Morphological identification of ticks (Acari: Ixodidae) infesting donkeys (*Equus asinus*) in Maputo Province, Mozambique

Carolina Esculudis[1], Carlos De Matos[2], Aida Cristina Cala[2], Marvelous Sungirai[3], Maxime Madder[4], Milton Mapatse[1]*

[1] Veterinary Faculty, University Eduardo Mondlane, Maputo, Mozambique

[2]Agricultural Research Institute of Mozambique, Central Veterinary Laboratory, Maputo, Mozambique

[3] Department of Animal and Wildlife Sciences, Midlands State University, Gweru, Zimbabwe

[4] Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa

***Correspondence:** Milton Mapatse, E-mail: miltonkitovet@gmail.com

Abstract

Donkeys (*Equus asinus*) are rustic animals, but in Africa's poorest regions, they can present multiple health problems, including tick infestation. The study's objective was to determine the species composition of ticks that infest donkeys in Maputo Province (Mozambique). Ticks were collected in five conveniently selected southern districts of Maputo Province (Moamba, Matutuíne, Marracuene, Boane, and Matola) and were identified to species level using a stereoscopic microscope with the aid of dichotomous identification keys. In total, 500 ticks were collected from all 88 selected donkeys. Three genera of ticks were identified, namely *Rhipicephalus* (97.2%; 486/500), *Amblyomma* (2.2%; 11/500), and *Hyalomma* (0.6%; 3/500). Seven species were identified, of which *Rhipicephalus evertsi evertsi* with 50.4% (252/500) was the most prevalent, followed by *Rhipicephalus appendiculatus* (27.4%; 137/500), *Rhipicephalus turanicus* (11.6; 10/500), *Rhipicephalus* (*boophilus) microplus* (6.8; 20/500), *Amblyomma hebraeum* (2.2%; 11/500), *Rhipicephalus sanguineus* (1%; 5/500) and *Hyalomma truncatum* (0.6%; 3/500). *Rhipicephalus evertsi evertsi* occurred in all locations, whereas *Hyalomma truncatum* occurred only in the Boane district. Males were the most prevalent (67.2%; 336/500). The study revealed that donkeys in Maputo Province were infested with seven tick species of which *R. evertsi evertsi* was the main species.

Key words: *Rhipicephalus evertsi evertsi*, *Rhipicephalus turanicus*, *Rhipicephalus* (*boophilus) microplus*, *Amblyomma hebraeum*, *Rhipicephalus sanguineus*, *Hyalomma truncatum*, donkeys, Maputo province

Introduction

Donkeys (*Equus asinus*) are domestic animals distributed throughout Asia, Africa, and Latin America, where they are kept for work, transportation of people and goods, and ploughing the soil, especially in rural communities (Saul et al., 1997; Starkey et al., 2000; Ibrahim et al., 2011). Some determining factors for the increased use of donkeys in rural agriculture are their low purchase costs, fast work, easy training, and tolerance to drought and vector-borne diseases (Starkey, 2001; Porter, 2002; Starkey et al., 2002; Oudman, 2004). In general, donkeys are cheap and easier to keep compared to horses and mules. Despite the many tasks assigned to donkeys, their health and welfare are not always taken into account by their owners, which ends up impairing their usage and performance (Porter, 2002). The rusticity and welfare of donkeys are threatened by traumatic wounds leading to secondary infections due to overloading, poor harness and saddles, endoparasites, infectious diseases (rabies, anthrax), and tumours (Oudman, 2004; Getnet et al., 2014; Björkengren, 2016). One of the major problems afflicting donkeys are ticks, whose importance in tropical and subtropical regions, including Mozambique, is that they are vectors of agents causing babesiosis and theileriosis, not only in asinine but also in other domestic animals (Kumar et al., 2009; Tefera et al., 2011; Laus et al., 2015). Ixodid ticks also cause annoyance, uneasiness, pruritus, myiasis, and hide/skin damage (Jani et al., 1997). Of all ixodids infesting donkeys in developing countries of Africa, the most common belong to the genera *Rhipicephalus*, *Hyalomma*, and *Amblyomma* (Ferede et al., 2010; Halajian et al., 2018; Kyari et al., 2019). They cause economic losses resulting from the death not only of donkeys but also of other domestic animals. The treatment and control of hemoparasites and other microorganisms they transmit is quite expensive (Saul et al., 1997; Jongejan et al., 2004; Dantas-Torres, 2009).

