and no treatment against babesiosis was applied. Tetracycline preparations were used frequently on both farms to treat various other diseases. The data of both farms were pooled for the purpose of this analysis.

Host infection rate

Haematological techniques

A total of 154 blood samples were screened with PCR for the presence of haemoparasites. Monthly single and multiple infections are presented in table I. Calves carrying a single infection of *A. marginale* showed the highest average prevalence during the study period (37%) followed by *B. bovis*/*A. marginale* infected calves (21%) and by calves carrying the three haemoparasites (14%). Single infections with *B. bigemina* were not found. Twenty-one percent of samples tested negative for the three haemoparasites.

Highest prevalence rates were found for *A. marginale*, varying from 55 to 88% of blood samples positive in a particular month. Infection rates for *B. bovis* ranged from 6 to 56%. Lowest prevalence rates were detected for *B. bigemina*, ranging from 0 to 50%. Highest infection rates for the three haemoparasites occurred from October to January.

Blood taken from the uninfected blood donor was consistently negative for the three haemoparasites. All positive blood controls tested positive for the three haemoparasites.

Of 17 calves under investigation, 133 time intervals (t_0 to t_0+1, 3 to 5 weeks between measurements) were recorded. Haemoparasite detection of individual animals varied over the months. Of the calves detected positive for *B. bovis*, *B. bigemina* and *A. marginale* at one visit (t_0), 16, 14 and 17% were not detected positive during the next observation (t_0+1), respectively, while 24, 8 and 62% were still positive at the next observation (t_0+1), respectively (table II).

Serological techniques

A total of 154 serum samples were examined for the presence of antibodies against *B. bovis* and *B. bigemina*. Monthly numbers of seropositive calves for *B. bovis* and/or *B. bigemina* are presented

Table I

PCR positive (+) and negative (-) calves for a combination of three haemoparasites (*B. bovis*, *B. bigemina* and *A. marginale*) per total number of calves observed per month in the South Coast of Guatemala

Month	Num. of calves	+ + +	+ + -[1]	+ - -	+ - +	- - -	- + -	- - +	- + +
September	17	3	0	0	5	4	0	4	1
October	16	6	0	0	3	2	0	3	2
November	17	4	1	0	3	1	0	5	3
December	17	4	0	1	3	2	0	7	0
January	17	1	0	1	6	2	0	7	0
February	17	2	0	0	5	3	0	7	0
March	16	0	0	0	1	4	0	11	0
April	14	0	0	0	3	5	0	6	0
June	11	0	0	0	1	5	0	4	1
July	12	1	0	1	2	5	0	3	0
Average		14%	1%	2%	21%	21%	0%	37%	5%

1. + + - = PCR positive for *B. bovis* and *B. bigemina*, PCR negative for *A. marginale*

Table II

Number and percentage of PCR positive (+) and negative (-) calves for one of the three haemoparasites (*B. bovis, B. bigemina* and *A. marginale*) in the South Coast of Guatemala, at one visit (t_0) and the next observation (t_0+1)

Situation at t_0	Situation at t_0+1	*B. bovis*	*B. bigemina*	*A. marginale*
+	+	32 (24%)	10 (8%)	82 (62%)
+	-	21 (16%)	18 (14%)	23 (17%)
-	-	63 (47%)	91 (68%)	9 (7%)
-	+	17 (13%)	14 (11%)	19 (14%)
Total		133	133	133

in table III. Seropositivity for both *B. bovis* and *B. bigemina* ranged from 35% of the calves in January to 71% in September and October (end of the rainy season). Seropositivity for either *B. bovis* or *B. bigemina* ranged from 17 to 53% and 0 to 18% of the calves, respectively.

Of 17 calves under observation, 131 time intervals (t_0 to t_0+1) were recorded. The serological status of individual animals varied over time. Calves seropositive for *B. bovis* at one visit (t_0) were seropositive at the next observation (t_0+1) in 82% of the recordings. For *B. bigemina*, of calves seropositive at one visit (t_0), 37% were still seropositive at the next observation (t_0+1)(table IV).

