



Frequency of endo-globularhemotropic parasites in short haired sheep in Córdoba-Colombia

BERNARDO REYES B¹, JOSÉ CARDONA Á² and DONICER MONTES V³

Faculty of Veterinary Medicine, University of Córdoba, Montería, Colombia

Received: 17 January 2019; Accepted: 4 April 2019

ABSTRACT

The infection caused by hemoparasites is one of the diseases of greater presentation within the tropical and subtropical countries in the world, anaplasmosis and babesiosis are diseases caused by intracellular hemotrophs that make up the complex of parasitic sadness. Hemotropic agents (*Anaplasma ovis*, *Anaplasma marginale*, *Trypanosoma vivax*, *Trypanosoma melophagium*, *Babesia ovis* and *Babesia motasi*) are common cause of blood infections in sheep. A prospective descriptive type study was carried out. For convenience, a simple random probabilistic sampling type was used. 25 sheep farms were selected from small producers (who had 25 females and 1 male) representative of the department, who manage sheep as a production system. The technique of capillary microcentrifugation was used, where the percentage volume of blood cells was determined red blood cells (Hematocrit), by reading on a Hawksley microhaematocrit reader table, subsequently, they were made blood smears that were stained with WRIGTH dye and were evaluated under the light microscope. It can be concluded that the prevalence found in the study for *Anaplasma* (24.4%) and *Babesia* (18.4) in short hair sheep in the department of Córdoba, is not a significant value because the agro-ecological conditions of this area are conducive to the development of specific vectors of these microorganisms, based on the results of the study, it is timely to implement prophylactic measures to reduce the incidence of these agents, because they are directly related to economic losses in the world sheep population, considerably deteriorating the health status of the animals and in some cases causing death.

Key words: *Anaplasma* sp, *Babesia* sp, Endo-globularhemaetogenous, Sheep

The sheep in Colombia exist since the time of the conquest and were brought from Europe. The wool breeds were crossed and mixed, which resulted in the well-known Colombian Creole (CC), among other races; then the African hairy breeds came, which are known as camuros; these two racial types are the most predominant in the country. The raising of sheep has been developed in the Colombian territory, mainly in the departments of La Guajira, Magdalena, Cesar, Boyacá, Nariño, Córdoba and Cundinamarca, places where sheep are widely used (Lozano 2014).

The infection caused by hemoparasites is one of the diseases of greatest presentation in tropical and subtropical countries in the world, anaplasmosis and babesiosis are caused by intracellular hemotrophs that make up the complex of parasitic sadness (Blanco *et al.* 2015).

The diseases caused by hemotrophs that have a higher

incidence in animals in production systems in the tropics are: Babesiosis caused by protozoa of the genus *Babesia* spp, Anaplasmosis by Rickettsia of the genus *Anaplasma* spp, and trypanosomiasis by flagellated protozoa of the genus *Trypanosoma* spp (Avila *et al.* 2013).

In endemic areas, *Anaplasma ovis*, *Anaplasma marginale*, *Babesia ovis* and *Babesia motasi* are transmitted to sheep and goats by arthropod vectors, including several species of *Stomoxys calcitrans*, *Haematobia irritans* (*Anaplasma ovis*) and ticks of the species *Rhipicephalus bursa* (*Anaplasma ovis* and *Babesia* sp., *R. tyranice* and *R. revertis* (*Babesia ovis*), *Demacentor marginatus* (*Anaplasma ovis*) (Avila *et al.* 2013).

Hematropic agents (*Anaplasma ovis*, *Anaplasma marginale*, *Babesia ovis* and *Babesia motasi*) are common cause of blood infections in sheep, 73.7% were reported for *Anaplasma* in goats in a study conducted in Antioquia (Avila *et al.* 2013).

The genus *Anaplasma* are gram-negative, alpha protobacteria, obligate intracellular parasites of eukaryotic cells, and was discovered in 1910 by Sir Arnold Theiler (family Anaplasmataceae), in the order Rickettsiales, and includes 6 species: *Anaplasma marginale*, *Anaplasma centrale*, *Anaplasma ovis*, *Anaplasma phagocytophilum*,

Present address: ¹Veterinary Doctor Zootechnician (bernardo47@hotmail.com), Research Group in Large Animal Medicine (MEGA); ²Professor (cardonalvarez@hotmail.com), Group MEGA, University of Córdoba, Montería, Colombia. ³Professor (donicer.montes@unisucra.edu.co), Department of Zootechnics, Faculty of Agricultural Sciences, University of Sucre, Colombia.