According to the 2009-2010 Agri-livestock census (The National Statistics Institute, 2011), the donkey population was 18,337 animals in Mozambique. Maputo Province has 487 donkeys, whereas Maputo City has 28 donkeys. To date, and to our knowledge, no studies have been carried out in Mozambique to identify hard ticks infesting donkeys. An exhaustive study of ectoparasites in Mozambique was carried out by Horak et al. (2009) to determine the species of ticks infesting only cattle, goats and dogs in Maputo Province. Understanding

ixodid ticks infesting donkeys and the negative impact they cause, especially concerning the diseases they may transmit, is essential. Donkeys are not given due healthcare attention by the local veterinary practitioners, regarding community education programs addressing health management and donkeys' welfare. Nevertheless, in rural, poor, and deprived regions of Africa, agricultural, social and economic policies are already beginning to contemplate the donkeys' massive use for the advantages already mentioned (Jongejan & Uilenberg, 2004). Through the knowledge of ticks that infest donkeys in Mozambique, this study will contribute to developing better strategies for the control of ixodid ticks, including the treatment of the possible diseases they transmit.

Methods

Study area

The study was conducted in five districts (Moamba, Marracuene, Matutuíne, Boane, and Matola) of Maputo Province, southern Mozambique (Fig. 1) between April 2015 and July 2017. The districts were selected by convenience (Dohoo et al. 2003), taking into account information of the significant presence of donkeys in the regions (according to the District Services for Economic Activities and previous surveys conducted on donkeys in Maputo Province; Nabetse, 2015) and the possibility of logistical support during fieldwork.

Maputo Province is the southernmost of the provinces of Mozambique. It has an area of 22,693 km², and is divided into eight districts (Matutuíne, Boane, Manhiça, Matola, Namaacha, Magude, Moamba and Marracuene). It is bordered to the north by the Province of Gaza, to the east by the Indian Ocean, to the south by the South African province of KwaZulu-Natal, and to the west by Eswatini and the South African province of Mpumalanga (Government of Maputo Province, 2017). The vegetation along the coast is described as shrubland with patches of wetland, mangroves, and deciduous trees along riverbanks and dunes (De Matos, 2008; Horak et al., 2009). Inland it is dominated by woodland and savannah and mopane associations (*Colophospermum mopane*) in the north-west district of Magude (De Matos, 2008; Horak et al., 2009). In the south, inland vegetation is dominated by forest from the mountain district of Namaacha to the southern Mozambican border (De Matos, 2008; Horak et al., 2009). Annual summer and winter rainfall vary between 500 and 750 mm and between 125 and 250 mm, respectively. Midsummer mean daily temperatures vary between 25 and 27.5°C and midwinter temperatures between 15 and 20°C (De Matos, 2008; Horak et al., 2009).

