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Epidemiology of bovine anaplasmosis in dairy herds from Costa Rica

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ABSTRACT

Bovine anaplasmosis is endemic and occurs in almost all areas of livestock production of Costa Rica. The aim of this study was to determine the seroprevalence and risk factors of anaplasmosis in dairy farms of Costa Rica by the recombinant truncated MSP-5 (rMSP-5) enzyme-linked immunosorbent assay (ELISA). Serum samples were obtained from 733 cattle from 20 commercial dairy herds of Costa Rica. The overall seroprevalence was 37.2% and herd seroprevalence ranged from 20.0 to 72.0%. The age-specific seroprevalence was 49.3% in young and 33.4% in adult animals. The main risk factors associated with seroprevalence were season of occurrence of clinical cases (rainy season) (OR = 22.8), presence of tabanids (OR = 9.5) and stable flies (OR = 6.2), stable flies control measures (OR = 3.2), non-use of ear tattoos (OR = 2.8), interval of veterinary visit (≤60 days) (OR = 2.7), altitude of the farms (<800 masl) (OR = 2.6) and age (<2 years) (OR = 1.8). The results indicated that exposure of cattle to *Anaplasma marginale* is common in dairy herds of Costa Rica and endemic instability situation probably is due to inadequate vector control.

1. Introduction

Anaplasma marginale (Rickettsiales, Anaplasmataceae) is the causative agent of bovine anaplasmosis which is endemic in tropical and subtropical areas of the world. The disease is the most prevalent haemoparasitoses in Latin America, responsible by economic losses estimated in $875 million per year (Kocan et al., 2003). An annual loss of $64 million in dairy herds ($60 per cow) was estimated in Costa Rica due to anaplasmosis (McCauley and Pérez, 1980). Anaplasmosis is characterized by fever, severe anemia, weight loss, decreased milk production and abortion (Kocan et al., 2003; Urdaz-Rodríguez et al., 2009). After the first infection with *A. marginale*, cattle remain persistently infected carriers and serve as a reservoir for the maintenance of the organism within a herd (Kocan et al., 2003; Urdaz-Rodríguez et al., 2009).

A. marginale is transmitted by *Rhipicephalus* (*Boophilus*) *microplus* ticks and many hematophagous diptera are implicated as mechanical vectors for transmission of *A. marginale*, including *Stomoxys calcitrans*, *Haematobia irritans* and *Tabanus* spp. (Guglielmone, 1995; Kocan et al., 2003; Urdaz-Rodríguez et al., 2009). Moreover, iatrogenic transmission by blood-contaminated fomites, including needles, ear tagging, dehorning and castration instruments is considered to be important in the epidemiology of

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anaplasmosis in areas of Central and South America, Africa, and the US (Morley and Hugh-Jones, 1989; Alonso et al., 1992; Guglielmone, 1995).

The knowledge of prevalence is an important component in the assessment of levels of herd immunity to this disease (Herrero et al., 1998; Urdaz-Rodríguez et al., 2009). In the previous studies using the rapid-card agglutination test (card-test), the seroprevalence of anaplasmosis in Costa Rica ranged from 72.4% to 86.4% (Pérez et al., 1980, 1994a, 1994b). Nevertheless, Pérez et al. (1994b) noted the possibility of an endemic instability in Costa Rican dairy herds due to the excessive tick control. Successful management of anaplasmosis depends on adequate knowledge of seroprevalence for *A. marginale* and the risk factors associated with the transmission (Alonso et al., 1992; Swai et al., 2005). The aim of this study was to determine the seroprevalence of and risk factors of anaplasmosis in dairy herds of Costa Rica by using the recombinant truncated MSP-5 (rMSP-5) enzyme-linked immunosorbent assay (ELISA). This study formed part of an epidemiological survey of haemoparasitic diseases in dairy herds from Costa Rica.

