Short Communication

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Identification and prevalence of ticks (Acari, Ixodidae) in bovines in five municipalities of the Popayán plateau (Colombia)[¤]

Identificación y prevalencia de garrapatas (Acari, Ixodidae) en bovinos en cinco municipios de la meseta de Popayán (Colombia)

Identificação e prevalência de carrapatos (Acari, Ixodidae) em bovinos em cinco municípios no planalto de Popayán (Colombia)

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Abstract

Background: Ticks and tick-borne diseases are one of the major problems of public and animal health worldwide. Tick infestation causes economic losses in cattle farms of the Popayán plateau (Cauca, Colombia) due to costs associated with preventive management, decreased production of milk and meat, as well as transmission of diseases such as babesiosis and anaplasmosis. **Objective:** To determine the type and degrees of tick infestation in cattle of the Popayán plateau and its correlation with erythrogram and blood parasites frequency. **Methods:** One-thousand four-hundred forty cattle were evaluated in 144 farms distributed in Popayán, Cajibio, Timbio, Morales, and Piendamó. In each farm ticks were collected, quantified, and identified from 10 bovines of 1 year of age or older regardless of sex or breed. Additionally, a blood sample was taken from five of the animals to evaluate the erythrogram and determine the presence of blood parasites. **Results:** Tick prevalence was 79.5%; of these, 99.6% were *Rhipicephalus microplus* and 0.4% were *Dermacentor nitens*. Most cattle (58%) presented medium and high degrees of tick infestation and red blood cell counts, and no relationship between the degree of tick infestation and red blood cell counts, and no relationship between the degree of tick infestation with *R. microplus*.

Keywords: Cauca, hemoparasites, infestation, Rhipicephalus microplus.

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Resumen

Antecedentes: Las garrapatas y enfermedades transmitidas por estas son uno de los grandes problemas de salud pública y veterinaria en el mundo. En la meseta de Popayán (Cauca, Colombia) la infestación por garrapatas causa pérdidas económicas en las explotaciones ganaderas debido a mayores costos por manejo preventivo, disminución en la producción de leche, carne y pieles, y transmisión de enfermedades como babesiosis y anaplasmosis. **Objetivo:** Determinar el tipo de garrapatas y grado de infestación en bovinos de la meseta de Popayán y correlacionarlo con el eritrograma y presencia de hemoparásitos. **Métodos:** Se evaluaron 1.440 bovinos en 144 fincas de los municipios de Popayán, Cajibio, Timbio, Morales y Piendamó. En cada finca se colectaron, cuantificaron, e identificaron las garrapatas de 10 bovinos mayores de 1 año, independientemente de sexo o raza, y a cinco de ellos se les tomó muestra de sangre para realizar eritrograma y determinar presencia de hemoparásitos. **Resultados:** La prevalencia de garrapatas fue 79,5%; de ellas, 99,6% eran *Rhipicephalus microplus* y 0,4% *Dermancentor nitens*. La mayoría de los bovinos (58%) presentaron infestación media y alta (11 a 50, y más de 50 garrapatas, respectivamente). Se encontró una baja relación entre grado de infestación y nivel de glóbulos rojos, y no se encontró relación entre grado de infestación y presencia de hemoparásitos. Conclusión: Las ganaderías de la meseta de Popayán presentan un grado medio-alto de infestación por garrapatas, principalmente por *R. microplus*.

Palabras clave: Cauca, hemoparásitos, infestación, Rhipicephalus microplus.

Resumo

Antecedentes: Os carrapatos e as doencas transmitidas por eles são um dos principais problemas de saúde pública e animal em todo o mundo. No planalto de Popayán, departamento de Cauca (Colômbia), a infestação por carrapato é uma importante causa de perdas econômicas nas explorações pecuárias devido aos custos de manejo preventivo, diminuição da produção de leite, carne e peles, além da transmissão de doenças como babesiose e anaplasmose. Objetivo: Determinar o tipo de carrapato e o grau de infestação em bovinos no planalto de Popayán e correlacioná-los com os achados no eritrograma e a presença de hemoparasitas. Métodos: Foram avaliados 1.440 animais em 144 fazendas distribuídas nos municípios de Popayán, Cajibio, Timbio, Morales e Piendamó. Em cada propriedade foram coletados, quantificados e identificados carrapatos de 10 bovinos com mais de 1 ano de idade independentemente do sexo ou raça; de cinco deles foram colatadas amostras de sangue para realização de eritrograma e determinação da presença de hemoparasitas. Resultados: A prevalência de carrapatos foi de 79,5%; 99,6% dos carrapatos identificados eram da espécie Rhipicephalus microplus e 0,4% Dermancentor nitens. A maioria dos animais (58%) apresentou média e alta infestação (11 a 50 e mais do 50, respectivamente). Foi encontrada uma baixa relação entre o grau de infestação por carrapatos e número de células vermelhas no sangue, e não foi encontrada nenhuma relação entre grau de infestação por carrapatos e a presença de hemoparasitas. Conclusão: Os bovinos no planalto de Popayán apresentam um grau de infestação por carrapatos médio-alto, principalmente por R. microplus.

