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Occurrence of Haemoparasites and Prevalence of Antibodies against Ehrlichia ruminantium in Goats from Smallholder Farms in Mozambique

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1. Abstract

A survey was carried out to determine the prevalence of haemoparasites and of heartwater - Ehrlichia ruminantium infection in goats in four ecological regions of Mozambique, from November 2016 to October 2017 in Tete and Cabo Delgado, from November 2016 to October 2018 in Maputo, and from November 2016 to May 2018 in Gaza. It has been demonstrated in this study that infections by A. ovis and T. ovis are common in goats in Mozambique. Although the literature indicates low or nonpathogenicity, further studies on the role that these parasites play in goats from smallholder farms should be considered. Serological testing to detect antibodies against E. ruminantium showed the presence of high levels of infection rates by this organism in Maputo, Gaza and Cabo Delgado. In Tete, however, a low prevalence of cowdriosis was observed, which probably indicates that goats are highly susceptible to the disease. In conclusion, the epidemiological data provided here on haemoparasites in goats of Mozambique demonstrate that the occurrence of A. ovis and T. ovis are common in goats in Mozambique. Its importance, however, was not determined. The importance of cowdriosis could be related to the impossibility to transfer animals from Tete Province, where they exist in large numbers, to the other regions of the country where cowdriosis is endemic, since no vaccination against cowdriosis is applied routinely in this country.

2. Keywords: Prevalence; Anaplasma ovis; Ehrlichia ruminanitum; Theileria ovis; Goats; Smallholder farms

3. Introduction

The prevalence of tick-borne diseases and their occurrence of various diseases is linked to the

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importance varies from one region to another, since spatial distribution of their vectors, which depends largely on climatic factors [1]. In many areas of Africa, haemoparasites, especially Ehrlichia ruminantium (formely Cowdria ruminantium,) an obligate intracellular Gram-negative bacterium, pleomorphic coccus in the family Anaplasmataceae and order Rickettsiales [2], causing heartwater, a fatal disease of domestic ruminants in sub-Saharan Africa and eastern Caribean [3], cause high mortality in cattle, sheep and goats [4-6].

Theileria ovis is considered non-pathogenic while Anaplasma ovis causes ovine and caprine anaplasmosis. Although [7] and [8] refer that the disease is, of relatively little importance in southern Africa, Papadopoulos, Brossard and [9] observed that clinical disease caused by A. ovis occurred in improved breeds, introduced in endemic areas.

During the acute stage of the infection, the diagnosis of A. ovis in small ruminants is usually made on the basis of clinical signs, the presence of the parasite in stained blood smears and haematological changes [10].

Heartwater, known as cowdriosis or ehrlichiosis, is a serious economic problem wherever it occurs, in an enormous area covering most of sub-Saharan Africa [2], and has long been recognized as one of the main constraints for goat breeding in Mozambique [11, 12], and two vector ticks are of importance in their transmission in this country, namely Amblyomma hebraeum in the South and Amblyomma variegatum in the North of the country, the latest authors suggest. The objective of this survey, therefore, was to determine the prevalence of haemoparasites and of heartwater - Ehrlichia ruminantium infection in goats in four ecological regions, in order to obtain epidemiological data on the occurrence of these parasites.

4. Materials and Methods

4.1. Study locations and animals

The study was conducted in four of the ten provinces of Mozambique, namely Cabo Delgado, Tete, Maputo and Gaza, where 24,1%, 15%, 10,1% and 5,8% of the small ruminant population, respectively, occur, from November 2016 to October 2017 in Cabo Delgado and Tete, and from November 2016 to October 2018 in Maputo and Gaza, respectively. The climate in Mozambique consists of two distinct seasons namely a hot and rainy season, and a relatively cool and dry season. In Tete region, however, the climate is subarid and hot, and the rainy season spans a period of a few months, extending from December to about mid-March. The dry season is characterized by almost complete drought and normally extends from April to the beginning of December. The Tete survey area, influenced by the intertropical convergence zone, is too far inland to experience the moderating influence of the sea, so that temperatures are high and rainfall is low [13].

The study areas were chosen because of distinct ecological conditions. In each province both lowland (areas within river valleys) and upland zones (areas located outside of the river valleys), were included in the study. The mean distance of the areas varied 35 to 70 km from the capital cities of each province, namely Maputo for Maputo Province, Xai-Xai for Gaza Province, Tete for Tete Province and Pemba for Cabo Delgado Province. This arrangement was chosen to facilitate the access by car for specimen collections and the subsequent processing at the laboratory.