2. Materials and methods

2.1. Design and population studies

A cross-sectional serological study was carried out between January and December 2008 in 20 commercial dairy herds of the Cooperativa de Productores de Leche Dos Pinos R.L., located in the provinces of Alajuela, Heredia and San José (Fig. 1). Clinical cases of anaplasmosis have been frequently reported in all these commercial dairy herds.

The number of animals on each farm was calculated according to Cameron and Baldock (1998), and young (<2 years) and adult (>2 years) cattle from varied breed were included in this study. The animals for testing were randomly selected.

A standard questionnaire was applied on dairy owners and veterinarians on each farm at the time of blood collection. The information gathered concerned farm and animal events, farm husbandry practices, seasonality of clinical cases, veterinary assistance (interval of visits at the farms and instrumentals used), presence of ticks and biting flies, acaricide/insecticide application method, and acaricide/insecticide application frequency (Table 1).

2.2. Collection of samples and serological assay

Blood samples were taken from the coccygeal vein using vacutainer tubes and transported to the laboratory on the same day on ice. The serum was then removed and frozen at −20 °C until tested.

An indirect enzyme-linked immunosorbent assay (ELISA) based on recombinant truncated major surface protein 5 of *A. marginale* (rMSP-5) (sensitivity 96.9%, specificity 100%) was used to detect antibodies to *A. marginale* (Araújo et al., 2005; Melo et al., 2007).

Table 1
Description of the questionnaire parameters to analyse management factor, not related to the external parasites control and specifically related to the external parasites control, in 20 commercial dairy herds of Costa Rica from January to December 2008.

Variable	Total of farms
Season of clinical cases	
All year	3
Rainy season	10
Dry season	1
Transition period (dry/rainy)	6
Veterinary assistance	
Veterinary visit interval	
0–60 days	11
>60 days	9
Veterinary instruments	
Individual needles	
No	12
Yes	7
Sometimes	1
Ear tattoos	
No	15
Yes	5
Presence of ectoparasites	
No	1
Yes	19
Ticks	
Rhipicephalus (*Boophilus*) *microplus*	18
Amblyomma cajennense	0
Biting flies	
Stable flies (*Stomoxys calcitrans*)	18
Horn flies (*Haematobia irritans*)	0
Tabanids (*Tabanus* spp.)	1
Tick control	
No	1
Yes (spraying)	19
Tick control interval (spraying)	
15 days	9
22 days	5
30 days	4
45 days	2
Stable flies control	
No	3
Spraying	10
Pour-on	5
Spraying and pour-on	2

2.3. Statistical analysis

Descriptive statistics about the farm characteristics and overall prevalence were performed using SAS/STAT ver. 6.12. To assess the relationship of the seroprevalence with characteristics of the hosts and environmental factors logistic regression with herd as the random effect was used. The logistic procedure consisted of 2 steps: (1) univariable analysis and (2) multivariable analysis. In the second step, all variables with $p < 0.25$ in the univariable analysis were included. A backward building model was followed based on the odds ratios (OR) test (Hosmer and Lemeshow, 1989). The process of exclusion–inclusion of each variable into the multivariable model tested confounding and interaction by comparison of the estimated coefficients in the new model with the estimated coefficients and OR of the old model. Confounding was deemed present if at least one coefficient changed more than 0.1 (if the coefficient had a value between −0.4 and 0.4) or if at least one coefficient changed more than 25% (if the coefficient had a value <−0.4 or >0.4).