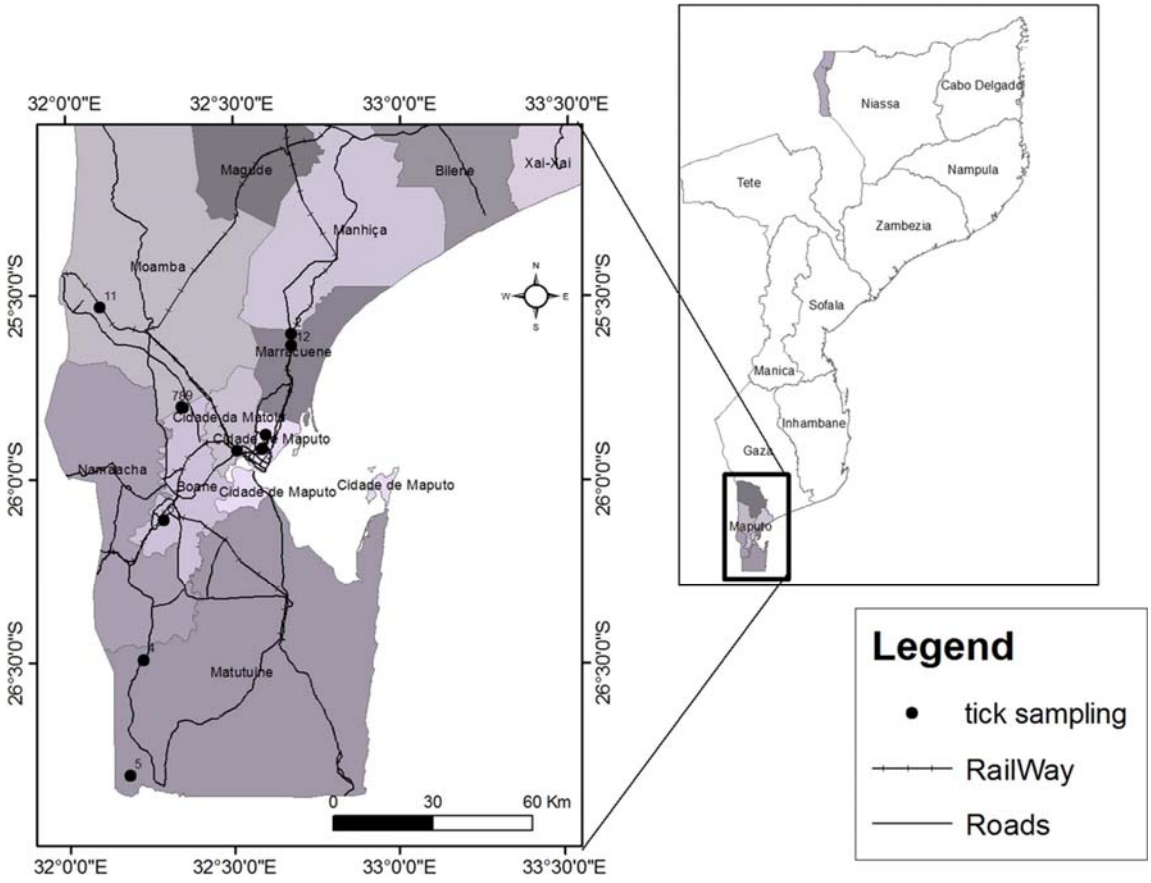

Figure 1. Maputo Province map showing the study area

Moamba district

It is located in the northern part of Maputo Province. The district has a dry steppe-type climate, with a vast area of plains. The average annual temperature of the district oscillates between 23 and 24ºC and has two seasons, one rainy season with annual rainfall between 580 and 590 mm, which runs from October to March and another cool and dry season from April to September (Ministério de Administração Estatal, 2005d; Fundo de Desenvolvimento Agrário, 2018).

Marracuene and Matutuíne districts

Marracuene district is located in the eastern part of Maputo Province and is crossed in the north-south direction along an extensive plain by the Incomati River, whereas Matutuíne is located in the extreme south. In both districts, the climate is tropical, characterized by high temperatures, with an annual average of over 20°C. Relative humidity varies between 55 and 75%, and rainfall is moderate, with an average of 500 mm inland and 1000 mm on the coast.

The rainy season is from October to April, with 60–80% of rainfall concentrated between December and February (Ministério de Administração Estatal, 2005b; Ministério de Administração Estatal, 2005c).

Boane district

It is located in the southeast of Maputo Province and is characterized by three distinct units of landforms: a coastal plain with sandy soils, medium plateaus (at 200–500 m elevation), and high erosive plateaus (500–1000 m) (Mubai, 2014). The climate is sub-humid (steppe) and cold. The annual average temperature is 23.7ºC, the coldest months are June and July and the hottest are January and February. The average annual relative humidity is 80.5%, and the average annual rainfall is 752 mm (Ministério de Administração Estatal, 2005a).

Matola district

It is located in the south of the country. The district's climate is the dry tropical type with two seasons, the rainy season between November and March and the dry season between April and October. The average annual temperature is 23.4ºC, the relative humidity is 77%, and an average annual rainfall of <500 mm (NEMUS África, 2017).

Study animals, design and sampling

In total, 88 donkeys (male and female) were selected of local breeds, managed extensively by local farmers. Details of the donkey population are given in Table 1.

Table 1 Details of the donkey population: sex and number (% in parentheses) per district of Maputo Province, Mozambique

	District					Total
	Matutuíne	Marracuene	Moamba	Boane	Matola	
Male	4 (19)	10 (58.8)	10 (33.3)	8 (47.1)	3 (100)	35 (39.8)
Female	17 (81)	7 (42.2)	20 (56.7)	9 (52.9)	0 (0)	53 (60.2)
Total	21	17	30	17	3	88

A cross-sectional study was conducted from April 2015 to July 2017 to assess the ocurrence of ticks in donkeys of the above-mentioned districts of Maputo Province preceded by oral consent from the owners. With the help of technicians from the District Economic Activities Services, a mobilisation campaign was conducted so that all those with donkeys would bring them to the pre-arranged areas. These concentration points included communal

grazing areas, grain milling points, acaricide tanks and goods loading stations. All donkeys found were selected (Thrusfield, 2007) followed by a visual inspection for the presence of ticks In order to include donkeys that for various reasons were not covered in the first visit, we made at least two visits to each study site (Table S1).