Palavras chave: Cauca, hemoparasitas, infestação, Rhipicephalus microplus.

Introduction

Ticks affect 80% of cattle in the world and are considered the most economically significant ectoparasite on farms. According to Guglielmone *et al.* (2010), 896 tick species are recognized worldwide. Colombia, due to its tropical climate, presents an ideal environment for tick development; about 80 species of ticks have been identified, the most important being *Rhipicephalus microplus* (Canestrini, 1888; Betancourt, 1973; Lopez, 2013). The presence of *R. microplus* in Colombia was documented from 0 to 2,600 m.a.s.l. until the last century. However, studies in 2010 reported its presence at 2,903 m.a.s.l. in areas not considered previously suitable for tick survival (Cortes *et al.*, 2010).

The negative economic impact of *R. microplus* in cattle farming is due to direct and indirect effects. Direct consequences of tick infestations include: Damage of animal skin at tick feeding sites, decreased performance, and blood loss. The indirect effects are associated with tick-borne diseases caused by transmitted pathogens such as the blood parasites *Babesia* spp. and *Anaplasma* spp. (Betancourt, 1973; Lopez, 1990; Jongejan and Uilenberg, 1994; Lima

et al., 2000; Bock *et al.*, 2008; Kocan *et al.*, 2008; Lopez, 2013). Economic losses caused by ticks in Colombia, as reported by Garcia in 1983, were \$5,000 million COP (equivalent to \$1.6 million USD) without taking into account the cost of treatments (Polanco-Echeverry and Rios-Osorio, 2016). Furthermore, there are reports of tick resistance to acaricides including organochlorines, formamidines, organophosphates, synthetic pyrethroids, and ivermectin (Benavides *et al.*, 2000; Abbas *et al.*, 2014; Araque *et al.*, 2014; Villar *et al.*, 2016).

An important aspect of tick control is the frequency of acaricide application, taking into account the life cycle of ticks (1, 2, or 3 hosts). In 2003, Rodriguez and Betancourt reported R. microplus (one-host tick) was the predominant cattle tick in the Cauca province (Colombia). However, the effects of climate change and the influx of cattle from other regions of the country where three-host ticks such as Amblyomma spp. have been reported, make it necessary to update the knowledge on ticks in cattle of the Popayán plateau. This can be achieved by determining the degree of tick infestation and its relationship to changes in the erythrogram, as well as the presence of blood parasites. This information is essential for ongoing studies in the development of new strategies for tick control and for improving cattle farm productivity in the Cauca province.

Materials and methods

Ethical considerations

The Ethical and Animal Welfare Committee of Universidad Antonio Nariño (Colombia) approved the protocol for sample collection and animal handling.

Location of the study

The Popayán plateau is an inter-Andean valley located in the central part of the Cauca province (Colombia), between 1,200 and 1,900 m.a.s.l.. Its area is 1,565 Km². It includes Popayán, Cajibio, Piendamó, Morales, Caldono, Silvia, El Tambo, and Timbio municipalities (IGAC, 2009). The location of the municipalities included in the study is presented in Figure 1. Annual rainfall is 2,110 mm, with 78% relative humidity, and 19 °C average temperature (IDEAM, 2016). Vegetation in the Popayán plateau is varied, including pastureland, forests, and bushes. The soil is mainly acidic and grasses include kikuyu (*Pennisetum clandestinum*), pasto estrella (star grass: *Cynodon plectostachyus*), brachiaria (*Brachiaria decumbens*), and natural grasses (*Cynodon dactylon*), which are low-growing. Some grasses grow in association with legumes such as maní forrajero (*Arachis pintoi*), and brush such as helecho (*Pteridium aquilinum*), escobilla (*Hyptis suaveolens*), mora silvestre (*Rubus niveus*), dormidera (*Mimosa púdica*), venturosa (*Lantana cámara*), coquito (*Cyperus rotundus*), salvia (*Euphatorium odoratum*), and espartillo (*Scirpus* spp.; Bernal, 2008; IGAC, 2009).