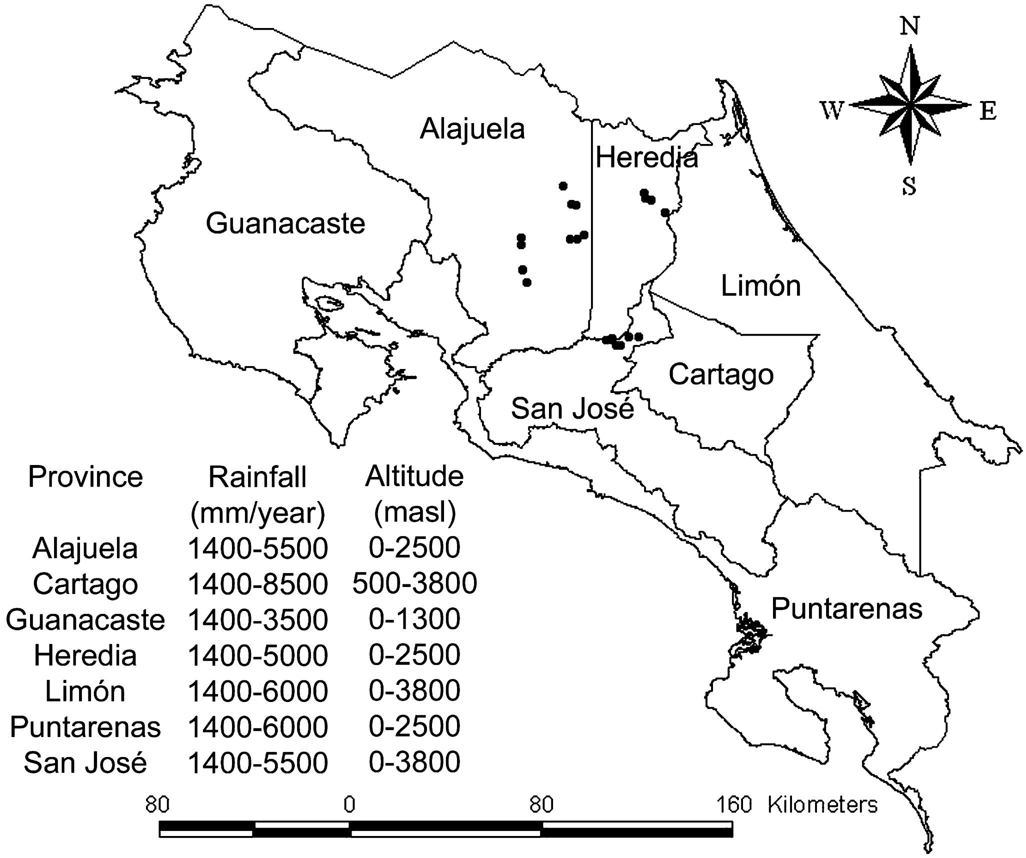

Fig. 1. Geographical distribution of 20 commercial dairy herds of Costa Rica.

Finally, simple correlation calculations were used to check for collinearity with the variables in the final model in order to avoid potential confounding. Analyses were carried out using EGRET (SERC, 1990; McDermott and Schukken, 1994; McDermott et al., 1994).

Due to the high number of independent variables, they were categorized into three groups: (1) related to the herd and environmental, (2) general management factors not related to the external parasite control, and (3) specifically related to the external parasite control.

3. Results

3.1. Descriptive statistics

Farms were located in following provinces: 50% in Alajuela, 30% in San José and 20% in Heredia (Fig. 1). Fifty-five percent of the farms were located at altitudes of 0–800 masl and 45% at 801–1600 masl (Table 2). Seventy-five percent of the farms had 100 or fewer animals (farms #1, 2, 3, 6, 7, 8, 10, 11, 12, 13, 14, 16, 18, 19 and 20); 15% had 101–200 animals (farms #4, 5, and 9), and 10% had 201–300 animals (farms #15 and 17) (Table 2). The number of animals per farm, farm location and altitude, and presence of ectoparasites are listed in Table 2.

The diagnosis of disease was based on clinical manifestations (mainly fever and anemia) and response to therapy with oxytetracycline. In some clinical cases the confirmation of the infection was done by microscopic examination of *A. marginale* in blood smears.

Clinical cases occurred mainly in the rainy season (50%); transition period (dry/rainy) (30%); during all year (15%), and dry season (5%) (Table 1). In 55% of the farms the interval of veterinary visit was ≤60 days (Table 1). Non-use of ear tattoos was reported in 75% of the farms; on the other hands, in 60% of them individual needles are not used (Table 1).