Anaplasma bovis and *Anaplasma platys* (Battilani *et al.* 2017).

Anaplasma ovis is the main causative agent of ovine anaplasmosis, which is an intra-erythrocytic rickettsial pathogen of sheep, goats and wild ruminants. The infection in sheep and goats is usually asymptomatic. Clinical signs usually develop in the case of immunosuppression in sheep, which is similar to the infection of *Anaplasma marginale* in cattle characterized by severe anemia, fever, weight loss, abortion, paleness of the mucous membrane and ichthyosis. Anaplasmosis mainly occurs in tropical and subtropical areas (Yousefi *et al.* 2017).

The taxonomic classification of *Babesia* spp. places them in the phylum Apicomplexa (also called Sporozoa), class Aconoidasida (Piroplasma), and the order Piroplasmida (Homer *et al.* 2000)

The objective of the present study was to determine the prevalence of endo-globular hemotropic parasites in short haired sheep in the department of Córdoba, Colombia.

MATERIALS AND METHODS

Type of sampling and calculation of sample size: A simple random probabilistic sampling type was used. 25 sheep farms were selected from small producers (who had 25 females and 1 male) representative of the department, who manage sheep as a production system. For the choice of farms and animals, the data of the properties registered in the pilot program of the government of Córdoba (governing agreement, University of Córdoba and Umatas de San Carlos and Ciénaga de Oro) were used.

We evaluated 10 animals per farm, chosen at random, giving a total of 250 adult sheep, of both sexes, different weights, ages and productive stages.

Location: The study was conducted in the department of Córdoba, Colombia, located between coordinates 7° 23' and 9° 26' north latitude and 74° 52' and 76° 32' longitude west of the Greenwich meridian, at a height of 30 m with average annual temperature of 28°C, relative humidity of 82%, average annual rainfall of 1,400 mm and belongs to the climatic formation of tropical rain forest. Two well-defined seasons are presented (rainy season and dry season) (Vela and Pérez 2012).

Collection of information: For the collection of the samples, field trips were made in the Medical-Surgical Large Animal Clinic area, with a total of 25 outings, where the animals were randomly chosen to sample each of the farms and data were collected as identification number, age, race and sex.

We proceeded with the collection of each blood sample by puncture of the jugular vein in vacutainer tubes of 5 cc with EDTA duly identified with the number of the samples sampled, refrigerated and later taken to the Veterinary Clinical Laboratory of the Faculty of Veterinary Medicine and Zootechnics.

The technique of capillary microcentrifugation was used, where the percentage volume of red blood cells (Hematocrit) was determined, by reading in a table

Hawksley microhaematocrit reader (Dill and Cost, 1974). Subsequently, blood smears were made which were stained with WRIGTH dye and evaluated under a light microscope with immersion objective according to the procedure, which allows to evaluate intracellular parasitic forms morphologically compatible with *Anaplasma* and *Babesia* (López *et al.* 2014).

A formula was applied to find the incidence of the etiological agent of the diseases by endo-globular hemotropic parasites, which was:

$$\frac{\text{Number of animals positive to endoglobular hemotropics}}{\text{Total sampled animals}} \times 100$$

To relate the prevalence found in endo-globular hemotropic parasites with the variable body condition in sheep of the department of Córdoba following formula was used:

$$\frac{\text{Number of positive animals with body condition scoring}}{\text{Total sampled animals}} \times 100$$

The body condition in animals was measured on a scale of 1 to 5 with intervals of 0.5 between one titration and the next.

where a 1 on the scale is considered a skinny animal and a score of 5 is an obese animal and an optimal state was a score between 3 to 4. Body condition will be classified in a table in qualitative ranges: bad, fair and good. Where bad are animals with a score of 2 to 2.5, regular is an animal with a score of 3 to 3.5 and a good body condition is given to animals with a score of 4 to 4.5.