A total of 440 goats of both sexes and various ages were selected from the family sector for the study in the four provinces; 74 in Maputo, 134 in Gaza, 100 in Tete and 132 in Cabo Delgado, respectively. A total of twenty flocks, consisting of five flocks in each province, each with 8 to 34 goats, were involved in the survey. The animals were reared under extensive systems in communal pastures during the day and kept in corrals at night in all smallholder farms. The animals were mainly a local breed, the Landim goats,

and animals were identified by ear tags with code numbers. All the farms were visited once a month and blood smears prepared.

4.2. Examination of blood smears

A total of 4 963 blood smears, 1 495 from Maputo, 1 446 from Gaza, 903 from Tete and 1 119 from Cabo Delgado, respectively, were made according to standard methods [14]. Thin smears were made from blood collected directly from a puncture wound made with a hypodermic needle on the ear of the animal. The smears were air-dried and subsequently fixed in methyl alcohol for 5 minutes and stained with 10% Giemsa's solution for 20 minutes. All smears were examined under a standard microscope at 100x magnification.

4.3. Collection and testing of sera

Blood was collected in August/September from the survey goats directly from jugular vein using a plain vacuum tube (Vacutainer). The tubes containing all blood were left to stand until complete clot was formed, and serum was decanted into a vial for storage. Sera were stored at –20ºC until processed. A total of 361 sera, 83 from Maputo, 82 from Gaza, 105 from Tete and 91 from Cabo Delgado province, respectively, were submitted to the Microbiology Laboratory at the Veterinary Faculty in Maputo, where they were tested for the presence of antibodies against Ehrlichia antibodies using a MAP1b – ELISA test. This test is an indirect enzyme linked immuno-assay for the detection of antibodies to recombinant MAP1b antigen of E. ruminantium. The kit was supplied by Dr Franz Jongejan, Utrecht University, Department of Parasitology and Tropical Veterinary Medicine, The Netherlands.

4.4. Histopathology of nervous tissue

A total of 139 tracer goats kept in the herds studied throughout the study period were killed by euthanasia, and brain samples were collected from cerebrum and cerebellum during the necropsies, and preserved in 10% formalin. Subsequently, they were submitted to the Pathology Division at Animal Sciences Directorate where histopathological examination was performed in order to detect E. ruminantium in the capillary endothelial cells. The tissues were trimmed and processed by conventional paraffin embedding, and sections cut 4-5 microns thick, were stained with toluidine blue for rickettsiae. E. ruminantium occurs as clumps of reddish-purple to blue, coccoid to pleomorphic organisms inside capillary endothelial cells [2].

5. Results

A. ovis and T. ovis were the haemoparasites identified in this survey, and the results of blood smears are shown in below tables.

Table 1: Prevalence of haemoparasites in goat blood smears examined from November 2016 to October 2018 in Maputo Province, and from November 2016 to May 2018 in Gaza Province.

Maputo Province				Gaza Province			
A. ovis and T. ovis				A. ovis and T. ovis			
Month	Number examined	Number of positive	Prevalence (%)	Month	Number examined	Number of positive	Prevalence (%)
Nov-16	20	6	30,0	Nov-16	134	69	51,5
Dec-16	74	30	40,5	Dec-16	123	55	44,7
Jan-17	71	22	31,0	Jan-17	128	65	50,8
Feb-17	68	49	72,0	Feb-17	120	62	51,6
Mar-17	62	34	54,8	Mar-17	*	*	*
Apr-17	79	56	70,9	Apr-17	108	68	63,0
May-17	74	49	66,2	May-17	*	*	*

Jun-17	68	51	75,0	Jun-17	99	73	73,7
Jul-17	66	47	71,2	Jul-17	97	75	77,3
Aug-17	76	41	53,9	Aug-17	96	65	67,7
Sep-17	82	46	56,1	Sep-17	91	76	83,5
Oct-17	81	56	69,1	Oct-17	*	*	*
Nov-17	79	35	44,3	Nov-17	53	48	90,6
Dec-17	78	51	65,4	Dec-17	*	*	*
Jan-18	67	36	53,7	Jan-18	87	50	57,5
Feb-18	65	38	58,5	Feb-18	81	53	65,4
Mar-18	61	45	73,8	Mar-18	78	53	67,9
Apr-18	59	40	67,8	Apr-18	78	52	66,6
May-18	53	28	52,8	May-18	71	34	46,6
Jun-18	41	22	53,7	**Total**	**1 446**	**898**	**61,2**
Jul-18	64	33	51,6	* - No data were available during these months.			
Aug-18	43	25	58,1	-	-	-	-
Sep-18	46	31	67,4	-	-	-	-
Oct-18	18	10	55,5	-	-	-	-
Total	**1 495**	**881**	**58,9**	-	-	-	-

Table 2: Prevalence of haemoparasites in goat blood smears examined from November 2016 to October 2017 in Tete and Cabo Delgado Provinces.