In order to confirm the information about presence of ectoparasites, some specimens were collected and transported to laboratory for identification. *R. microplus* and *Stomoxys calcitrans* (stable flies) were present on the majority of farms (90%) while *Tabanus* sp. (horse flies or tabanids) was observed in 10% of these farms (Tables 1 and 2).

The most farmers (>85%) used control measures of ectoparasite (Table 1). External parasites control was exclusively acaricide and insecticide-based (pyrethroids, organophosphates and amidines) including: spraying (used in backpack sprayer) and/or pour-on formulations (Table 1). In 75% of the farms, tick control occurred when infestation level was <20 ticks/animal.

3.2. Seroprevalence and risk factors

The overall seroprevalence was 37.2% (273/733) and herd seroprevalence ranged from 20.0 to 72.0% (Table 2

Table 2
Seroprevalence to *Anaplasma marginale* and ectoparasites species in 20 commercial dairy herds of Costa Rica from January to December 2008.

Farms	Province	Altitude (masl)	Total of animals	Animals sampled	Seropositives (seroprevalence %)	Ectoparasites
#1	Alajuela	1.550	75	45	14 (31.1)	*Rhipicephalus microplus*, *Stomoxys calcitrans*
#2	Alajuela	1.400	30	20	9 (45.0)	*R. microplus*, *S. calcitrans*
#3	Alajuela	680	70	42	12 (28.6)	*R. microplus*, *S. calcitrans*
#4	Alajuela	85	172	50	16 (32.0)	*R. microplus*, *S. calcitrans*
#5	Alajuela	1.300	148	50	17 (34.0)	*R. microplus*
#6	Alajuela	450	58	41	18 (43.9)	*R. microplus*, *S. calcitrans*
#7	Alajuela	460	89	45	23 (51.1)	*R. microplus*
#8	Alajuela	330	85	45	21 (46.7)	*R. microplus*, *S. calcitrans*
#9	Alajuela	460	190	54	15 (27.8)	*R. microplus*, *S. calcitrans*, *Tabanus* sp.
#10	Alajuela	85	30	15	9 (60.0)	*R. microplus*, *S. calcitrans*
#11	Heredia	40	35	25	8 (32.0)	*R. microplus*, *S. calcitrans*
#12	Heredia	40	30	20	9 (45.0)	*R. microplus*, *S. calcitrans*
#13	Heredia	40	69	40	15 (37.5)	*R. microplus*, *S. calcitrans*
#14	Heredia	40	35	25	18 (72.0)	*R. microplus*, *S. calcitrans*
#15	San José	1.400	300	50	13 (26.0)	*R. microplus*, *S. calcitrans*
#16	San José	1.400	60	36	10 (27.8)	*R. microplus*, *S. calcitrans*
#17	San José	1.450	210	50	10 (20.0)	*R. microplus*, *S. calcitrans*
#18	San José	1.600	36	24	16 (66.7)	*S. calcitrans*
#19	San José	1.400	85	41	12 (29.3)	*R. microplus*, *S. calcitrans*
#20	San José	1.600	30	15	8 (53.3)	*S. calcitrans*
Total	–	–	–	733	273 (37.2)	–

and Fig. 1). The age-specific seroprevalence was 49.3% in young (<2 years) and 33.4% in adult animals (>2 years).

Herd and environmental factors with a clear association with seroprevalence included: season of occurrence of clinical cases (rainy season) (OR = 22.8); altitude of the farms (0–800 masl) (OR = 2.6) and age (<2 years) (OR = 1.8) (Table 3).

On the other hand, general management factors that were associated with seroprevalence included: non-use of ear tattoos (OR = 2.8); interval of veterinary visit (≤60 days) (OR = 2.7) and non-use of individual needles (OR = 2.1) (Table 4).

The odds of seropositivity tended to increase with the presence of tabanids (OR = 9.5) and stable flies (OR = 6.2) (Table 5). Combined use of spraying and pour-on formulations for stable flies control showed an increased likelihood of seropositivity (OR = 3.2) when used at intervals of up to 60 days (OR = 3.3). Spraying for tick control (OR = 2.7) at dipping intervals of 22 and 45 days (OR = 1.1, respectively) appears to increase the likelihood of seropositivity (Table 5).