Tick collection and identification

After a thorough visual inspection, adult ticks were collected from all body regions of donkeys, taking into account the predilection sites, i.e, perineum, tail base, scrotum, abdomen and udder. All ticks were removed with the aid of a 'duckbill' dissection clamp or manually. Ticks from each animal were placed in plastic sample bottles containing 70% ethanol. The plastic containers were identified (date, place, and body region of the collection) for further identification.

A stereomicroscope (Zeiss, Stemi DV4-Germany-2000) was used to identify each tick to the genus and species level with the aid of a dichotomous key (Walker et al., 2003). Attention was paid to size, mouthparts, ad-anal plates, and the presence or absence of eyes. The developmental stage of ticks was determined based on the number of legs and the presence of genital aperture.

Data analysis

Data were exported and analysed using IBM-SPSS Statistics for Windows, v.18.0 (IBM, Armonk, NY, USA) to calculate frequencies, averages, and ratios according to genera, species, sex, body region, and geographical area of ticks' occurrence. The identification and validation of the results were carried out at the Directorate of Animal Sciences (DCA), Maputo.

Results

Genera and species

In total, 500 ticks were collected. The distribution of ticks by genus was as follows: *Rhipicephalus* 97.2% (486 specimens; 95% CI = 95.2–98.4%), *Amblyomma* 2.2% (11; 95% CI = 1.2–4.0%) and *Hyalomma* 0.6% (3; 95% CI = 0.16–1.9%).

The identified ixodid species are illustrated in Table 2. The most prevalent species were *Rhipicephalus evertsi evertsi* (50.4%; n = 252) and *R. appendiculatus* (27.4%; n = 137).

Table 2 Ticks collected from 88 donkeys in Maputo Province, Mozambique (total n = 500)

Species (no. specimens)	Sex		% prevalence [95% confidence interval]	No. donkeys infested	Mean infestation intensity (%)
	Male	Female			
Amblyomma hebraeum (11)	8	3	2.2 [1.16-4.02]	3	3.4
Hyalomma truncatum (3)	3	0	0.6 [0.16-1.90]	1	1.1
Rhipicephalus microplus (34)	0	34	6.8 [4.82-9.47]	20	22.7
R. appendiculatus (137)	112	25	27.4 [23.58-31.57]	50	56.8
R. evertsi evertsi (252)	188	64	50.4 [45.93-54.86]	88	100
R. sanguineus (5)	2	3	1 [0.37-2.46]	1	5.7
R. turanicus (58)	23	35	11.6 [8.99-14.81]	10	11.4

Distribution of ticks by sex, collection site and donkey body region

Table 3 illustrates the distribution of ticks by sex. Males were the most prevalent (67.2%; n = 336). All *H. truncatum* ticks were male, whereas all 34 *R. microplus* was female. Overall, the ratio of male to female was 2:1. *Rhipicephalus evertsi evertsi* occurred in all sites covered by this study (overall prevalence 50.4%), whereas *R. sanguineus* (1%) and *H. truncatum* (0.6%) were present only in Marracuene and in Boane districts, respectively (Tables 2 and 3).

The donkey body regions where the ticks were collected are indicated in Table 4. *Rhipicephalus evertsi evertsi* was found everywhere except on the ears, and *H. truncatum* was present only in the perianal region.

Table 3 Prevalence of ixodid ticks – percentage (number) [95% confidence interval] – on donkeys according to collection site

District	*A. hebraeum*	*H. truncatum*	*R. microplus*	*R. appendiculatus*	*R. evertsi evertsi*	*R. sanguineus*	*R. turanicus*
Boane	0 (0) [0]	2.4 (3) [0.63-7.50]	3.3 (4) [1.05-8.62]	0 (0) [0]	94.3 (116) [88.2-97.48]	0 (0) [0]	0 (0) [0]
Matutuíne	1.4 (3) [0.37-4.44]	0 (0) [0]	0 (0) [0]	60.7 (128) [53.7-67.23]	12.8 (27) [8.75-18.24]	0 (0) [0]	25.1 (53) [19.53-31.63]
Matola	10 (1) [0.52-45.89]	0 (0) [0]	0 (0) [0]	10 (1) [0.52-45.89]	80 (8) [44.22-96.46]	0 (0) [0]	0 (0) [0]
Marracuene	6 (7) [2.67-12.48]	0 (0) [0]	5.2 (6) [2.12-11.38]	6.9 (8) [3.24-13.56]	77.6 (90) [68.72-84.59]	4.3 (5) [1.60-10.26]	0 (0) [0]
Moamba	0 (0) [0]	0 (0) [0]	40 (24) [27.83-53.45]	0 (0) [0]	27.5 (11) [9.93-30.85]	0 (0) [0]	12.5 (5) [0]

