Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i13.7258

PREVALENCE OF ENDOGLOBULAR HEMOTROPIC PARASITES IN OPC SHEEP IN THE MUNICIPALITY OF SINCELEJO AND SAVANNA SUB-REGION, DEPARTMENT OF SUCRE-COLOMBIA

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Submitted: 2 February 2023; Accepted: April 2023; Published: 6 June 2023: jptcp (ISSN: 1710-6222)

ABSTRACT
Infection caused by hemoparasite is one of the most prevalent diseases in tropical and subtropical countries in the world. Anaplasmosis and babesiosis are diseases caused by intracellular hematopoietic agents that are part of the parasitic complex. Hematotropic agents (Anaplasma ovis, Anaplasma marginale, Trypanosoma vivax, Trypanosoma melophagium, Babesia ovis and Babesia motasi) are common causes of hematopoietic infections in sheep. Thus, the aim of the present study was to determine the frequency of endoglobular hemotropic parasites in Colombian hair sheep (OPC) in municipality of Sincelejo and Savanna Sub-region of the department Sucre- Colombia. A cross-sectional study was carried out at convenience, according to availability in each farm during the years 2020-2022, totaling 400 animals. The capillary microcentrifuge technique was used, where the percentage volume of red blood cells (Haematocrit) was determined by reading on a Hawksley microhaematocrit reader table, then blood smears were made, stained with WRIGTH dye and evaluated under a light microscope. Incident variables in the parasitaemia of the animals were considered, such as sex, location and Body Condition Score, looking for a relationship with the parasite positivity that the animals could present. The results of the analysis showed 165 positive samples, representing a prevalence for Anaplasma spp of 14.26%; Babesia spp 14.5% and for both agents 12.5% in OPC. There was a significant relationship ($p<0.05$) between animal origin, gender (Male-Female) and Body Condition Score, with the degree of prevalence that the animals possessed. In conclusion, Anaplasma spp and Babesia spp, is present in the municipality of Sincelejo and Savanna Sub-region (Sucre- Colombia), and these can occur in OPC animals from different localities, sex and Body Condition Score.

Key words: hemoparasite, sheep, Babesia spp, Anaplasma spp, endoglobular.

INTRODUCTION

In Colombia there is a diversity of sheep, exploited under a traditional production scheme, the largest population of animals correspond to the short-haired breed, known as Camuras, currently OPC, highly adapted to diverse agro-climatic conditions (Flórez et al., 2020; Noriega et al., 2022). Sheep production in Colombia, especially on the north coast, is developed by small producers, basing its development specifically on the OPC breed, which has a series of limitations for optimal performance (infrastructure, reproductive, nutritional, genetic and sanitary management), reflected in low production indices (Hernández et al., 2023). Studies by Al Kalaldeh at al. (2019), showed that parasite infections were one of the most important problems in sheep and goats, causing severe production constraints in small ruminants, especially those raised by marginal producers in a low external input system. In this sense, infections caused by hemoparasite have a high rate of occurrence in tropical and subtropical countries (Torres et al., 2021; Nyifi et al., 2023; Torres et al, 2023), with the highest incidence in animal production systems being caused by Babesiosis (Babesia spp), Anaplasmosis (Anaplasma spp) and Trypanosomiasis (Trypanosoma spp), causing a considerable reduction in meat and milk production in livestock (Avila et al., 2013, Torre et al., 2021).

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Haematopoietic agents (Anaplasma ovis, Anaplasma marginale, Trypanosoma vivax, Trypanosoma melophagium, Babesia ovis and Babesia motasi), are a common cause of blood borne infections in sheep, Avila et al, (2013) reported the presence of Anaplasma spp. in 73.7%, in a sheep population, studied in the department of Antioquia-Colombia. The identification of haemoparasitic disease is of great help and allows better targeting of intervention (Plaza et al., 2019). Haemoparasitic disease is suspected when there is fever, accompanied by anaemia, animal death and/or abortion, presence of oedema, haemoglobinuria or jaundice. The department of Sucre (Colombia) is one of the thirty-two departments of Colombia, located in the northern part of the country. It is made up of 26 municipalities and 275 townships, which, taking into account the criteria of geographical location, political-administrative division, economic vocation, inter-municipal relations, historical and socio-cultural links, are grouped into five physiographic Sub-regions: Gulf of Morrosquillo, Mountains of María, Savannas, San Jorge and La Mojana, the majority of the population lives in rural areas and depends on agriculture as the main economic activity. Thus, the objective of the present study was to determine the frequency of endoglobular hemotropic parasites in OPC sheep from the municipality of Sincelejo and Savannas Sub-region (department of Sucre, Colombia).