Table 3
Herd and environmental factors associated with the seroprevalence to *Anaplasma marginale* in 20 commercial dairy herds of Costa Rica from January to December 2008.

Term	OR	CI95%
Age[a]		
<2 years	1.8	1.3–2.7
Altitude[b]		
0–800 masl	2.6	1.9–3.7
Season of clinical cases[c]		
All year	6.3	1.8–27.2
Rainy season	22.8	6.6–94.5
Transition period (dry/rainy)	3.5	0.9–15.5

[a] >2 years used as a reference factor.
[b] 801–1600 masl used as reference factor.
[c] Dry season used as reference factor.

Table 4
General management factors associated with the seroprevalence to *Anaplasma marginale* in 20 commercial dairy herds of Costa Rica from January to December 2008.

Term	OR	CI95%
Interval of veterinary assistance[a] ≤60 days	2.7	1.5–5.5
Ear tattoos[b]		
No	2.8	1.8–4.5
Individual needles[b]		
No	2.1	1.5–3.0

[a] >61 days used as reference factor.
[b] Yes used as reference factor.

Table 5
External parasites control associated with the seroprevalence to *Anaplasma marginale* in 20 commercial dairy herds of Costa Rica from January to December 2008.

Term	OR	CI95%
Ticks[a]		
Yes	2.7	0.9–8.2
Stable flies[a]		
Yes	6.2	3.2–13.5
Tabanids[a]		
Yes	9.5	5.8–14.1
Tick control[b]		
Yes (spraying)	2.7	0.9–8.33
Tick control interval (spraying)[c]		
22 days	1.1	0.6–1.8
30 days	0.4	0.2–0.6
45 days	1.1	0.7–1.9
Stable flies control[d]		
Spraying and pour-on	3.2	1.4–5.5
Pour-on	0.8	0.4–1.5
None	2.9	1.9 to −4.1
Stable flies control interval[e]		
0–60 days	3.3	0.1–6.3

[a] No used as reference factor.
[b] None used as reference factor.
[c] 15 days used as reference factor.
[d] Spraying used as reference factor.
[e] >60 days used as reference factor.

4. Discussion

The Overall seroprevalence (37.2%) was similar to observed in Tanzania (20% and 37%) (Swai et al., 2005) and Puerto Rico (27.4%) (Urdaz-Rodríguez et al., 2009), but was lower compared to that one reported in previous seroepidemiological studies in Costa Rica (72.4–86.4%) (Pérez et al., 1980, 1994a, 1994b), Brazil (97%) (Barros et al., 2005), Colombia (90.3%) (Patarroyo et al., 1978), Kenya (89%) (Maloo et al., 2001), El Salvador (78.5%) (Payne and Scott, 1982) and St Lucia (70%) (Hugh-Jones et al., 1988b). The different seroprevalence ratios can be explained by sensitivity and specificity of serological tests used in these studies.

According to Perry (1996), endemic stability is defined as the state where the relationship between host, agent, vector and environment is such that clinical disease occurs rarely or not at all. In endemic areas, cattle are exposed to infection by *A. marginale* at an early age and develop immunity that favors the maintenance of infection in a natural cycle in which tick infection levels remain low, creating a situation in which few clinical cases occur (Mahoney and Ross, 1972; Callow, 1984; Guglielmone, 1995). Although the seroprevalence and the occurrence of clinical cases suggest an endemic instability situation of anaplasmosis in studied dairy herds, endemic stability to anaplasmosis in Costa Rican dairy cattle was demonstrated in previous seroepidemiological studies (Pérez et al., 1980, 1994b). However, despite of seroprevalence Pérez et al. (1980, 1994b) noted the possibility of the existence of an artificial endemic instability due to excessive tick control practices. This conclusion was based mainly on the occurrence of several clinical cases of anaplasmosis during the rainy season. This is the first report of instability to anaplasmosis in Costa Rican dairy herds, which agree with Pérez et al. (1980, 1994b).