Host infection: combined haematological and serological results

▪ *B. bovis*

Calves, both PCR- and seropositive for *B. bovis* ranged from 6 to 56% for a particular month during the study period. The highest monthly percentages were found at the start of the study. Calves seropositive for *B. bovis* but not positive in PCR ranged from 44 to 82%. During the study this percentage showed a slight increase (table V). A positive result with PCR but negative with ELISA was found in eight samples obtained from five different calves.

Table III

Number of seropositive (+) and seronegative (-) calves for a combination of two haemoparasites (*B. bovis* and *B. bigemina*) per total number of calves observed per month in the South Coast of Guatemala

Month	Num. of calves	+ +	+ -[1]	- +	- -
September	17	12	5	0	0
October	17	12	5	0	0
November	17	7	9	1	0
December	17	9	6	0	2
January	17	6	7	3	1
February	16	9	5	1	1
March	17	10	3	3	1
April	12	8	2	0	2
June	12	7	4	0	1
July	12	5	5	1	1
Average		55%	33%	6%	6%

1. + - = seropositive for *B. bovis* and seronegative for *B. bigemina*

Table IV

Number and percentage of seropositive (+) and seronegative (-) calves for *B. bovis* or *B. bigemina*, in the South Coast of Guatemala, at one visit (t_0) and the next observation (t_0+1)

Situation at t_0	Situation at t_0+1	*B. bovis*	*B. bigemina*
+	+	107 (82%)	49 (37%)
+	-	8 (6%)	31 (24%)
-	-	10 (8%)	21 (16%)
-	+	6 (5%)	30 (23%)
Total		131	131

Table V

Number of PCR positive and seropositive calves for *B. bovis* per total number of calves observed per month in the South Coast of Guatemala

Month	Num. of calves	+ +	+ -	- +	- -
September	17	8	0	9	0
October	16	9	0	7	0
November	17	8	0	8	1
December	17	7	1	8	1
January	17	4	4	9	0
February	17	6	1	9	1
March	17	1	0	12	4
April	13	2	0	9	2
June	11	1	0	9	1
July	12	2	2	8	0
Average		31%	5%	57%	6%

+ + = PCR positive and seropositive
+ - = PCR positive and seronegative
- + = PCR negative and seropositive
- - = PCR negative and seronegative

Table VI

Number of PCR positive and seropositive calves for *B. bigemina* per total number of calves observed per month in the South Coast of Guatemala

Month	Num. of calves	+ +	+ -	- +	- -
September	17	3	1	9	4
October	16	5	3	6	2
November	17	4	4	4	5
December	17	0	4	9	4
January	17	1	0	8	8
February	16	0	2	10	4
March	17	0	0	13	4
April	12	0	0	8	4
June	11	0	1	6	4
July	12	1	0	5	6
Average		9%	10%	51%	30%

+ + = PCR positive and seropositive
+ - = PCR positive and seronegative
- + = PCR negative and seropositive
- - = PCR negative and seronegative

▪ *B. bigemina*

During the last seven months of the study only two calves were detected seropositive in combination with a positive PCR. Calves seropositive but negative in PCR ranged from 24 to 76% (table VI). Positive PCR results but seronegativity were found in 15 samples obtained from nine different calves.

Relationship between age and PCR/serology results

PCR and serological results were compared for various age groups of calves. Although numbers per age group varied and were small (2-18), the reason for which statistical analyses were not carried out, some trends could be observed.

For *B. bovis*, high infection rates based on PCR (50-54%) were observed for calves younger than 10 months. After the age of 10 months, these rates decreased, ranging from 14 to 39%. PCR data for *B. bigemina* and *A. marginale* did not show remarkable differences between the two age groups.

All calves younger than 10 months were seropositive for *B. bovis* but seroprevalence rates decreased (to 67% at the age of 14 months) when calves grew older. For *B. bigemina* no age difference was apparent.