Tete Province				**Cabo Delgado Province**			
A. ovis and T. ovis				**A. ovis and T. ovis**			
Month	**Number examined**	**Number of positive**	**Prevalence (%)**	**Month**	**Number examined**	**Number of positive**	**Prevalence (%)**
Nov-16	100	39	39,0	Nov-16	132	64	48,5
Dec-16	86	44	51,2	Dec-16	129	70	54,3
Jan-17	70	29	41,4	Jan-17	125	64	51,2
Feb-17	29	14	48,3	Feb-17	122	59	48,4
Mar-17	*	*	*	Mar-17	121	75	62,0
Apr-17	102	81	79,4	Apr-17	118	89	75,4
May-17	97	65	67,0	May-17	116	84	72,4
Jun-17	92	73	79,3	Jun-17	114	77	67,5
Jul-17	91	60	65,9	Jul-17	107	83	77,6
Aug-17	85	46	54,1	Aug-17	101	75	74,3
Sep-17	82	59	72,0	Sep-17	98	58	59,2
Oct-17	69	50	72,5	Oct-17	96	63	65,6
Total	**903**	**560**	**62,0**	**Total**	**1 119**	**861**	**76,9**

A. ovis and T. ovis were present in 3,200 (64.5%) of the 4,963 blood smears examined in the four provinces. Of these, 1 663 (52.0%) had A. ovis, 1 072 (33.5%) T. ovis and 465 (9.4%) had a mixed infection. The overall prevalence was 58.9% in Maputo, 62.1% in Gaza, 62% in Tete and 76.9% in Cabo Delgado.

The total prevalence of these parasites varied in different months in different provinces during the study period, however, no seasonal variation was observed in any of the provinces.

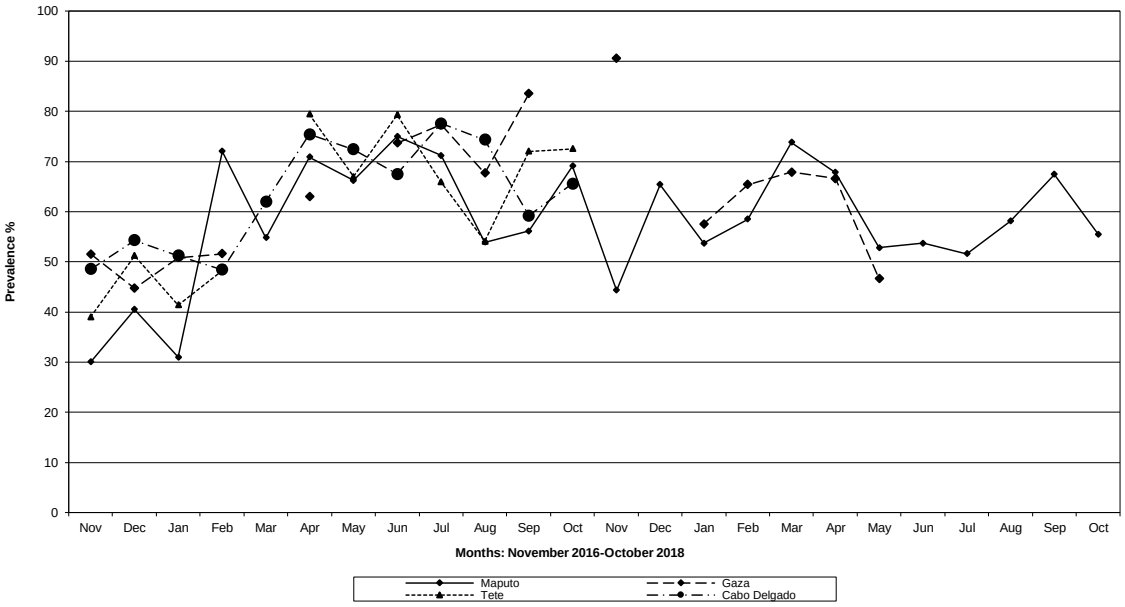

Figure 1: Prevalence of haemoparasites in blood smears of goats examined in Maputo, Gaza, Tete and Cabo Delgado during the study period. No data were available for Gaza in March, May, October and December 2017 and for Tete in March 2017.