2. MATERIALS AND METHODS

2.1 Type of study. A descriptive cross-sectional study was conducted on OPC animals at convenience, according to availability on each farm during the years 2020-2022 (Manterola & Otzen, 2014).

2.2 location. OPC sheep farms in the Savannas Sub-region of the department of Sucre (Municipalities of Sincé, El Roble, San Pedro, Sampués, Los Palmitos, Galeras, Buenavista, Corozal, San Juan de Betulia) and Sincelejo, Colombia (Figure 1). The life zone of the study site is classified as tropical dry forest (bs-t), with temperatures ranging between 25.5 and 28.7°C, precipitation fluctuating between 990 and 1275 mm per year, relative humidity of 80% (Montes et al., 2022).

2.3 Sample size and study population. Considering that municipality of Sincelejo and the Savannas Sub-region have a sheep census to 2023 (ICA-2023) of 12,151 head, a sample size of 400 animals was calculated (confidence level of 95%; α= 0.05; Z= 1.96 and a margin of error of 5%), distributed in municipality of Sincelejo and the 10 municipalities that make up the Savannas Sub-region. A stratified random sampling was carried out, to take 40 samples per municipality, for a total of 400 animals. The farms per municipality (4 in total) were subject to selection by convenience, taking into account the geographical distribution of the municipality, and 10 animals were taken at random from each farm. The selected production units usually carried out traditional management for the breeding and exploitation of sheep.

2.4 Inclusion and exclusion criteria. The animals were sampled from three months of age, between males and females, no younger animals were considered, due to maternal acquired immunity, the selected animals were numbered with Ear Tags Numbered.

2.5 Data collection techniques and instruments.

2.5 1 Blood sampling and diagnosis. Blood samples were collected by venipuncture of the jugular vein. Then, 5 ml of peripheral blood was extracted in a vacuum vacutainer tube without anticoagulant and with anticoagulant (EDTA), duly identified with the number of the specimen sampled, kept refrigerated, and then processed in the laboratory.