To determine the prevalence ratio of positive animals to endo-globular hemotropics with hematocrit, the following statistical equation was implemented in animals that are positive for *Anaplasma* and *Babesia*, or that are negative for both.

$$\frac{\text{Hematocrit of animals positive for endoglobular hemotropic parasites}}{\text{Total sampled animals}} \times 100$$

For greater ease of analysis, the obtained hematocrits were classified into ranges for tabulation. The ranks managed were the following:

Hematocrit ranging from 20 to 30, 31 to 40, 41 to 50 and hematocrit greater than 50, which were divided into animals positive to either hemotropic parasites, *Anaplasma*, *Babesia*, or both negative to these parasites.

Statistical analysis: The data was organized in tables and analyzed in a descriptive way in the Software Statistic 8.0 (2008).

RESULTS AND DISCUSSION

During the period between September 2016 and December 2017, 250 blood samples were collected from sheep of different sheep farms of small producers in the department of Córdoba, to determine the frequency of endo-globular hemotropic parasites in Creole hair sheep. Obtained frequency of endo-globular hemotropics in sheep from

Córdoba was 53.2% (133/250), of which 24.4% were positive for *Anaplasma* spp, 18.4% were positive for *Babesia* spp, and 10.4% were positive for both agents (Table 1).

One of the possible reasons why the study observed a smaller number of animals parasitized by *Babesia* spp with respect to *Anaplasma* spp may be that baths are usually carried out for the control of ticks frequently, taking into account that *Rhipicephalus* spp. It is the only vector of *Babesia*, and eliminating the vector decreases the presence of the parasite in animals.

One of the most important indicators of the general state of the animals is the body condition, although it is not a specific and strict parameter in its measurement, it is a great help in the clinical evaluation of an animal, since it allows taking into account the condition of feeding without leaving aside the time of year and also the possible disturbance of the health of the animal, in this way it is necessary to evaluate the body condition taking into account the time of year, topography of the area, presence of vectors as well as availability of water sources, everything, in order to guide the evaluation to possible causes of the state of Body Condition (BC)

Table 2. describes the relationship of animals diagnosed positive to endo-globular parasites with respect to body condition, 24.4% of the animals sampled were positive for *Anaplasma*, of which, 14% had a bad body condition, 6% had a regular CC and only 4.4% of the animals had a good

body condition, taking into account that more than half of the *Anaplasma* positive sheep had a bad body condition, this endo-globular bacteria can be related to the decay of BC. 18.4% of the study animals were positive for *Babesia* spp, of these 12.4% had a bad body condition, which can be attributed to the inappetence experienced by the animals that occur with this disease and that is given due to the secretion of some substances that disturbs the feeding process of the animal, 4.8% presented regular body condition, and only 1.2% equivalent to 3 animals, which were positive to *Babesia* spp had a good BC, showing the influence of the presence of the parasite on the body condition of the animal, likewise, 10.4% of the total of animals in the study were positive for both agents, of these, 5.6% had a bad CC, 2.8% a regular body condition and 2% a good BC.

In Table 3, the hematocrit-haematropic agent relationship is described, it can be found that 62.3% of the animals that were positive to *Anaplasma* had a hematocrit between 20 and 30, this may be due to the fact that this bacterium produces intra-splenic hemolysis by reticular endothelial system which causes a notable decrease in the hematocrit, 24.6% of the animals positive to *Anaplasma* presented a hematocrit value between 31 and 40, and only 13.1% of these animals presented hematocrit in the range between 41 and 50, given the above, it is presumable that this intra-erythrocytic bacteria is the direct cause of anemia in these animals. 91.3% of *Babesia*-positive animals were found in the range between 20 and 30, this is attributable to the pathogenesis of *Babesia* that, apart from directly producing

Table 1. Total positive and negative animals sampled

Property	No. Sheep sampled	Positives <i>Anaplasma</i>	Positive <i>Babesia</i>	Positive to <i>Babesia</i> and <i>Anaplasma</i>
1	10	2	2	1
2	10	4	1	0
3	10	3	3	1
4	10	5	1	0
5	10	3	2	1
6	10	2	2	0
7	10	7	4	5
8	10	1	1	0
9	10	3	2	2
10	10	1	3	2
11	10	2	1	1
12	10	4	1	1
13	10	4	1	3
14	10	5	1	0
15	10	2	2	1
16	10	3	2	2
17	10	1	3	2
18	10	2	1	0
19	10	2	1	1
20	10	1	2	3
21	10	0	6	0
22	10	0	4	0
23	10	1	0	0
24	10	2	0	0
25	10	1	0	0
Total	250	61	46	26

Table 2. Relationship of animals diagnosed positive to endo-globular parasites with respect to body condition.