Thirty-four of the 139 goat brains examined for E. ruminantium were from Maputo, 36 from Gaza, 41 from Tete and 28 from Cabo Delgado. Only two samples from Cabo Delgado were found positive for this rickettsia, which represents a prevalence of 7.1% for this province. The remaining samples were negative for E. ruminantium.

The death of eight animals due to acute ehrlichiosis in the herds involved in this study was confirmed in DCA / IIAM. Of these, one goat was from Boane, two from Mahubo, Maputo, and two were from Chicumbane and three were from Barra, both places in Gaza. A goat from Cabo Delgado showed clinical signs of nervous disorders, probably due to ehrlichiosis, but symptoms disappeared and the animal recovered after treatment with a single intramuscular injection of 10 mg / kg bodyweight long-acting oxytetracycline (Curamycin LA, Agriculture (Bayer AH), South Africa).

Of the 361 sera tested, 142 (39.3%) were positive, comprising 56 sera of 83 (67.5%) from Maputo, 44 of 91 (48.3%) from Cabo Delgado, 37 of 82 (45.1%) from Gaza, and 5 out of 105 (4.8%) from Tete. Thus, the prevalence of ehrlichiosis was high in Maputo, moderate in Cabo Delgado and Gaza, and low in Tete Province.

6. Discussion

Some tick species that are likely to be responsible for the transmission of A. ovis and T. ovis, namely Rhipicephalus evertsi, Rhipicephalus simus, Rhipicephalus (Boophilus) microplus, Rhipicephalus (Boophilus) decoloratus and Hyalomma spp. occur in Mozambique. The E. ruminantium vectors that occur in this country are A. hebraeum in the south, and A. variegatum in the center and north of the country. The description of their distribution is provided by [15] as follows below. The A. hebraeum tick is exclusively a Southern African tick, occurring in the warm and humid coastal areas of the Eastern Cape, Kwazulu-Natal and Southern Mozambique. Its distribution also extends inland, namely Swaziland and East Botswana. In Botswana, the distribution of this tick is limited by dryness, and in northern Zimbabwe and Central Mozambique the

distribution is limited by interspecific competition with A. variegatum.

A. variegatum is widely distributed throughout West, Central and East Africa, and in Southern Africa it extends to Zambia, Northeast Zimbabwe, Central and Northern Mozambique.

R. (Boophilus) microplus is present in certain areas along coastal areas of the Eastern Cape and Kwazulu-Natal Provinces, South Africa, and Mozambique. Inland it is present in certain areas of Mpumalanga, parts of eastern and central Zambia provinces, and in Malawi. It spread to the interior of Zimbabwe in the 1970s and replaced R. (Boophilus) decoloratus in many areas. R. (Boophilus) decoloratus is distributed throughout the wettest parts of southern Africa, except in areas where it has been replaced by R. (Boophilus) microplus and cold mountainous areas such as Drakensburg and parts of Lesotho. It is absent from the most arid parts of southern Africa with an average annual rainfall of less than 380 mm.

The R. evertsi tick tolerates a wider range of Ambrosio & De Waal, 1991, cited by Stoltsz, [16] in South Africa.

The 62% prevalence of haemoparasites in Tete, a semi-arid region, is surprising since the number of ticks, Rhipicephalus spp. and Hyalomma spp. in goats it was low. The presence of ticks on animals was most noticeable during the rainy season. In Maputo, Gaza and Cabo Delgado high loads of Rhipicephalus spp. and Hyalomma spp. and Amblyomma spp. were observed throughout the year, reaching peaks, however, during the peak of the rains. The high prevalence of haemoparasites in these provinces was therefore expected.

The effects of A. ovis in Boer goat nannies in weather conditions and is present throughout most of southern Africa. The main limiting factor for its western distribution is increasing aridity, with a critical rainfall level of 250 to 280 mm per year. R. simus is widely distributed throughout areas with moderate to high rainfall in southern Africa. Hyalomma spp. it is widespread in southern Africa, in both high rainfall and low rainfall areas.