It is possible that in the studied herds the ectoparasites control practices (exclusively acaricide and insecticide-based) results in a low vector population, insufficient for maintaining constant transmission of *A. marginale* (Mahoney and Ross, 1972; Callow, 1984; Hugh-Jones et al., 1988a,b; Pérez et al., 1994b; Guglielmone, 1995). In this case, an imbalance caused by the parasite–host relationship due to infrequent transmission creates an artificial endemic instability condition, where clinical cases occur seasonally coinciding with the maximum activity period of the vectors (Mahoney and Ross, 1972; Pérez et al., 1980; Callow, 1984; Hermanns et al., 1994).

Unfortunately, in current study was not possible to make the confirmation of the infection of *A. marginale* by microscopic examination of blood smears of all sick animals. For this reason, the numbers of outbreaks and number of animals affected per outbreak cannot be associated with seropositivity in the farms. In studied farms the diagnosis of clinical cases of anaplasmosis was carried out based on clinical signs and positive response to therapy with oxytetracycline. Diagnosis based in nonspecific clinical signs (fever and anemia) could lead to misdiagnosis with other diseases, as babesiosis and trypanosomiasis. In Costa Rica, anaplasmosis and babesiosis are the major health problem in dairy herds, but according to Pérez et al. (1980) and Oliveira et al. (2009) *A. marginale* is the most common pathogen of cattle in the country. In one of the evaluated farms the infection by *Trypanosoma* (*Duttonella*) *vivax* was diagnosed in blood smears of animals with clinical signs such as fever, anemia, abortion or preterm birth and marked weight loss (Oliveira et al., 2009). Some animals showed mixed infection with *A. marginale* and *T. vivax*, and *Babesia bovis* and *T. vivax* (Oliveira et al., 2009). For this, farmers and veterinarians were advised about the importance to improve the diagnosis of disease through of laboratory techniques (blood smears) to confirm clinical diagnosis of haemoparasitoses.

Successful management of anaplasmosis depends on adequate knowledge of prevalence and the risk factors associated with the transmission (Alonso et al., 1992; Swai et al., 2005). However, according to Urdaz-Rodríguez et al. (2009) it is difficult to understand clearly the factors associated with acquisition of infection by a cross-sectional study of antibody prevalence.

In this survey, seroprevalence was higher in younger animals, consistent with previous reports in Costa Rica and Brazil (Pérez et al., 1994b; Barros et al., 2005). Young animals probably become infected early but overtime lack of vectors precludes reinfections, becoming susceptible adults to the disease. For this, seroprevalence was lower in adult animals, which were most affected in clinical cases, according to reported by veterinarians and farmers. It is important to clear up that in majority of studied farms younger animals are not exposed to infestation by ticks because are kept in stables until 8–10 months of age. Age is a determining factor in the immunity of animals to *A. marginale*, since young animals are more resistant to primary infection (Mahoney and Ross, 1972; Callow, 1984; Hugh-Jones et al., 1988a,b; Kocan et al., 2003; Barros et al., 2005). According to Mahoney and Ross (1972) and Callow (1984), if at least 75% of calves were exposed to infection by 6–9 months of age the disease incidence would be very low and a state of natural endemic stability would exist.

There was an association between the season of occurrence of clinical cases (rainy season) and seroprevalence, also reported in Costa Rica (Pérez et al., 1994b). The pattern of increased seropositivity during the rainy season perhaps is associated with an increase in the population of vectors (Hermanns et al., 1994; Pérez et al., 1994b). The altitude of the farms was also associated with seroprevalence in areas below 800 masl, which was expected because *R. microplus* is present in Costa Rican territory at altitudes up to 2000 masl (McCauley and Pérez, 1980; Hermanns et al., 1994; Herrero et al., 1998). McCauley and Pérez (1980) demonstrated that 85% of cattle in low altitude areas (<2000 masl) of the country had antibodies anti-*A. marginale*, while cattle at higher altitudes are highly susceptible to infection. Environmental factors are very important to epidemiology of anaplasmosis, but must be analysed in association with other factors as local variation in grazed cattle densities and external parasites control measures (Swai et al., 2005).