Tick infestation rate

A total of 1752 ticks were detached from calves and all were identified to be *B. microplus*. No "standard" ticks were encountered in 27 (18.5%) of the 146 observations made on 17 individual calves. Monthly average numbers of "standard" female *B. microplus* ticks collected on one side of the calf are presented in figure 2. Monthly infestation rates (number of calves infested with ticks per total number of calves examined) varied from 58 to 94%.

By using the dragging technique only larvae were collected. At farm 1 in April, June and July 94, 138 and 36 larvae were found, respectively. At farm 2 in April and June, 169 and 76 larvae were found, respectively. All larvae examined were *Boophilus* spp.

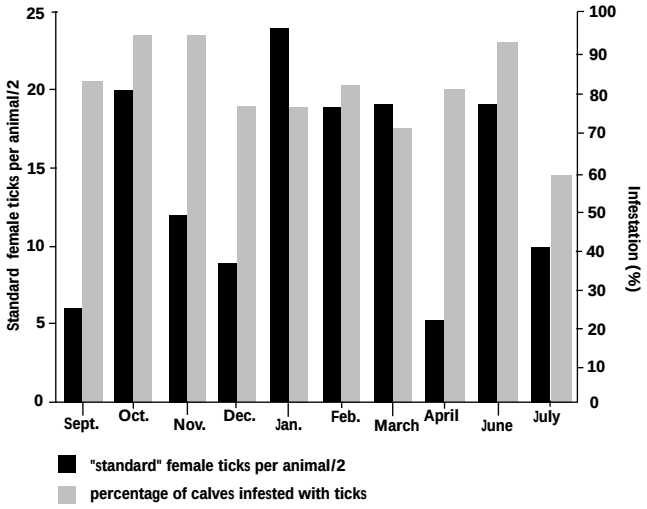

***Figure 2**: monthly average numbers of "standard" female* Boophilus microplus *(> 4.5 mm and < 8 mm) ticks collected on one side of the calf and monthly infestation rates (percentage of the calves under investigation infested with ticks) in the South Coast of Guatemala.*

Tick infection rate

Detached ticks: the majority of 170 ticks investigated by PCR were negative (72%). Infection rates of the ticks with *B. bovis*, *B. bigemina* and *A. marginale* were 2, 21 and 18%, respectively. Eleven percent of the ticks were infected with *B. bigemina* only and 10% were infected with both *B. bigemina* and *A. marginale*. Single infections with *A. marginale* were found in 6% of the samples. Single infections with *B. bovis* were not found (table VII).

Table VII

PCR positive (+) and negative (-) ticks for a combination of three haemoparasites (*B. bovis*, *B. bigemina* and *A. marginale*) per total number of ticks investigated per month in the South Coast of Guatemala

Month	Num. of ticks	+ + +	+ - -	+ + -[1]	+ - +	- - -	- + -	- - +	- + +
October	9	0	0	0	0	6	0	1	2
November	16	0	0	0	0	14	2	0	0
December	13	0	0	0	0	12	1	0	0
January	30	1	0	0	1	24	3	1	0
February	27	0	0	0	1	19	0	3	4
March	15	0	0	0	0	11	2	0	2
April	11	0	0	0	0	6	1	2	2
June	30	0	0	0	0	18	5	2	5
July	19	0	0	0	0	12	4	1	2
Average		1%	0%	0%	1%	72%	11%	6%	10%

1. + + - = PCR positive for *B. bovis* and *B. bigemina*, PCR negative for *A. marginale*

All negative blood and tick controls tested negative. The positive blood samples tested consistently positive for the three haemoparasites.

Ticks were found to be infected with *B. bovis* in January and February only. The lowest infection rates for *B. bigemina* and *A. marginale* were detected in November and December, at the end of the rainy season.

Haemolymph: microscopic examination of 190 haemolymph samples showed three samples positive for *Babesia* spp. (1.6%). Examination of 152 haemolymph samples by PCR showed two to be positive for *B. bovis* (1.3%) and one for *B. bigemina* (0.7%). The samples positive in both methods did not originate from the same tick. *A. marginale* was not found in the haemolymph samples examined by PCR.