Figure 1. Location of the five municipalities where the study was conducted.

Farm selection

The foot and mouth disease vaccination records of year 2012 by Federación Colombiana de Ganaderos (FEDEGAN) from five municipalities in the Popayán plateau were used as the sampling frame. A stratified random sampling was conducted. Briefly, sample size was established by assuming 15% infestation with a 95% confidence interval, according to the following formula for finite populations:

$$n = \frac{Z_a^2 \times p \times q}{d^2}$$

Where:

n = required sample size.

Z = confidence level (95%).

p = proportion of the population having the characteristic (90%).

q = 1- p (10%).

d = error (5%).

A total of 144 cattle farms were randomly selected from the different municipalities according to their weight in the sample frame: Popayán 47 farms, Cajibio 40 farms, Piendamó 17 farms, Morales 15 farms, and Timbio 25 farms. Surveys and animal sampling were conducted between January 2013 and August 2015.

Animal selection

Standard ticks (larger than 4 mm) were collected from 10 bovines per farm. Infestation degree was classified according to the number of ticks per animal: 0 (no infestation), 0 to 10 (low), 11 to 50 (medium), >50 (high; Rodriguez and Betancourt, 2003). Each animal was adequately restrained and thoroughly examined. Visual inspection and palpation was performed starting with the ears and head, followed by the neck, dewlap and back to the rear and perineum, the tail to its tip, continuing with the ventral perineal region, udder, scrotum, inner thigh, belly, legs, and flank on the left side of the animal (Walker, 1977).

Tick removal was done by hand with the greatest possible care to avoid damaging the mouthparts. Once detached, ticks were stored in 70% ethyl alcohol for identification by Control Genético en Salud Animal Research Group at Universidad Nacional de Colombia.

Blood collection and analysis

Five of the 10 bovines were randomly selected for blood sampling. Each animal was properly restrained for venipuncture of the coccygeal vein using a vacutainer tube with EDTA as anticoagulant (Greiner Bio-One, Monroe, North Carolina, USA). Samples were processed in a VET2800 hematology analyzer (Mindray Bio-medical Electronics, Shenzhen, China) to evaluate erythrogram parameters (i.e. hematocrit, red blood cell count, and hemoglobin).

A blood film prepared with capillary blood from the tip of the tail and stained with Hemacolor[®] (Merck, Darmstadt, Germany) was examined at high magnification (1,000X) for the presence of blood parasites. The parasitemia was determined following the recommendations by Benavides *et al.* (2012).

Survey

The researchers interviewed farmers during farm visits. The survey included questions regarding tick control practices and problems related to tick infestation in their farms.

Statistical analysis

A descriptive analysis of the data was performed and the Chi-square test with the Goodman and Kruskal's tau coefficients, in addition to the uncertainty and Cramér's V coefficients, were used to determine the relationship between nominal and ordinal variables. The Somers' delta test was used to determine the relationship between ordinal variables. The statistical software PSPP version 0.7.9 (Free Software Foundation Inc., Boston, Massachusetts, USA) was used for analyses.

Results

The presence of ticks on 144 cattle farms in five municipalities of the Popayán plateau (location presented in Figure 1) was evaluated. Of these, 9% were beef farms, 38% dual-purpose (beef and milk) farms, and the majority (53%) were dairy farms. Most cattle evaluated were *Bos taurus* (79%), 12% were crosses, and 9% *Bos indicus*.

The estimated tick prevalence was 79.5%, as follows: 82.4% in Popayán, 71% in Cajibio, 85.9% in Timbio, 77.7% in Morales, and 81.2% in Piendamó. The 9,252 collected ticks were predominantly identified as *R. microplus* (99.6%) and 0.4% as *Dermacentor nitens*. Most cattle evaluated had a medium degree of infestation (34%) followed by high (24%), low (21%), and without infestation (21%). The

estimated prevalence and degree of tick infestation in the different municipalities are presented in Table 1.

