First report of sheep naturally infected with *Trypanosoma* sp. in Ecuador

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ABSTRACT: The prevalence of trypanosomiasis in sheep is of worldwide concern. It is a hemoparasitic disease caused by the protozoan *Trypanosoma* (T) spp., and the biological cycle of transmission involves susceptible hosts and certain hematophagous flies, such as Tabanids and Stomoxys. The objective of this study was to determine the presence of *Trypanosoma* sp. in sheep (*Ovisaries*). Through an applied, descriptive-prospective-cross-sectional qualitative study conducted between October 1, 2018, and January 29, 2019, blood samples extracted from the jugular vein were analyzed by the blood smear method using 2 Romanowsky staining techniques (Giemsa and Diff-Quick). Animals studied were between ages of 3 and 10 years, coming from 3 farms in the city of Colimes (Ecuador). Of 100 sampled and processed animals, 2 (2%) were positive for *Trypanosoma* sp., constituting the first report of this hemoparasite in sheep in Ecuador, and 1 case of Babesia spp. (1%) and 4 cases of Anaplasma marginale (4%) were also identified. However, none of these cases presented symptomaticity of any hemotropic-parasitic disease. Subsequently, the positive animals were dewormed. The presence of these parasites was confirmed at the study site and, thus, could become a serious animal health problem.

Key words: Ovisaries, Trypanosomiasis, Romanowsky staining, *Trypanosoma* sp.

INTRODUCTION

The domestic sheep (*Ovisaries*) in Ecuador is an animal 90% adapted to extreme climate and management conditions, and its rearing is underexploited in the llanuras (plains) and sub-plains of high elevation mountains that in some cases are abandoned and others poorly used. The production of sheep is generally in the hands of marginal farmers, who, through them, obtain meat, wool, milk, skins, natural fertilizers and other products that provide subsistence for many Ecuadorian families (ANCO, 2019).

However, there is little information about the risk of animals contracting hemoparasitic diseases, such as trypanosomiasis, through domestic sheep breeding, representing a serious problem for the development of the livestock industry in Africa, Asia and Latin America (PARRA-GIMENEZ & REYNA-BELLO, 2019).

Trypanosomiasis in sheep is caused by unicellular flagellar protozoa belonging to the family *Trypanosomatids* and the genus *Trypanosoma*; the latter includes many species that cause diseases in domestic and wild animals as well as humans (AREGAWI et al., 2019).
There are 3 species of trypanosomes of veterinary importance in South America: *Trypanosoma evansi*, the causative agent of surra, transmitted mechanically by blood-sucking insects such as tabanids; *T. vivax*, also mechanically transmitted by some hematophagous diptera such as Tabanids and/or Stomoxys; and *T. equiperdum*, a tissue parasite adapted for sexual transmission and the causative agent of dourine, a distinctive disease affecting only equines (PARRA-GIMENEZ & REYNA-BELLO, 2019).

Although *Trypanosoma evansi* preferentially affects horses and camels, it can also affect donkeys, dogs, cats, cows and buffalo (DESQUESNES et al., 2013), but in sheep, experimental infections have resulted in a chronic and often cryptic disease, with low parasitemia and, in some cases, self-healing (ONAH et al., 1996; AUDU et al., 1999).

*Trypanosoma vivax* causes a critical and often deadly disease in ruminants such as cattle, buffalo, sheep and goats. Among clinical signs, fever, wasting, anemia, anorexia, immunosuppression, thrombocytopenia, formation of microthrombi, and hemorrhage suggestive of disseminated intravascular coagulation have been reported (PARRA-GIMENEZ & REYNA-BELLO, 2019; MAGEZ & RADWANSKA, 2014).

Importantly, in the world in recent years, there has been little information on trypanosomiasis in sheep; therefore, there is little literature on the subject. In Ecuador, cases of trypanosomiasis in sheep have not been identified, but in 2017, the first report of *T. vivax* in bovines was described (REYNA BELLO, 2017).

The objective of this preliminary study was to determine the presence of *Trypanosoma* sp. in sheep from a rural area on the Ecuadorian coast.

### MATERIALS AND METHODS

#### Study area and time period

This study was conducted on 3 farms in the rural sector of the city of Colimes, located in the northern part of the province of Guayas, on the Ecuadorian coast. Its geographical coordinates are 01° 32’ 43.9” South and 80° 01’ 00” West. The city has 25,167 inhabitants, with 6,352 households, and is divided into an urban zone and a rural zone, with 77.7% of the population residing in the rural zone.

The country has an altitudinal range of 32-232 meters above sea level (m.a.s.l) and a humid-tropical climate, with marked differences between winter (rainy) and summer (dry), with temperatures ranging from 25 to 26 °C. An applied, qualitative, descriptive-prospective-cross-sectional study was conducted between November 1, 2018, and June 30, 2019.