Table 4 Distribution of ticks according to body region of the donkey: percentage (number) [95% confidence interval]

Body region	*A. hebraeum*	*H. truncatum*	*R. microplus*	*R. appendiculatus*	*R. evertsi evertsi*	*R. sanguineus*	*R. turanicus*
Ear	0 (0) [0]	0 (0) [0]	27 (24) [18.36-37.59]	33.7 (30) [24.24-44.6]	1.1 (1) [0.06-6.98]	5.6 (5) [2.09-13.22]	32.6 (29) [23.25-43.44]
Perineum	3.1 (7) [1.35-6.49]	1.3 (3) [0.34-4.11]	0 (0) [0]	43.9 (100) [37.36-50.57]	43 (98) [36.51-49.69]	0 (0) [0]	8.8 (20) [5.57-13.42]
Tail base	6.2 (4) [1.99-15.79]	0 (0) [0]	0 (0) [0]	0 (0) [0]	80 (52) [67.88-88.52]	0 (0) [0]	13.8 (9) [6.91-25.17]
Abdomen	0 (0) [0]	0 (0) [0]	21.3 (10) [11.2-36.07]	14.9 (7) [6.69-28.92]	63.8 (30) [48.48-76.94]	0 (0) [0]	0 (0) [0]
Scrotum	0 (0) [0]	0 (0) [0]	0 (0) [0]	0 (0) [0]	100 (40) [89.09-99.77]	0 (0) [0]	0 (0) [0]
Udder	0 (0) [0]	0 (0) [0]	0 (0) [0]	0 (0) [0]	100 (31) [86.27-99.71]	0 (0) [0]	0 (0) [0]

Discussion and conclusions

Most published studies on the morphological identification of ticks in Mozambique focused on cattle, small ruminants, and canines. To our knowledge, this is the first and most up-to-date study investigating hard ticks infesting donkeys in the country. Three genera of ixodid ticks were identified – *Amblyomma*, *Hyalomma* and *Rhipicephalus* –, the same genera were also reported on donkeys and horses in South Africa (Horak et al., 2017), denoting their importance in southern Africa. As observed in neighboring South Africa, there was greater infestation by males. After copulation, females usually fall to the ground to oviposit whereas males remain attached to the host for some time. For *R. microplus* no males were seen but this might be due to their small size. Normally males are always attached below the female, but if the female is removed first, the male cannot be found anymore.

Rhipicephalus species are among the most widespread ixodid ticks in Africa (Walker et al., 2003; Jongejan et al., 2018), and some of them, including *R. evertsi evertsi*, have horse, donkey, cattle and sheep as their preferred hosts (Walker et al., 2003). Therefore, it was foreseeable that this species would be present in large numbers in the donkey population in all the districts under study. De Matos (2008) reported its presence in the same districts, where the average annual rainfall is >250 mm, which is considered by Walker (1991) as an essential factor for its proliferation. Adults of *R. evertsi evertsi* have the perianal and inguinal region as their place of predilection, whereas the immature stages can be found in the deepest parts of the auricular pavilion and the external auricular (Walker et al., 2003; De Matos, 2008; Horak et al., 2017). However, in this study, besides in the perianal region, adults were also found on scrotum, abdomen, and udder, which shows that more attention should be paid to its 'new' pattern of predilection. *Rhipicephalus evertsi evertsi* transmits *Babesia caballi* and *Theileria equi*, the causative organisms of equine piroplasmosis (De Waal & Potgieter 1987; Norval & Horak 2004), and *Theileria separata*, the causative organism of ovine theileriosis (Jansen & Neitz 1956). It also transmits *Anaplasma marginale*, the causative organism of anaplasmosis in cattle (Potgieter 1981). Engorging *R. evertsi evertsi* females secrete a paralysis-inducing toxin that affects lambs born during spring in the Highveld regions of Mpumalanga, the eastern Free State and the north-eastern region of the Eastern Cape Province (Gothe 1981).