The results of this study indicate that A. ovis and T. ovis infections are common in Mozambican goats. Rates of infection by both parasites varied slightly in the four provinces. In all four provinces A. ovis was more prevalent than T. ovis, which is in line with results found by Arnold [4], who found infections by Anaplasma spp. and by T. ovis throughout the year in goats from Maputo Province. A prevalence of A. ovis between 0 and 90%, and T. ovis ranging from 0 to 80%, respectively, were reported by these authors.

A prevalence of A. ovis ranging from 33 to 90% has also been reported (Stoltsz, 1990, Visser,

South Africa were investigated by Barry & Van Niekerk [17], who concluded that A. ovis is transmissible to goats, is capable of transplacental migration to the fetus, and is likely to cause abortion in areas where it is prevalent.

Although anaplasmosis appears to be widespread, the extent of the infection and the loss of productivity in small ruminants remain poorly understood, probably because the infection with A. ovis was neglected since it is considered to induce only mild clinical symptoms and thus being of minor economic importance [18].

In a study of A. ovis infection in goat flocks in [19] observed that there was no correlation

between infection and clinical signs, thus, A. ovis in goats does not seem to pose a significant problem because of being endemic, and mostly it is a subclinical infection in the goat population. However, previously, Naqid [20] stated that acute A. ovis infections can cause severe clinical symptoms and might lead to significant economic losses in small ruminant flocks.

The low prevalence of E. ruminantium in the examined brains was expected since organisms are usually detected in endothelial cells during clinical stages of the disease. Vink & Wapenaar [21] examined brain smears from 130 goats from Tete and in no case a clear diagnosis was possible. They claim that brain smears, therefore, seem to have little value in determining prevalence during epidemiological surveys. Figueroa & Buening [22] also indicate that carrier animals generally have very low numbers of organisms in the bloodstream and that these cannot easily be demonstrated by examination of blood or brain smears with traditional methods.

Despite the low prevalence of positive cases found, ehrlichiosis has been responsible for goat deaths in endemic areas of Mozambique. A similar situation has been reported in endemic regions of South Africa, where 3.8% mortality in small ruminants was observed throughout 1993 [16]. It indicated that mortality due to ehrlichiosis is three times higher than the combined deaths caused by babesiosis and anaplasmosis in endemic areas in South Africa [23].

Some studies had also been conducted on ticks and tick-borne diseases in goats in Africa, with special reference to ehrlichiosis. The seasonal fluctuation of ixodid ticks on a herd of indigenous goats at Oodi, Botswana, during a two-year period, indicated that the infestation rate was 37,7% with an average of 3,5 ticks per goat [24]. Rhipicephalus evertsi was the most abundant tick collected (90,4%) and is of importance as vector for A. ovis. Other ticks present in small numbers were A. hebraeum, R. (Boophilus) decoloratus and Hyalomma truncatum. As in Mozambique, A. hebraeum is the most important tick of goats as it is the vector of heartwater which is regarded as the major infectious disease of goats in Botswana [24].

The high prevalence of antibodies against E. ruminantium in Maputo and Gaza agrees with the findings of Asselbergs et al. [11] who used the indirect immunofluorescence test (IFAT) for antibody detection. Vink & Wapenaar [21] also found a prevalence of 65.6% in Maputo and a prevalence of 50.8% in Chibuto (Gaza province) using the MAP-1b ELISA test.

The low prevalence of antibodies against E. ruminantium reported in Tete is in agreement with the results of Asselbergs et al. [11], who found a prevalence of 2.2%, and also those of Vink & Wapenaar (1998), who found a prevalence of 6.1% in goats of the family sector in this province. This confirms the absence of enzootic stability for ehrlichiosis also found in this study. [25] It indicated that in areas where no ticks were observed in the animals under study, a low prevalence of E. ruminantium of about 4% was observed while in areas of high tick prevalence it reached 86%. Therefore, animals from tick-free areas often succumb to clinical ehrlichiosis if moved to areas with high Amblyomma loads. Asselbergs et al. [11] stated that the transfer of goats from Tete to the southern region may have serious implications, and [21] reported a mortality rate of about 50%

in a herd of goats one month after being transferred from Tete to Maputo.

The prevalence in Cabo Delgado disagrees with the low prevalence of 11.1% reported by Asselbergs et al. [11] in the same province. Note that the sera tested during this study were collected in herds of small breeders where ticks are normally not controlled. Thus, animals are highly exposed to ticks, resulting in protective infection with E. ruminantium. Asselbergs et al. [11] collected many of the sera they tested in a state farm as well as in private farms where tick control programs are implemented. Payne, Scott & Osorio [26, 27] observed that in farms where a high proportion of animals were positive for tick-borne diseases such as anaplasmosis and babesiosis, no tick control was performed, while only a small percentage were positive when tick control was implemented.