Intestines: of 120 intestine samples investigated by PCR, one sample was found positive for *B. bigemina* and one for *A. marginale*. *B. bovis* was not detected.

■ DISCUSSION

The present study confirms the assumed widespread presence of *B. bovis*, *B. bigemina* and *A. marginale* in the South Coast of Guatemala. Using PCR for the examination of blood samples, *A. marginale* was detected more frequently than *Babesia* parasites. Furthermore, the infection rate of calves for *B. bovis* was higher than for *B. bigemina*, which was confirmed serologically.

High prevalence rates for the three haemoparasites and absence of clinical cases indicate that anaplasmosis and babesiosis are endemic in the region. It is likely that stability is maintained by exposure of young animals, protected by colostral antibodies and age-resistance, to infective ticks. Furthermore, the frequent use of tetracyclines on the selected farms could theoretically have curtailed the occurrence of anaplasmosis.

In general, it is assumed that following infection animals become carriers, characterized by persistent infection with fluctuating levels of parasitaemia. With the PCR technique and its high sensitivity, carrier animals with very low parasitaemias can also be detected. Figueroa *et al.* (7) introduced the multiplex PCR for detection of three haemoparasites in one single blood sample. However, the multiplex PCR assay showed a decreased sensitivity as compared to the single PCR assay. Fahrimal *et al.* (3) reported a more than 95% detection rate of carrier animals infected with *B. bovis* when analyzing two consecutive samples taken one to two weeks apart.

In the present study, consecutive blood samples, taken three to five weeks apart, showed considerable variation in detection of the parasites in the blood. This variation might be attributable to various factors. These include the length of interval between two samples, not corresponding with the duration of parasitaemia levels detectable by the multiplex PCR, or a possible superinfection with new strains of the parasite. Furthermore, the last step of the multiplex PCR technique, eye-reading of membranes, causes an undesirable variation in detection.

Similarly, variation in the detection of antibodies directed against *B. bovis* and *B. bigemina* in consecutive samples could be due to a combination of a weak antibody response by a low level of parasitaemia and the preset cut-off point as determined for the utilized kit, which could be inappropriate for the situation in Guatemala.

The only tick species found in this study was *B. microplus*, whereas others also mentioned the presence of *Amblyomma cayennense* and *B. annulatus* in the South Coast of Guatemala (12, 19). *B. microplus* was found all year round.

Monthly percentages of calves infested with ticks and average numbers of “standard” female *B. microplus* ticks per animal as found in the present study, correspond with the results observed in Costa Rica by Hermans *et al.* (11). However, compared to the situation in Costa Rica, the present study revealed lower infection rates in ticks for all three haemoparasites.

A proportion of the infected ticks, as determined with PCR, were infected by alimentary infection and should therefore reflect the host infection rate. For *B. bigemina*, the infection rate of the ticks (21%) coincided with that detected by PCR in the calves (20%). However, a high infection rate found in calves for *B. bovis* (38%) was neither associated with a high proportion of infected ticks (2%), nor with the presence of *vermiculi* in the haemolymph (1.3%). Similarly, a high infection rate for *A. marginale* (77%) in calves was not reflected by a high infection rate in ticks (18%).

In the study of Hermans *et al.* (11) it was proposed to dissect the tick to obtain more information about the localization of the parasite and the epidemiology of the disease. In the present study, for *B. bigemina* and *A. marginale*, the PCR results of gut and haemolymph samples did not coincide with the infection rate in the ticks. An analysis by PCR of other tick organs, including salivary glands, is therefore suggested. Additionally, the infection rate of the larvae found in the field could be determined, especially for *B. bovis* which is exclusively transmitted by larvae and not by nymphs and adults (8).

■ CONCLUSION

This study confirmed the suspected endemic situation of bovine babesiosis and anaplasmosis in the South Coast of Guatemala. Using multiplex PCR, *B. bovis*, *B. bigemina* and *A. marginale* were detected in both cattle and ticks. *B. microplus* was the most important tick species infesting cattle in the South Coast of Guatemala.