#### Sampling

Before obtaining samples, the research protocol was analyzed and approved by the Research Coordination of the Faculty of Veterinary Medicine of the University of Guayaquil, following the protocols of authors such as (AL-KHALIFA et al., 2009; ADEJINMI et al., 2010; SEBELE et al., 2015). After approval, the inhabitants of the studied farms were informed about the importance of conducting the research and about the risk of the disease in the infected animals. Then, informed consent was requested from the domestic sheep owners, and with prior acceptance, non probabilistic directed sampling was performed, in which blood samples were taken directly from the jugular vein of 100 sheep from 3 farms in the rural zone (Entrance to Colimes, Loss Enclosure and The Guabito) of the city of Colimes. The following sampling procedure was performed. The animal was restrained, excess hair was shaved, complete antisepsis was performed on the sample extraction area, and pressure was exerted on the vacutainer tube containing EDTA (ethylenediaminetetraacetic acid) anticoagulant. Approximately 4 ml of blood was vacuum-extracted and then transported in a cooler, maintained at temperature between 4 and 8ºC using cooling gel, to the Laboratory of Animal Diagnostics of the Phytosanitary and Zoosanitary Regulation and Control Agency (AGROCALIDAD) of Guayaquil, where the samples obtained were analyzed.

Additionally, in the present study, the following were recorded: symptoms, body condition, temperature and conjunctiva status. Regarding body condition, the following clinical parameters were analyzed: state 1 = extremely low weight animal; state 2 = low weight animal; state 3 = animal in good condition; state 4 = overweight animal; and stage 5 = obese animal (ROMERO, 2015).

The body temperature of the sheep studied was classified using the following parameters: >40 °C = fever-pyrexia (hyperthermia); 39-40 °C = normal; 37-39 °C = moderate hypothermia; and <37 °C = severe hypothermia (MENDOZA et al., 2013).

Conjunctiva status was determined using criteria published by MENDOZA et al. (2010): status 1 = optimal; status 2 = normal, acceptable; status 3 = intermediate; status 4 = dangerous; and status 5 = fatal.
Laboratory analysis

Blood smears were performed, with Giemsa and Diff-Quick staining separately, as described by AL-KHALIFA et al. (2009). Samples were analyzed under a microscope (100× objective) using immersion oil to identify *Trypanosoma* sp.

RESULTS

Of the 100 samples processed by the 2 methods, 2 animals were positive for *Trypanosoma* sp., resulting in a prevalence of 2%.

During the study, the following were also observed: 1 animal positive for *Babesia* sp. (1%) and 4 animals positive for *Anaplasma marginale* (4%) (Table 1). The infected animals provenance of two farms which are Entrance to Colimes and Loss Enclosure, with a distance of 30 km between them; also, only one sheep was introduced, because it was bought in the Province of Manabí, the others were from the sector studied, likewise, the presence of vectors was observed in all farms.

Also, results indicated Hemoparasitic coinfection of *Trypanosoma* sp. and *Anaplasma marginale* in sample-039 and of *Babesia* sp. and *Anaplasma marginale* in sample-047 (Table 1).

Animals that were positive did not present any symptomatology of hemoparasitism during the study period but showed slight anemia through a biochemical test; likewise, all presented body state 3 (good condition), a normal temperature of 39 °C, and conjunctiva status 1 (normal) (MENDOZA et al., 2013) (Table 2).

Deworming with diminazene aceturate was performed on all positive animals intramuscularly with a single dose of 3.5 mg/kg of weight (equivalent to 1 ml/20 kg of weight), besides vitamins and immune support. Additionally, periodic evaluations were conducted for 2 months to rule out the presence of any kind of hemoparasite (DEMESSIE & DERSO, 2015).

DISCUSSION

In the present study, the presence of *Trypanosoma* sp. was identified in 2 animals (2%), and in Paraguay, 1 case has been reported (TOMASSI et al., 2018). The prevalence, however, is below that reported in countries such as Iraq (3.3%) (HASAN, 2012), Nigeria (7.4%) (DANIEL et al., 1994), Venezuela (9.75-62.3%) (MORA & CASTRO, 2015), Brazil (approximately 25%) (BATISTA et al., 2009), Kenya (25.3%) (NG’AYO et al., 2005), Colombia (46.2%) (AVILA et al., 2013) and Nicaragua (37-47%) (MORA & CASTRO, 2015). In addition, DESQUESNES et al. (2013) claimed that *T. evansi* was observed for the first time in Paraguay in 1847 and that *T. vivax* was described nearby Pantanal region of Brazil (CAMPIGOTTO et al., 2015).

Also, natural infections with *T. evansi* in sheep have been reported in countries such as: Egypt, Ethiopia, Sudan, India, Guyana and Spain. In these mentioned, an accumulated prevalence between 1 to 27% is reported (AREGAWI, 2019).

In this study, the presence of *Trypanosoma* sp. was identified in sheep from Ecuador, reporting a low prevalence (2%), presumably at early detection of the parasite and a limited interaction of transmission between parasites - hosts - and environment.