CC	<i>Anaplasma</i>		<i>Babesia</i>		<i>Anaplasma</i> and <i>Babesia</i>	
	#	%	#	%	#	%
Bad (2,5)	35	14	31	12.4	14	5.6
Regular (3–3,5)	15	6	12	4.8	7	2.8
Good (4–4,5)	11	4.4	3	1.2	5	2
Total	61	24.4	46	18.4	26	10.4

#CC, Corporal condition.

Table 3. Concentrations of hematocrit in animals positive for *Babesia* and *Anaplasma* in ovines of the Colombian low tropic.

Hematocrit	Positive <i>Anaplasma</i> (A)	%	Positive <i>Babesia</i> (B)	%	Positive (A and B)	%
20–30	38	62.3	42	91.3	23	88.5
31–40	15	24.6	3	6.5	3	11.5
41–50	8	13.1	1	2.1	–	–
>51	–	–	–	–	–	–
Total	61	100	46	100	26	100

lysis on the red blood cell, also causes the immune system to destroy other red blood cells and in this way produces more severe anemia. 6.5% of the *Babesia* positive sheep had hematocrit values between 31 and 40, and only 2.1% was found in the range between 41 and 50. In spite of being the least diagnosed, *Babesia* in the study, the group of animals that were parasitized by this agent was the one that greater sanitary deterioration presented, finally, 88.5% of the sheep that were parasitized by both agents, I present values of hematocrit between 20 and 30, so it can be said that the *Babesia*-hematocrit ratio predominated than the *Anaplasma*-hematocrit ratio, finally it was found that 11.5% of these animals had values in the range between 31 and 40, it should be noted that the hematocrit is the most important measure to determine the degree of anemia in an animal, and one of the most important clinical signs of the diseases produced by these microorganisms is anemia, therefore, it is essential to know the Hematocrit-hematropic agent relationship.

In Table 4, The relationship between the colour of the mucous membranes with the presence of *Babesia* and *Anaplasma* is described, 93.5% of the 61 sheep that were positive for *Anaplasma* had pale mucous membranes, which is explained by the type of hemolysis that this produces, being carried out at the spleens (extravascular) does not increase the production of bile pigments by the liver, as a result the membranes turn to a whitish color, 6.5% of this group presented sub-icteric colouration that can be attributable to some other type of liver problem or the presence of *Babesia* without being observed in the samples, since the technique used in this study is dependent on the experience of the operator in the laboratory. Of the group of animals positive for *Babesia*, 63% had sub-icteric mucous membranes, this phenomenon can be caused by the intravascular hemolysis that produces *Babesia*, the free Heme group in blood, is converted into biliverdin by the Reticular endothelial system, and then conjugated in the

liver, up to bilirubin diglucuronide and its high levels cause yellowish pigmentation of the mucous membranes, the remaining 37% of this group presented congestive mucous membranes which could be related to dehydration of the animals, which prior to taking samples were in pens where in most cases there was no water available. Of the 26 animals that were positive for both agents, 30.7 presented congestive mucous membranes, 57.7 sub-icteric mucosa indicating a dominance of the clinical signs associated with *Babesia* on the clinical signs of *Anaplasma*, and 11.6% I present pale mucous. It is valid to remember that the hematocrit is directly related to the colour of the mucous membranes, this relationship being concordant in the study.