Tick control strategies included the following products: diamidines (29%), pyrethroids (28%), and organophosphates (27%). In several farms (36.8%), ivermectin was used as a tick control strategy. Furthermore, 8.3% of farms also reported the use of agricultural products. The frequency of spray baths in the majority (42%) of the farms was 22 days or more, followed by 15-21 days (39%), 8-14 days (14%), and 0-7 days (5%). The type of acaricide used and frequency of application in the different municipalities are presented in Table 1. Handspray was the most common method of acaricide application (76.4%), followed by pour-on

(14.3%), and others (9.3%). A 60% of the farms that used handspray applied the right amount of product per animal (4-5 L/adult animal) and only 15% of the farms, had water analysis information. Finally, 65% of respondent farmers reported that they had had problems related to ticks on their farms but only 24% reported animal deaths due to tick infestation. The majority of surveyed farmers (76%) did not send blood samples to the laboratory when cattle got sick.

When correlating the degree of infestation with acaricide application frequency, using the Chi square and Somers' d tests, a low degree of association (0.113) between variables was found; however, as acaricide application frequency increases degree of tick infestation decreases.

Table 1. Estimated tick prevalence, degree of tick infestation, type of acaricide used, as well as acaricide application frequency in five municipalities of the Popayán plateau.

Municipality	Estimated tick prevalence (%)	Degree of infestation* (%)	Type of acaricide used (%)	Frequency of acaricide application (days) and (%)
Popayán	82.4	N:18	Diamidine: 38.8	0-7: 2.3
		L:21	Pyrethroid: 18.4	8-14: 22.7
		M:32	Carbamate: 0	15-21: 50
		H:29	Organophosphate: 30.6	>22: 25
			Other: 12.2	
Cajibio	71.0	N:29	Diamidine: 35.7	0-7: 10.3
		L:22	Pyrethroid: 33.3	8-14: 5.1
		M:28	Carbamate: 0	15-21: 35.9
		H:21	Organophosphate: 14.3	>22: 48.7
			Other: 16.7	
Morales	77.7	N:22	Diamidine: 28.6	0-7: 0
		L:17	Pyrethroid: 33.3	8-14: 0
		M:32	Carbamate: 0	15-21: 41.7
		H:29	Organophosphate: 33.3	>22: 58.3
			Other: 4.8	
Timbio	85.9	N:14	Diamidine: 16	0-7: 9.1
		L:20	Pyrethroid: 32	8-14: 13.6
		M:43	Carbamate: 4	15-21: 45.5
		H:23	Organophosphate: 28	>22: 31.8
			Other: 20	
Piendamó	81.2	N:19	Diamidine: 5.9	0-7: 0
		L:27	Pyrethroid: 23.5	8-14: 30.8
		M:40	Carbamate: 0	15-21: 30.8
		H:14	Organophosphate: 41.2	>22: 38.4
			Other: 29.4	

*Overall estimated degree of infestation, where, N: No infestation, L: Low infestation, M: Medium infestation, H: High infestation.

Lastly, erythrogram evaluations were normal for 88% of the cattle tested and 12% had anemia. Only 22% of the cattle were positive for blood parasites; 15% were positive for *Anaplasma* spp. with parasitemias ranging between 0.01 and 1.5 %; 5% were positive for *Babesia* spp. with parasitemias ranging between 0.01 and 0.2, and 2% were positive for both pathogens with parasitemias ranging between 0.04 and 0.6% for *Anaplasma* spp. and 0.01 and 0.1% for *Babesia* spp., respectively. None of the cattle tested were positive for *Trypanosoma* spp. (Figure 2).

When correlating the degree of infestation with erythrogram findings, although the Chi square test indicated a relationship (p<0.05) between variables, this relationship was weak when the contingency coefficient (0.121) and Cramer's V coefficient (0.122) were applied. Regarding variables degree of infestation and presence of blood parasites, no relationship (p>0.05) was found.

Discussion

It has been over 10 years since Rodriguez and Betancourt (2003) studied the presence of ticks in the Cauca province. However, few differences with their results were found in this study; *R. microplus* infestation still prevails and *Amblyoma* spp. are not found, most cattle have a medium degree of infestation and the main control strategy against ticks is acaricide application (organophosphates, pyrethroids, or amitraz) with handspray. However, a higher number of farms without tick infestation (21%) or lowlevel infestation (21%) were found. In addition, the number of farms employing an inadequate amount (L/spray bath) of acaricide per animal has decreased (Rodriguez and Betancourt: 84 vs 40 % today). Still, it is necessary for farmers to become aware of the importance of water analyses (only 15% have done it) to optimize the action of acaricides. When acaricides are dissolved in water for application, as is the case with the handspray method, it is important to evaluate water quality in terms of pH, presence of organic matter, and presence of ions (hardness). Acaricide activity can be affected by alkaline pH and presence of cations (Leiva, 2010).