Hematophagous diptera play an important role in the transmission of *A. marginale* beyond of ticks in Latin America (Alonso et al., 1992; Guglielmone, 1995; Rodríguez-Vivas and Domínguez-Alpízar, 1998; Kocan et al., 2003; Barros et al., 2005). Although McCauley and Pérez (1980) indicated ticks play the most important role in the transmission of *A. marginale* in Costa Rica, there is an association between seroprevalence and presence of tabanids and stable flies in this survey. These results agree with Pérez et al. (1980) that found no relationship between anaplasmosis and *R. microplus* in cattle from Limón, Costa Rica and also in Argentina, where *R. microplus* tick was eradicated (Guglielmone, 1995). According to Rodríguez-Vivas and Domínguez-Alpízar (1998), low tick populations are enough for the transmission of the agent and to induce immunity in susceptible animals.

An association between seroprevalence and non-use of individual needles was determined in present study. Veterinary instruments as well as the interval of veterinary visit also play an important role in the transmission of *A. marginale* to susceptible cattle (Alonso et al., 1992; Guglielmone, 1995; Rodríguez-Vivas and Domínguez-Alpízar, 1998; Kocan et al., 2003; Rodríguez-Vivas et al., 2004). Perhaps the more frequent presence of the veterinarian on farms could result in an increased use of some instruments, such as needles, favoring iatrogenic transmission (Morley and Hugh-Jones, 1989; Rodríguez-Vivas et al., 2004). Surprisingly, the results did not demonstrate association between ear tattoos usage and seroprevalence, which is difficult to explain. According to Rodríguez-Vivas et al. (2004), interventional and experimental studies are needed to clarify this data.

The producers claimed to practice some form of ectoparasite control with variation in the frequency of use and the methods of application of acaricides/insecticides. The association between seroprevalence and ectoparasites control was clear in this study, similar to observed in Mexico (Rodríguez-Vivas et al., 2004). On the other hand, there is no corroboration with registered in Costa Rica (Pérez et al., 1994b), Tanzania (Swai et al., 2005) and Puerto Rico (Urdaz-Rodríguez et al., 2009). It is possible that the association between seroprevalence and control indicates that the vectors of *A. marginale* have developed resistance to pesticides. Improper use of equipment, inadequate dilution of a product, insufficient contact time (i.e. heavy rains after applications), and incomplete coverage of the animal can contribute to increase resistance in ticks and tick-borne diseases (George et al., 2004; Swai et al., 2005; Álvarez, 2010). In Costa Rican dairy herds, Álvarez (2010) recorded an incorrect use of acaricides/insecticides and Álvarez et al. (1999) registered the resistance of *R. microplus* to organophosphates and pyrethroids (Álvarez et al., 1999). In Puerto Rico farmers that participated in the certification program for use and application of insecticides were less likely to have high herd seropositivity for *A. marginale*.

Respect to intervals of acaricides/insecticides usage for ticks control, these were associated with higher odds at 22 and 45 days (OR = 1.1, respectively). On the other hand, a decreased likelihood of seropositivity at interval of 30 days (OR = 0.4) is difficult to explain. Contrarily, Pérez et al. (1994b) noted an increased likelihood of seropositivity to anaplasmosis at dipping intervals of 30 days but a decreased likelihood at intervals of 22 and 60 days, whereas Pérez et al. (1980) determined that dipping intervals of 11–14 days produced good tick control and decreased the likelihood of transmission of *A. marginale*. Therefore, it is important for farmers to recognize the benefits, limitations, and potential problems with each application method and product to ectoparasite control.

In conclusion, serological results indicated that exposure to *A. marginale* is common in commercial dairy farms of Costa Rica and endemic instability probably is due to inadequate vector control. Some risk factors were identified and quantified, which can guide the planning of successful control strategies to arthropods and bovine anaplasmosis in Costa Rica.

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