Despite the lack of studies in Mozambique portraying the main diseases transmitted by *R. evertsi evertsi* such as *B. caballi* and *T. equi* in donkeys and *A. marginale* in cattle, it should not be overlooked that such a situation is a reality, especially if we take into account that in South Africa, bordering the province of Maputo, there are reports of the occurrence of piroplasmosis in donkeys and cattle (Motloang et al., 2008; Mlangeni, 2016)

Rhipicephalus appendiculatus (brown ear tick) was the second most prevalent ixodid in the study, particularly in Matutuíne (n = 128; 60.7%), a district bordering Kwazulu-Natal (South Africa) and Eswatini, where this species was reported to occur (Walker, 1991; Walker et al., 2003; Horak et al., 2009). Hence, the same agro-ecological factors, including the collection season (rainy season), may have contributed to their distribution in Matutuíne. Jongejan and Uilenberg (2018) also pointed out the species occurrence in Mozambique's central region (Angónia and Chimoio), which shows that through animal movements, *R. appendiculatus* can invade other regions. Although donkeys are not the preferred hosts of *R. appendiculatus*, studies have identified their presence on donkeys in South Africa and Zimbabwe (Horak et al., 2009, 2017). The presence of domestic and wild ruminants (preferential hosts) in the study areas may have contributed to a spillover through cross infestation of asinines. Although they have a preference for ears, in donkeys they also occurred on perineum and abdomen – according to De Matos (2008), invasion of other body regions may occur in cases of massive infestations.

Rhipicephalus appendiculatus is a three-host tick, which is the vector of *Theileria parva* as well as buffalo-derived *T. parva*, the causative organisms of East Coast fever and Corridor disease, respectively, in cattle and buffalo (Lawrence et al., 2004; Norval & Horak 2004). The veterinary importance of this tick in Mozambique in general and in Maputo Province in particular, should not be underestimated.

Rhipicephalus turanicus was the third most abundant ixodid species. It is easily confused with *R. sanguineus*. De Matos (2008) and Horak et al. (2009) reported its presence in Magude, Boane, Namaacha and Matutuíne districts. The fact that it has been identified in the Moamba district may be related to the livestock movement (trade) from the places where this hard tick is most abundant, thereby contributing to a spillover. *Rhipicephalus turanicus* was present in the ears, perineum, and tail. However, its place of predilection is not yet clear. Nevertheless, Walker (1991) mentioned that ears could be massively infested in sheep and goats. From the study carried out by Horak et al. (2009), *R. turanicus* were mostly collected on dogs. Nonetheless, infested dogs may cohabit with donkeys, posing a risk to the latter. Infestation with this ectoparasite has been reported in horses in South Africa (Walker et al., 2003; Horak et al., 2017). There are few reports of donkey infestation in sub-Saharan Africa, especially in Mozambique, where it seems to be the first time it has been reported in asinines. Donkeys infested by *R. turanicus* were also reported in the districts of the Oromia region in central Ethiopia (Gizachew et al., 2013). *Rhipicephalus turanicus* is a vector suspected of transmitting *B. caballi* and *T. equi*, the causative agents of equine piroplasmosis (Scoles et al.,

2015; Jongejan & Uilenberg, 2018). Because *R. turanicus* has frequently been confused with *R. sanguineus*, reports of the transmission of equine piroplasmosis by *R. sanguineus* could have referred to *R. turanicus*, but transstadial transmission of *T. equi* and *B. caballi* from nymphs to adults with a South African strain of *R. turanicus* have been unsuccessful (Scoles & Ueti, 2015; Jongejan & Uilenberg, 2018).

No *R. decoloratus* were found in this study. The invasive *R. microplus* have replaced the indigenous *R. decoloratus* populations in Maputo province (De Matos, 2008; Horak et al., 2009), as has been reported in other geographical areas throughout West, East, and southern Africa (Tonnesen et al., 2004; Madder et al., 2011; Jongejan & Uilenberg, 2018). Climatic factors, reproductive capability, interspecific competition on the host, adaptation to the environment, different resistance patterns to acaricides, and a shorter life cycle of *R. microplus* than of *R. decoloratus* are possible explanations for the displacement (Madder et al., 2011).

Rhipicephalus microplus (blue tick) infests cattle (its chief host), although sheep, goats, and horses may also be targeted (Walker, 1991; Jongejan & Uilenberg, 2018). However, a study in Mpumalanga (South Africa) by Menks (2014), identified large numbers of adult *R. microplus* on donkeys, suggesting that donkeys can be an alternative host for this tick. The fact that horses and donkeys share the same grazing areas as cattle can also result in an accidental infestation of the latter. Furthermore, Ferede et al. (2010), in a study in Ethiopia, only mentioned the genus *Rhipicephalus* (*Boophilus*) infesting donkeys without specifying the main species involved. This species was present in the same geographical regions covered by De Matos (2008) and Horak et al.'s (2009) studies on the occurrence of ticks in cattle, small ruminants, and canines. *Rhipicephalus microplus* is a vector of *Babesia bovis*, *B. bigemina*, *A. marginale*, and *Borrelia theileri*, agents responsible for Asian redwater, African redwater (babesiosis) in cattle, as well as anaplasmosis and borreliosis, respectively (Barbour et al., 1986; De Vos et al., 2004; Rikhotso et al., 2005).