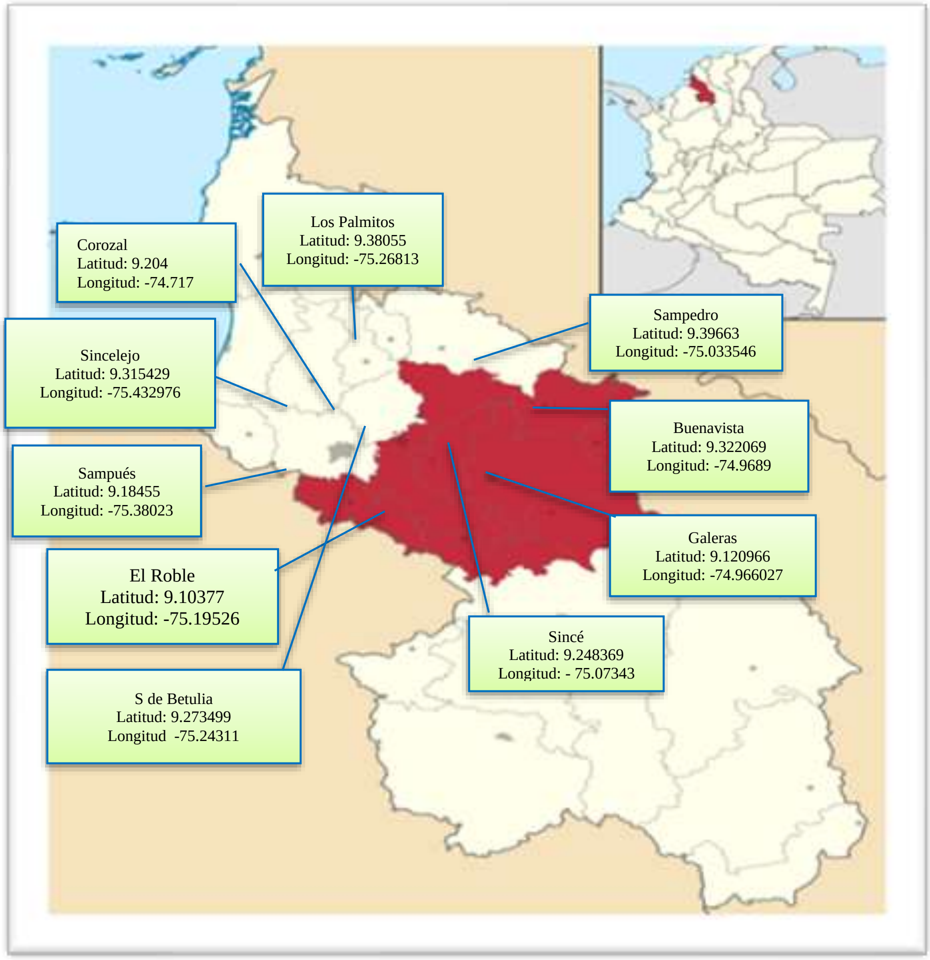

Figure 1. Study area: municipality of Sincelejo and Savanna Sub-region (Department of Sucre, Colombia). Source: Taken from Montes et al., (2022).

2.5.2 Data processing and analysis. The capillary microcentrifugation technique (TMS, described by Woo 1969) was used, where the percentage volume of red blood cells (haematocrit) was determined by reading on a Hawksley micro-haematocrit reader (Dill and Cost, 1974). Afterwards, a blood smear and staining with wrigth dye was performed, and evaluated under a light microscope with an immersion objective, according to the procedure, which allows the evaluation of intracellular parasitic forms morphologically compatible with Anaplasma spp and Babesia spp (López et al., 2014; Calderón et al., 2016).

With the results obtained, a database was elaborated with the evaluated variables where sheep without haematozoa and with haematozoa were compared; where the latter category was established with at least the diagnosis of only one group. The comparison of the groups was implemented with the t-student test for quantitative variables; all these calculations were carried out using the statistical software R. 4.3.02.
Equation 1 was applied to find the incidence of the etiological agent of endoglobular hemotropic parasite diseases.

$$\text{Equation 1.} = \frac{\text{Number of endoglobularhemotropic positive animals}}{\text{Total animals sampled}} \text{ x } 100$$

Equation 2 was applied to relate the prevalence of endoglobular hemotropic parasites with the variable Body Condition Score (BCS) in OPC sheep from the municipalyty of Sincelejo and Savanna Sub-region (Sucre – Colombia).

$$\text{Equation 2.} = \frac{\text{Number of positive animals with BCS } (1-5)}{\text{Total animals sampled}} \text{ x } 100$$

BCS was determined by palpation of the lumbar region based on a scale of 1-5, (with an increment of 0.5), where 1: very thin and 5: obese (Russel et al., 1969; Canul et al., 2022), 2022), with a score between 3 and 4 being considered optimal. The qualitative BCS classification would be in the order of: obese (> 4.0), fat (3.5-4.0), normal (3.0-3.5), thin (2.5 - 3.0), very thin (< 2.5).

To determine the prevalence ratio of endoglobular hemotropic positive animals to haematocrit, equation 3 was implemented for the resulting

$$\text{Equation 3.} = \frac{\text{Haematocrit of endoglobular hemotropic parasite} - \text{positive animals}}{\text{Total animals sampled}} \text{ x } 100$$

For ease of analysis the haematocrit obtained were classified into ranges for tabulation. The ranges used were as follows: Haematocrit ranging from 20 to 30, from 31 to 40, from 41 to 50 and haematocrit greater than 50. The animals were divided into those positive for haematopoietic parasites, either Anaplasma spp, Babesia spp or both, or those negative for these parasites. The ocular mucosa was evaluated according to the color of the mucosa, taking into account the following classification: Congestive; Sub-Icteric; Pale; Normal.

2.4.3 Survey of farm owners. In each sampled herd, the respective owners were surveyed in order to obtain animal information such as age (category), sex, zoning and presence of ticks, this information was used in the data analysis. The surveys were open-ended and the variables to be considered were nominal and binary.

3. RESULTS AND DISCUSSION

Taking into account the main objective of the present work, the frequency of endoglobular hemotropic in OPC sheep in the municipality of Sincelejo and Savanna Sub-region, department of Sucre, was 41.25% (165/400), being the main pathogen association Anaplasma spp. and Babesia spp. or co-infected by both (Table 1).