Awa [25] carried out a serological survey of heartwater relative to the distribution of the vector A. variegatum and other tick species in North Cameroon. He found that the disease appeared to be endemic and the mean prevalence of antibodies to E. ruminantium was 61-67%. The antibody prevalence was highly associated with the presence of A. variegatum.

In their study of the seasonal distribution of ticks and tick-borne haemoparasites of sheep and goats in southern Mozambique, Arnold & Travassos Dias [4] found that tick populations consisted mainly of R. evertsi evertsi, R. appendiculatus, A. hebraeum and R. (Boophilus) decoloratus. The examination of blood slides indicated various degrees of the prevalence of protozoa and rickettsia. T. ovis was found throughout the year in goats, with the mean prevalence of 38,1%. Anaplasma spp. was also present throughout the year, with the mean prevalence of 40,2%, as were Trypanosoma vivax and Trypanosoma congolense. The presence of A. hebraeum throughout the year is of great significance both in sheep and goats in southern Mozambique as E. ruminantium, is one of the major constraints to the production of small ruminants in this area.

In a preliminary study of the parasitism patterns of goats in the family sector in Mozambique, Atanásio & Boomker [28] also observed that haemoparasites were present in 50,2% of blood smears examined. Of these, 51% had Theileria spp., 45,7% had A. ovis, and 3,3% had a mixed infection.

The serological prevalence of E. ruminantium antibodies in goats in Mozambique was studied by Asselbergs, Jongejan, Langa, Neves & Afonso [11]. In the northern provinces a low prevalence of 10% of antibodies to Ehrlichia was found as opposed to the 63,5% of goats in the south of the country. In the north of the country cases of heartwater are rare and animals are probably fully susceptible, while in southern Mozambique, although the high seroprevalence suggests enzootic stability, clinical cases are found frequently, especially in kids between 2 and 12 months of age.

Although it is reported that the MAP-1b ELISA test occasionally gives false positive results [29], the absence of enzootic stability in Tete Province, and the existence of endemic stability for ehrlichiosis in Maputo and Gaza has been confirmed, and the existence of enzootic stability in Cabo Delgado was observed in this study. The occurrence of E. ruminantium cross reactions with Ehrlichia spp. using the MAP-1b ELISA has been demonstrated by several authors

(Allsopp, Visser, Du Plessis, Vogel & Allsopp [30] Mahan [29] Savadye, Kelly & Mahan, [31]. Serological testing with high specificity and sensitivity, such as polymerase chain reaction (PCR) may be of value for epidemiological studies.

Vink & Wapenaar [21] did a field study and serological survey on heartwater in indigenous goats in southern Mozambique. They tested 326 sera using the Ehrlichia MAP 1b – ELISA and found an overall prevalence of 65,6% while in a similar study conducted in Tete Province, only 8,2 % out of 320 sera tested were positive. This confirmed the findings of Asselbergs et al. [11].

The occurrence of heartwater in sheep and goats in Botswana, using brain crush smears stained with Giemsa, was investigated at the National Veterinary Laboratory between 1984 and 1993. The mean annual case incidence was about 80,3 and the highest number of cases, 17,1% of the total was recorded in 1990. Goats accounted for 91% of the cases that were confirmed throughout the year [6].

However, reported the occurrence of an apparently non-pathogenic E. ruminantium variants in a heartwater-free area and vector tick-free area of South Africa using an IFA and ELISA test as well as a PCR assay based on Ehrlichia ruminantium 16 S gene sequences [32].

The results of this study and those of previous studies [11] on the prevalence of ehrlichiosis in Cabo Delgado are contradictory. Thus, the presence or absence of enzootic stability for the disease in this province, involving different production systems, should be investigated.

7. Conclusion

It was shown in this study that infections by A. ovis and T. ovis are common in goats from Mozambique. Its importance, however, has not been determined. Although the literature indicates low or non-pathogenicity, further studies on the role these parasites play in goats from smallholder farms should be taken into account. A low prevalence of ehrlichiosis was observed in Tete, which probably indicates that goats in this region are highly susceptible to the disease. Its importance may be related to the difficulty of transferring animals from this province, where they exist in large numbers, to other regions of the country where ehrlichiosis is endemic, since vaccination against this disease is not routinely done in this country, probably due to economic reasons.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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