Farmers surveyed expressed concern about acaricide resistance and, because of this, some of them support the use of complementary methods to control ticks; such as the application of ivermectin and the use of agrochemicals not approved for animal baths. This is worrying due to negative impact on the environment and public health. Two methods of acaricide resistance development by ticks are known; one is metabolic resistance and the other is target site insensitivity

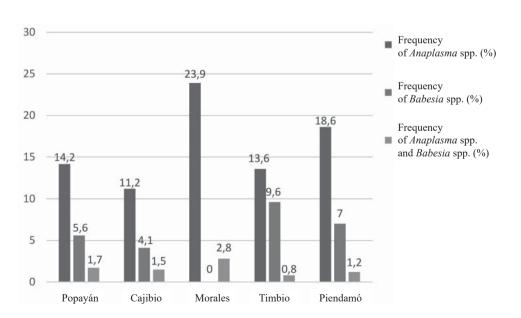


Figure 2. Frequency of blood parasites in cattle in the Popayán plateau.

(Diaz, 2012; de Oliveira Souza Higa *et al.*, 2015). In Colombia, there are reports of resistance to acaricides including organochlorines, formamidines, organophosphates, synthetic pyrethroids, and ivermectin (Benavides *et al.*, 2000; Abbas *et al.*, 2014; Araque *et al.*, 2014; Villar *et al.*, 2016). Information gathered in this study regarding estimated prevalence of ticks, degree of tick infestation, types of acaricide used and frequency of acaricide application (Table 1) suggest there is acaricide resistance in the Cauca province and highlights the need for further research on the subject in order to propose more pertinent strategies for tick control.

In addition to economic losses caused directly by tick infestation, there are those sustained by the blood parasites they transmit. In this regard, tick prevalence in Popayán plateau was high (79.5%), however, the overall prevalence of blood parasites was low (22%). Similarly, parasitemia levels were low (<1.5%) and none of the animals evaluated had clinical manifestations of disease. Studies in the Antioquia province have indicated possible enzootic stability for babesiosis based on the detection of pathogen directed antibodies (Ríos et al., 2010; Zapata et al., 2011). These studies were based on the concept proposed by Mahoney and Ross (1972), who stated that if there was 75% seroprevalence to Babesia spp. in calves under 9 months of age the clinical disease should be rare. In contrast, Jonsson et al. (2012) argue this concept is limited by the inability to guarantee sufficient exposure of cattle less than one year of age from year to year due to management, climatic and other environmental variability. Also, they consider it economically impractical since it would be necessary to continuously monitor the proportion of the herd that is immune. Studies on hemoparasites seroprevalence in the Cauca province are lacking.

In the Córdoba province, Herrera *et al.* (2008) reported a similar frequency of hemoparasites in bovines (22.5%) to that of the present study, the main difference being that they did find *Trypanosoma* spp. (30.9%) positive animals. The absence of *Trypanosoma* spp. positive animals in this study does not mean that the parasite is not in the region (if the limitations of the direct technique used for detecting blood parasites are taken into account). Higher sensitivity of molecular tests such as PCR-RFLP's

for detecting *Trypanosoma* spp. positive animals with low level parasitemias has been demonstrated (Cassalett *et al.*, 2011). Lower sensitivity of the direct technique can, in part, explain the weak relationship found between degree of tick infestation and presence of blood parasites.

Despite the high prevalence of ticks, few cattle had anemia. It has been reported that one adult female tick can imbibe between 0.5 and 3 mL of blood in its parasitic cycle, resulting in estimated blood losses of 40-50 L/animal/year (Soulsby, 1987). The absence of a relationship between degree of tick infestation and red blood cell count indicates that cattle in the region have developed resilience; however, additional studies are needed, in which production parameters are also evaluated to fully assess this.

The prevalence of ticks in cattle of the Popayán plateau is high and R. microplus still predominates. The medium to high degrees of infestation imply that ticks remain an important issue in regards to animal health in the Cauca province. Furthermore, results from this study suggest the existence of tick resistance to acaricides, and cattle have developed a certain degree of resilience to tick infestation and resistance to hemoparasite infection. Given the agroecological, sociocultural, and socioeconomic conditions of farmers in the Cauca province, we recommend responsible acaricide applications must continue as a means for tick control, but a reasonable amount of tick exposure should be also permitted. More research on ticks and tick-borne diseases is necessary in the southwest region of Colombia to develop integrated tick control programs in addition to permanent technical assistance to educate farmers on this subject.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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