Table1. Overall prevalence of endoglobular hemotropic in OPC sheep

		Positives		Negatives		
Specie	N° of samples	N°	%	N°	%	I.C.95%
Ovis aries (OPC)	400	165	41.25	235	58.75	0.024

Table 2 presents the overall infestation rates by species of endoglobular hemotropic, of which 14.26% (57/400) were positive for Anaplasma spp, 14.5% (58/400) were positive for Babesia spp, and 12.5% (50/400) were positive for both agents (co-infection). The possible reasons for the high number of parasitized animals observed in the study may be due to the poor parasite control carried out on the farms, sometimes these animals are bathed for tick control.

The results found in the present study differ from those reported by Martinez and Tatis (2001) in the department of Sucre - Colombia, where the frequencies of infection by Anaplasma spp., Babesia spp. and Trypanosoma sp. were 90%, 0% and 0% respectively. Likewise, Li et al (2020) reported prevalence of Babesia spp and Anaplasma spp (67.7%) in sheep from border regions of northwest China. In general, different prevalence values are reported in the literature (Rahravani et al., 2023; Arif et al., 2023; Taqadus et al., 2023; Onyiche & MacLeod, 2023), which may be attributed to the difference in micro and macro climate of each region, tick species, tick habitat and landscape between regions.

Table 2. Prevalence of endoglobular hemotropic species found in sheep OPC

N° Ovinos OPC	Positives Anaplasma spp.	%	Positives Babesia spp.	%	Positives Anaplasma spp. y Babesia spp.	%
400	57	14.26	58	14.5	50	12.5

A good indicator of the general state of the animals is the BCS, although it is not a specific and strict parameter in its measurement, it is of great help in the clinical and productive evaluation of an animal. In Colombia, livestock regions located in tropical areas are considered enzootic for the haemoparasites (González et al., 2014), animals become reservoirs, so it is necessary to have diagnostic techniques that allow the detection of carrier animals, in order to know the prevalence of the disease in the regions (Jaimes et al., 2017; Vargas et al., 2019).

Table 3 describes the relationship of OPC sheep diagnosed positive for endoglobular hemotropic parasites with respect to BCS. Of the 57 animals sampled for Anaplasma spp, 78.3% (45/57), presented a very thin BCS (< 2.5), 12. 3% (7/56) had a thin BCS and only 7% (4/57) of the animals had a normal BCS.

Of the 58 animals positive for Babesia spp, just over 90% of them had a poor BCS (Table 3), which can be attributed to the inappetence experienced by animals with this disease due to the secretion of some substances that disrupt the feeding process, In addition, more than 96% of the total number of animals in the study that tested positive for both agents had a BCS score below normal (3.0-3.5), resulting in low productive animals (Chochlakis, et al., 2009; Bauer et al.,2021; Onyiche et al., 2022; Onyiche & MacLeod, 2023). A significant difference (p= 0.039), for Anaplasma spp; Babesia spp (p= 0.029) and Anaplasma spp and Babesia spp (p= 0.042), was observed in the very thin BCS category (< 2.5) with respect to the other categories. In contrast, Mannat et al. (2023) reported a non-significant effect of BCS on animals diagnosed positive for endoglobular hemotropic parasites.

Table 3. Ratio of OPC sheep diagnosed positive for endoglobular hemotropic parasites to BCS.

	Positives Anaplasma spp.		Positives Babesia spp.		Positives Anaplasma spp. y Babesia spp.	
BCS	N°	%	N°	%	N°	%
Obese > 4.0	0	0.0	0	0	0	0

Fat (3.5-4.0)	1	1.8	1	1.7	0	0
Normal (3.0-3,5)	4	7.1	3	5.2	2	4
Slim (2.5 - 3.0)	7	12.5	6	10.3	5	10
Very thin< 2.5*	44	78.6	48	82.8	43	86
Total	57	100	58	100	50	100

*Significant differences ($p < 0.05$)

The results obtained evaluating the sex variable (Table 4), showed a significant difference (p= 0.036) of the sex in the OPC sheep, with respect to the prevalence of endoglobular hemotropic parasites, females have the highest prevalence 92. 7% (153/165), compared to males 7.3% (12/165), there is a relationship between the sex of the animal and the presence of endoglobular hemotropic parasites in OPC sheep, these results are concordant with those presented by Abdelsalam et al, (2023) and Eisawi et al., (2020). On the contrary, some results found in the literature state that the prevalence of endoglobular hemotropic parasites in sheep is not affected by gender (Naeem et al., 2023; Tamrat et al., 2023; Hamzah & Hasso,2019; Egbe-Nwiyi et al., 2018; Shah et al., 2017).