Additionally, in this study, 1 animal was positive for *Babesia* sp. (1%), and 4 animals were positive for *Anaplasma marginale* (4%). AL-KHALIFA et al. (2009), in a study conducted in sheep from Saudi Arabia, reported *Anaplasma ovis* in 2% of the animals studied and *Babesia* sp. in 4%; SEBELE et al. (2015), in a study conducted in Ethiopia, reported the presence of *Anaplasma ovis* in 1.8% of the animals studied; ADEJINMI et al. (2004), in a study conducted in Nigeria, reported a prevalence

| Positive sample  | Giemsa       | Diff-Quick | Hemoparasites identified                      |
|------------------|--------------|------------|-----------------------------------------------|
| Sample-035       | Negative     | Positive   | *Trypanosoma* sp.                            |
| Sample-039       | Positive     | Positive   | *Trypanosoma* sp. and *Anaplasma marginale*   |
| Sample-047       | Positive     | Positive   | *Babesia* sp. and *Anaplasma marginale*       |
| Sample-073       | Positive     | Positive   | *Anaplasma marginale*                         |
| Sample-075       | Positive     | Positive   | *Anaplasma marginale*                         |
of 1.9% for *Babesia* sp. and 12.2% for *Anaplasma* sp.; and MOHAMMED et al. (2018), in a study conducted in Sudan, detected *Babesia* sp. (46.70%) and *Anaplasma* sp. (19.82%).

Furthermore, this study detected hemoparasite coinfection with *Trypanosoma* sp. (2%) and *Anaplasma marginale* (4%), but there is little information on the prevalence of this coinfection, and with respect to a *Babesia* sp. (1%) and *Anaplasma marginale* (4%) found, AL-KHALIFA et al. (2009) described *Babesia* sp. (4%) and *Anaplasma ovis* (2%); ADEJINMI et al. (2004) determined *Babesia* sp. (1.9%) and *Anaplasma ovis* (12.2%) and RENNEKER et al. (2013) described *Babesia* sp. (1.5%) and *Anaplasma ovis* (62.6%).

It is important to highlight that all the animals positive for hemoparasites were asymptomatic, but with the presence of mild anemia; unlike those reported by IKEDE (1979), LOSOS & IKEDE (1972), MORA & CASTRO et al. (2015) and TOMASSI et al. (2018), where the following symptoms are described in infected sheep: fever, loss of appetite, weakness, loss of mass, progressive anemia, lymphadenopathy, pale oral and conjunctival mucosa, bruising in the inguinal region and mild bilateral serous nasal discharge. Likewise, bloody diarrhea, enlarged spleen, respiratory dyspnea, decreased pulse rate, difficulty opening the mouth and, in severe cases, neurological and eye damage, hypothermia and death usually appear.

In South America, the disease caused by *T. vivax* in ruminants is predominantly chronic, with clinical manifestations related to progressive wasting, anemia, and a variable increase in lymph nodes, with death observed only occasionally (MORA & CASTRO, 2015). The presence of signs and symptoms of disease in the sheep studied would presumably be due to the patent (asymptomatic) state of the infected host.

This study indicated that hemotropic parasites are present in the blood of sheep in the city of Colimes, which is influenced by climatic characteristics that favor the presence of vectors for the transmission of this parasitosis, but there is a potential risk of cases of the disease occurring in surrounding areas due to the presumption of parasitic circulation in infected vectors.

With these events exposed, we suggested that the blood samples from the animals be subjected to confirmatory tests (PCR) to determine the species of *Trypanosoma*; as described by N’DJETCHI (2017) in Africa. These data, in addition to enriching the research, are valuable information for local health authorities and the OIE.

**CONCLUSIONS**

In samples taken from domestic sheep from 3 farms in the rural area of the city of Colimes in Ecuador, *Trypanosoma* sp. (in 2% of the samples) and other hemotropic parasites (*Anaplasma maginale* and *Babesia* sp.) (in 5% of the samples) were identified through blood smears using 2 staining techniques, Giemsa and Diff-Quick, implying a potential risk of hemoparasites being present at the sites studied. This research was important because it showed that the detected parasites are present in the country and can cause an epizootic. Furthermore, this information is relevant to epidemiological surveillance systems and animal health.

Moreover, it is important to improve the dissemination and promotion of educational programs on these parasites and to establish effective control measures to reduce the infectious forms of parasites in animals.

Notably, coinfection with 2 hemoparasites was detected in the samples obtained. In addition, all the animals investigated did not present symptoms of hemoparasitic disease and had a good body condition, normal temperature and good conjunctival status.

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DECLARATION OF CONFLICTS OF INTERESTS

The authors covered the full funding of the research, therefore, there is no conflict of interest with any financial organization regarding the material discussed in the manuscript. In addition, those mentioned also contributed to the manuscript.

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