Amblyomma hebraeum was the fifth-lowest occurring species on donkeys in Maputo province. Binta et al. (2003), in a study conducted in Botswana, also observed a low occurrence of this species in donkeys. The low infestation observed in this study is probably because cattle are the primary hosts of *A. hebraeum* (Walker, 1991; Walker et al., 2003). The adults of *A. hebraeum* prefer large herbivores as hosts, whereas its immature stages and particularly the larvae infest the same hosts as the adults as well as smaller mammals, birds and tortoises (Horak et al. 1987; Horak, Golezardy & Uys 2007). The comparison of numerical fluctuations related to its occurrence among several studies (Horak et al., 2017) is

mainly due to differences in sample sizes. De Matos (2008) and Horak et al. (2009) also reported *A. hebraeum* in the same districts covered by this present study. The same authors also reported the perineal region, udder, genitalia and armpits as sites where *A. hebraeum* occurred. The tick is recognised as a vector of *Ehrlichia ruminantium*, the causative organism of heartwater or cowdriosis in domestic and wild ruminants in Southern Africa (Norval & Horak, 2004; Jongejan & Uilenberg, 2018).

Although *R. sanguineus* has dogs as its primary host almost exclusively, it can also infest other species, including cattle (Walker, 1991; Walker et al., 2003). Despite reports of donkey infestation occurring in other countries such as Sudan (Mohamed et al., 1990) and Ethiopia (Ferede et al., 2010), in Mozambique this is the first time that donkey infestation has been described. Possibly this occurrence was due to spillover from infested dogs. *Rhipicephalus sanguineus* occurred only in a single district (Marracuene), which is close to that of Manhiça, a region where this species was reported by De Matos (2008) and Horak et al. (2009). This tick is a vector suspected of *B. caballi* and *T. equi* transmission, causative agent of equine piroplasmosis (Scoles & Ueti, 2015).

Unlike the study conducted by Horak et al. (2017) where *Hyalomma truncantum* was the most abundant species (13.2%) on donkeys in South Africa, in this present study it was the least frequent. Horak et al. (2017) carried out tick collections over a longer period than we did, and in regions where they are abundant. Adults may feed on ruminants and other domestic animals, including donkeys (Walker, 1991; Hawkins et al., 2015). Some micro-ecological factors may have influenced their low occurrence and presence in only one district (Boane) as it is predominant in Libombos mountain range regions (De Matos, 2008; Horak et al., 2009). *Hyalomma truncatum* is the vector responsible for the transmission of *B. caballi* in horses, donkeys and mules (Rothschild, 2013; Scoles & Ueti, 2015).

Study limitations and recommendations

The constant mobility of rural households and their animals to other areas in search of livelihoods and better pastures and farming conditions limited the collection of more samples, especially when a second visit was planned to collect ticks from donkeys that escaped on the first visit. Lack of funds for molecular identification also limited the validation of morphologically identified species. Hence, there is a need for further studies on the occurrence of ixodid ticks in donkeys, including the related tick-borne diseases. A particular focus should be given to the most abundant species, *R. evertsi evertsi*.

Acknowledgements

We are grateful to the donkey owners for agreeing to participate in the interviews and also for allowing us to collect ticks from their animals. We appreciate the help and participation of staff of the District Services of Economic Activities (Dr Leonilde Chilundo and Dr Elvino Nabetse) and also of the Veterinary Faculty of Eduardo Mondlane University (Dr Alberto Dimande, Dr Narcisa Nhamitambo, Mónica De Rugeriis and Mr. Armando Inácio) during the fieldwork. A special thanks to Dr Abel Chilundo and Dr Jimis Deve for helping with the mapping of tick collection sites and the staff of the Laboratory of Parasitology of the Central Veterinary Laboratory of the Directorate of Animal Sciences in Maputo, for collaborating in the identification of ticks.

Declarations

Competing interests

The authors declare that they had no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

All authors discussed the results and commented on the manuscript. Caroline Esuludis: identified the ticks, compiled the gathered information and prepared the manuscript. Carlos De Matos and Cristina Aida Cala: were equally the study co-leaders, identified the ticks, wrote the manuscript. Marvelous Sungirai performed data analysis and contributed to the writing and revision of the manuscript. Maxime Madder: conceived the original idea and contributed to the writing and revision of the manuscript. Milton Mapatse: was the study leader, was in charge of overall direction and planning, collected the ticks and wrote the manuscript.