Table 4. Sex-specific prevalence of endoglobular hemotropic parasites in OPC sheep.

		Positivos		Negativos	
Sexo	N°	N°	%	N°	%
Males	82	12	7.3	70	29.8
Females*	318	153	92.7	165	70.2
Total	400	165	100	235	100

*Significant differences ($p < 0.05$)

The origin of the animal was evaluated with the possibility of zoning the distribution of the parasite in the localities of municipality of Sincelejo and Savanna Sub-region (Sucre-Colombia), finding significant differences (p= 0.042). The municipalities with the highest prevalence of endoglobular hemotropic parasites were Galera, San Pedro, Sampués, Sincelejo and Los Palmito with an overall prevalence equal to or higher than 45% (Table 5). In these municipalities, the presence and management of OPCs are closely related to cattle production, where animals tend to become infected with the pathological agent at an early age, allowing them to reach certain levels of coexistence between host and parasite. Noaman and Alireza, (2023), reported significant differences in parasite prevalence between the different geographical areas of their study (De la Fuente et al., 2007; Rahravani et al., 2023; Prajapati et al., 2023).

Table 5. Prevalence of endoglobular hemotropic parasites with respect to zoning by municipality in OPC sheep.

Municipalyty	N° de sites	Sample	Positive	%	Negative	%
Sincé	4	40	12	30.0	28	70.0
El Roble	4	40	16	40.0	24	60.0
San Pedro*	4	40	18	45.0	22	55.0
Sampués*	4	40	18	45.0	22	55.0
Los Palmitos*	4	40	19	47.5	21	52.5
Galeras*	4	40	22	55.0	18	45.0
Buenavista	4	40	12	30.0	28	70.0
Corozal	4	40	16	40.0	24	60.0
San Juan de Betulia	4	40	15	37.5	25	62.5
Sincelejo*	4	40	17	42.5	23	57.5
Total	40	400	165		235	

*Significant differences ($p < 0.05$)

The low prevalence in some localities (Table 5) may be attributed to frequent parasite control plans against ticks, which compensates for the decrease in the vector population. Similarly, the type of vegetation, landform, sampling areas and possibly the type of animal management may also influence the degree of prevalence of endoglobular hemotropic parasites. A statistically significant relationship between parasite treatments has been documented in the literature, favoring the acquisition of protective immunity from an early age and the absence of clinical signs in the animals over time (Reátegui et al., 2023).

able 6 describes the haematocrit concentration in OPC sheep positive for Babesia spp and Anaplasma spp. It can be observed that 61.4% (35/57) of the animals positive for Anaplasma spp. had a haematocrit between 20 and 30, possibly due to intrasplenic haemolysis by the endothelial reticulum system, produced by this type of bacteria. The 28% (16/57) of the animals positive to Anaplasma spp, presented haematocrit values between 31 and 40, and only 10.6% of these animals presented haematocrit in the range between 41 and 50, given the above, it is presumable that this intra-erythrocytic bacterium is the direct cause of anaemia in these animals. According to the literature, a goat or sheep infected with Anaplasma spp. that presents a haematocrit higher than 25% is considered an asymptomatic carrier, a condition commonly found in goats and sheep with anaplasmosis; which is also considered an epidemiological condition that makes the animals a reservoir for the microorganism (Avila et al, 2013; Arece et al., 2015), studies developed by Rahravani et al., (2023) and Nangru et al., (2023), found a statistically significant association between semi-yellow conjunctiva, mean red blood cell count, mean corpuscular haemoglobin and haemoglobin concentration in all haemoparasites infections.

Table 6. Haematocrit concentrations in Babesia spp. and Anaplasma spp. positive OPC sheep in OPC sheep.

Haematocrit	Positive Anaplasma spp	%	Positive Babesia spp	%	Positive Anaplasma spp y Babesia spp	%
20-30	35	61.4	48	82.8	43	86.0
31-40	16	28.0	8	13.8	6	12.0
41-50	6	10.6	2	3.4	1	2.0
>50	0	0.0	0	0.0	0	0.0
Total	57	100	58	100	50	100

Regarding the animals positive for Babesia spp, 82. This is attributable to the physiopathogenesis of Babesia spp, which apart from directly producing lysis on the red blood cell, also causes the immune system to destroy other red blood cells, resulting in severe anaemia. 13.8% of the OPCs positive for Babesia spp, had haematocrit values between 31 and 40, and only 3.4% were in the range between 41 and 50. The animals parasitized by this agent were the ones that showed the most sanitary deterioration, in relation to the animals parasitized by both agents, 86 % of the OPC sheep presented haematocrit values between 20 and 30, so it can be said that the Babesia-hematocrit ratio predominated over the Anaplasma-hematocrit ratio. Similarly, it was found that 12 % of these OPC sheep had values in the range between 31 and 40. It should be noted that the haematocrit is the most important measure to determine the degree of anaemia of an animal, and one of the most important clinical signs of the diseases produced by these microorganisms, therefore, it is essential to know the haematocrit-haematopoietic agent ratio (Nyifi & Bilbonga, 2023).