PCR and serological results of consecutive blood samples of the same animals showed considerable variation in antigen and antibody detection, respectively. This demonstrates the limitations of single tests and how carefully the results should be interpreted.

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Résumé

Van Andel J.M., Dwinger R.H., Alvarez J.A. Etude du taux d'infection du bétail et des tiques associées par *Anaplasma* et *Babesia* sur la Côte Sud du Guatemala

Babesia bovis, *B. bigemina* et *Anaplasma marginale* sont soupçonnés être très répandus sur la Côte Sud du Guatemala. L'épidémiologie des parasites a été étudiée en suivant mensuellement pendant un an 17 veaux dans deux fermes, et en prélevant et comptant toutes les tiques femelles "standard" sur une moitié du corps de tous ces animaux. Le sang et le sérum des veaux ont été examinés respectivement par la technique d'amplification en chaîne par polymérase (PCR) et des tests sérologiques. De plus, la technique de PCR a été appliquée sur l'hémolymphe et les intestins des tiques prélevées. *Boophilus microplus* était la seule espèce de tiques trouvée sur les veaux. La présence des trois parasites du sang a été confirmée chez les veaux et dans les tiques. Un taux d'infection élevé chez les veaux était associé à un taux d'infection élevé dans les tiques prélevées sur eux pour *B. bigemina*. Néanmoins, pour *B. bovis* et *A. marginale* cette association n'a pas été observée. En outre, les résultats de la PCR et des tests sérologiques des échantillons consécutifs des mêmes animaux ont montré une variation considérable respectivement des niveaux d'antigènes et d'anticorps. Durant la période de l'étude, il n'y a pas eu de cas cliniques de babésiose ni d'anaplasmose, ce qui concorde avec une situation endémique stable dans le cadre de la gestion actuelle des troupeaux.

Mots-clés : Bovin - Veau - *Boophilus microplus* - Metastigmata - *Anaplasma marginale* - *Babesia bovis* - *Babesia bigemina* - PCR - Immunologie - Guatemala.

Resumen

Van Andel J.M., Dwinger R.H., Alvarez J.A. Estudio de las tasas de infección de *Anaplasma* y *Babesia* en ganado y garrapatas asociadas en la Costa Sur de Guatemala

Se sospecha que *Babesia bovis, B. bigemina* y *Anaplasma marginale* se encuentran dispersas en la Costa Sur de Guatemala. Se estudió la epidemiología de los parásitos mediante el seguimiento de 17 terneros a intervalos mensuales, durante un período de un año, en dos fincas, así como por el conteo y desprendimiento de todas las garrapatas hembras "standard" recolectadas en una mitad de cada uno de los animales. Se examinaron muestras de sangre y suero bovino mediante la técnica de reacción en cadena de polimerasa (PCR) y por tests serológicos, respectivamente. Además, la técnica de PCR se aplicó a la hemolinfa e intestinos de las garrapatas desprendidas. *Boophilus microplus* fue la única especie de garrapata encontrada en los terneros. La presencia de los tres hemoparásitos se confirmó tanto en los terneros como en las garrapatas. Para *B. bigemina*, las altas tasas de infección en los terneros se asociaron con altas tasas de infección en las garrapatas desprendidas. Sin embargo, para *B. bovis* y *A. marginale*, esta asociación no fue demostrada. Los resultados de PCR y serológicos en muestreos consecutivos de un mismo animal, muestran variaciones considerables en el nivel de antígenos y anticuerpos, respectivamente. No se observaron casos clínicos de babesiosis y anaplasmosis durante el período en estudio, lo que coincide con una situación endémica estable bajo prácticas usuales de manejo del hato.

Palabras clave: Ganado bovino - Ternero - *Boophilus microplus* - Metastigmata - *Anaplasma marginale* - *Babesia bovis* - *Babesia bigemina* - PCR - Immunología - Guatemala.