It can be evidenced in table 5, that no sheep had a level of infestation of 0.07%, 8 of the 46 animals parasitized with *Babesia* had the maximum infestation of the study (0.06) as well as 2 animals that were positive for both agents, in 54% of the animals of the study that presented some degree of infestation, values of 0.05% were reported, and in only 4 infestation of 0.01% was reported, these sheep being infected with *Anaplasma*, 37% of the animals infected by *Anaplasma* had values equal to or less than 0.04%, in contrast to those parasitized by *Babesia*, only 10% of this group was found with infestations equal to or less than 0.04%, which may be indicative of severity of infestations by *Babesia* and *Anaplasma*.

It can be concluded that the prevalence founded in the study for *Anaplasma* (24.4%) and *Babesia* (18.4%) in short haired sheep in the department of Córdoba, is not a significant value because the agroecological conditions of this area are propitious for the development of the specific vectors of these microorganisms, however it is of paramount importance to continue conducting serological monitoring studies to monitor the presence and epidemiological behaviour of these hemotropic endo-globular agents, in order to take pertinent and preventive curative measures, which is a fact that greatly benefited the ovine producers

Table 4. Characteristics of the mucosa of the animals sampled during the study.

Character of mucous membrane	Positive <i>Anaplasma</i> (A)	%	Positives <i>Babesia</i> (B)	%	Positives A and B	%
Congestive	0	0	17	37	8	30,7
Sub-Icteric	4	6,5	29	63	15	57,7
Pale	57	93,5	0	—	3	11,6
Normal	—	—	—	—	—	—
Total	61	100	46	100	26	100

Table 5. Levels of infestation of animals positive to endo-globular hemotronics in sheep of the department of Córdoba.

	Level of infestation (%)							Total
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	
<i>Anaplasma</i> (A)	4	6	7	6	38	0	0	61
<i>Babesia</i> (B)	0	1	2	2	33	8	0	46
A and B	0	2	3	17	2	2	0	26

of the department of Córdoba.

The most predominant hemoparasite in the serological samples in short haired sheep that were analyzed in the clinical laboratory of University of Córdoba during the period 2016–2017 was the *Anaplasma*, of which 24 were positive samples, i.e. 4%.

Based on the results of the study, it is appropriate to implement prophylactic measures to reduce the incidence of these agents, because they are directly related to economic losses in the world sheep population, significantly deteriorating the health status of the animals and in some cases causing death.

REFERENCES

- Ávila P L, Restrepo A, Jurado G J, Echeverry D P, Velásquez V, and Zapata R. 2013. Hemoparasite infection in goats and sheep at five municipalities in north and northeastern Antioquia (Colombia). *Rev. CES Medicina Veterinaria y Zootecnia* **18**(1): 14–24.
- Battilani M, Arcangeli S, Balvoni A and Dondi F. 2017. Genetic diversity and molecular epidemiology of *Anaplasma*, infection. *Genetics and Evolution* **49**: 195–211.
- Blanco M R, Cardona Á J, Vargas V M. 2015. Prevalencia de parásitos hematótricos endoglobulares en bovinos gyr puros en Córdoba (Colombia). *Revue de Médecine Vétérinaire* **31**: 67–74.
- Homer M, Aguilar I, Telfort S, Krause P and Persing D. 2000. Babesiosis. *Clinical Microbiology Reviews* **13**(3): 451–69.
- Dill D, and Costill D. 1974. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology* **37**(2): 247–8.
- López J L. E, Hernández D M, Colín C C A, Ortega P, S, Cerón G G, and Franco C R. 2014. Las tinciones básicas en el laboratorio de microbiología. *Investigación en Discapacidad* **3**(1): 10–18.
- Lozano M H. 2014. Reproducción ovina en Colombia. *Revista Ciencia Animal* **8**: 67–83.
- StatSoft, Inc. 2008. Statistic(data analysis software system), version 8.0.
- Yousefi Ali, Sadegh Rahbari, Shayan Parviz, Sadeghi-dehkordi Zainab and Bahonar Alireza. 2017. Molecular detection of *Anaplasma marginale* and *Anaplasma ovis* in sheep and goat in west highland pasture of Iran. *Asian Pacific Journal of Tropical Biomedicine* **7**(5): 455–59.
- Vela M and Perez J. 2012. Murciélagos asociados a remanentes de bosque seco tropical en un sistema de ganadería extensiva (Colombia). *Chiroptera Neotropical* **18**(1): 1089–1100.