Table 7 describes the relationship between the color of the mucosal membranes in OPC sheep with the presence of Babesia spp and Anaplasma spp. 88 % of the 57 OPC sheep that were positive for Anaplasma spp, presented pale mucous membranes, which is explained by the type of haemolysis that this produces, being intrasplenic (extravascular) does not increase the production of bile pigments by the liver, as a consequence the membranes turn to a whitish color, 12 (7/57)% of this group, presented sub-icteric coloring, which can be attributable to some other type of hepatic problem or to the presence of Anaplasma spp, without being observed in the samples, since the technique used in this study is dependent on the expertise of the laboratory technician.

Table 7. Mucosal color of OPC sheep sampled during the study.

Hematocritos	Positive Anaplasma spp	%	Positive Babesia spp	%	Positive Anaplasma spp y Babesia spp	%
Congestive	0	0.0	20	34.5	11	22.0
Sub- Iteric	7	12	37	63.8	37	74.0
Pale	50	88	1	1.7	2	4.0
Normal	0	0.0	0	0.0	0	0.0
Total	57	100	58	100	50	100

Of the group of animals positive to Babesia spp, 63.8% (37/58) presented sub-icteric mucosa, possibly this could be caused by the intravascular haemolysis produced by Babesia spp, the free Heme group in blood is converted into biliverdin by the SRE, and then conjugated in the liver, to bilirubin diglucuronide and its high levels cause yellowish pigmentation of the mucosa, the remaining 34. The remaining 34.5% (20/58) of this group had congestive mucous membranes, which could be related to dehydration of the animals. Of the 50 animals that were positive for both agents, 22% (11/50) had congestive mucosa, and 74% (37/50) had sub-icteric mucosa, indicating a dominance of clinical signs associated with Babesia over clinical signs of Anaplasma. Several authors have related the conjunctival coloring of the eye with the presence of anaemia, finding association with parasite loads in animals (B. Adehanom et al., 2015; Adamu et al., 2020; Şahin et al.,2021; Coello-Peralta et al., 2022; Rahravani et al., 2023).

Table 8 shows the infestation level of OPC sheep positive for endoglobular hemotropic, none of the OPC evaluated presented an infestation level of 0.07%. Despite the high frequency of infection by Anaplasma spp and Babesia spp, no symptomatic animals were observed, which may be related to the low infestation levels (0.01) found in the positive animals. The samples tested showed an average infection and infestation percentage of 0.014% for Anaplasma spp; 0.017% for Babesia spp and 0.014% for Anaplasma spp and Babesia spp respectively.

Table 8: Infestation level (%) of endoglobular hemotropic positive OPC sheep.

Hemotropic	Level of infestation (%)							
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	Total
Anap	44	7	3	1	2	0	0	57
Bab	42	4	4	4	3	1	0	58
Anap y Bab	40	2	1	3	3	1	0	50

Anap= Anaplasma spp; Bab =Babesia spp; Anap y Bab = Anaplasma spp y Babesia spp

4. CONCLUSIONS

Anaplasma spp and Babesia spp are present in the municipality of Sincelejo and the Savanna Sub-region (Sucre-Colombia), and can occur in OPC sheep of different localities, sex and Body Condition Score. The agro-ecological conditions of the study area are conducive to the development of specific vectors of these microorganisms, so it is appropriate to implement prophylactic measures to reduce the incidence of these agents, because they are directly related to economic losses in the sheep population worldwide, significantly deteriorating the health status of animals and in some cases causing death.

5. CONFLICT OF INTEREST

There is no conflict of interest related to the subject matter of the work.

6. CONTRIBUTION OF THE

Conceptualization, data curation, formal analysis, research, methodology, supervision, validation, writing - original draft, writing - revision and editing: Alexander Pérez C, Donicer Montes V and Gabriela Flórez M.

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