Funding information

This study received no financial support for the research, authorship, and/or publication of this article.

Disclaimer

The views expressed in this manuscript represent our own, not an official position of the institution.

Ethical considerations

The study was approved through the protocol entitled: Morphological identification of hard ticks (Acari: *Ixodidae*) infesting donkeys (*Equus asinus*) in Maputo Province, Mozambique, approved by the Veterinary Faculty (University Eduardo Mondlane) scientific council

(reference 18.04.2018).

References

Barbour, A. G. & Hayes, S. F. (1986). Biology of Borrelia species. *Microbiological reviews, 50,* 381.

Binta, M. G., Chabo, R. G., Monnafela, L., & Mushi, E. Z. (2003). Seasonal fluctuation of parasitic infestation in donkeys (Equus asinus) in Oodi village, Kgatleng District, Botswana. *Journal of the South African Veterinary Association, 74,* 24-26.

Björkengren, J. (2016). *Assessment of donkeys' welfare in Addis Ababa and Ambo, Ethiopia.* MSc Thesis, SLU, Dept. of Animal Environment and Health.

Dantas-Torres, F. (2009). Ticks on domestic animals in Pernambuco, Northeastern Brazil. *Rev.Bras.Parasitol.Vet, 18,* 22-28.

De Matos, C. A. (2008). *Species composition and geographic distribution of ticks infesting cattle, goats and dogs in Maputo Province, Mozambique.* MSc. dissertation, University of Pretoria.

De Waal, D.T. & Potgieter, F.T. (1987) The transstadial transmission of *Babesia caballi* by *Rhipicephalus evertsi evertsi*. *Onderstepoort Journal of Veterinary Research* 54: 655–656.

De Vos, A. J., De Waal, D. T., & Jackson, L. A. (2004). Bovine babesiosis. *Infectious diseases of livestock, Volume One,* 406-424.

Dohoo, I.R., Martin, W., & Stryhn, H.E. 2003. *Veterinary epidemiologic research* Charlottetown, AVC Inc.

Ferede, B., Kumsa, B., Bsrat, A., & Kalayou, S. (2010). Ticks of donkeys in central Oromia regional state, Ethiopia. *Revue Méd Vét, 161,* 121-126.

Fundo de Desenvolvimento Agrário (2018). *Projecto de reabilitação do bloco I do regadio da Moamba, Província de Maputo* (Rep. No. Volume I). Impacto: Projectos e estudos Ambientais.

Getnet, F., Feyera, T., Alemu, F., Abera, T., & Niguse, A. (2014). Injuries in Donkeys and Mules: Causes, Welfare Problems and Management Practices in Amhara Region, Northern Ethiopia. *American-Eurasian Journal of Scientific Research, 9,* 98-104.

Gizachew, A., Schuster, R. K., Joseph, S., Wernery, R., Georgy, N. A., Elizabeth, S. K. et al. (2013). Piroplasmosis in DonkeysGÇöA Hematological and Serological Study in Central Ethiopia. *Journal of Equine Veterinary Science, 33,* 18-21.

Gothe, R., 1981, 'The toxic phase of paralysis inducing female *Rhipicephalus evertsi evertsi*

Neuman, 1897, during repletion and aspects on the patho-mechanism of this toxicosis', in G.B.

Whitehead & J.E. Gibson (eds.), *Tick biology and control.Proceedings of an international conference*, Rhodes University, Grahamstown,South Africa, January 27–29, 1981, p. 13.

Government of Maputo Province (2017). Geografia. *https://www.pmaputo.gov.mz/por/A-Provincia/Geografia2*.

Halajian, A., Palomar, A. M., Portillo, A., Heyne, H., Romero, L., & Oteo, J. A. (2018). Detection of zoonotic agents and a new Rickettsia strain in ticks from donkeys from South Africa: Implications for travel medicine. *Travel Medicine and Infectious Disease, 26,* 43-50.

Hawkins, E., Kock, R., McKeever, D., Gakuya, F., Musyoki, C., Chege, S. M. et al. (2015). Prevalence of Theileria equi and Babesia caballi as well as the identification of associated ticks in sympatric Grevy's zebras (Equus grevyi) and donkeys (Equus africanus asinus) in northern Kenya. *Journal of wildlife diseases, 51,* 137-147.

Horak, I. G., MacIvor, K. M., Petney, T. N. & De Vos, V. (1987). Some avian and mammalian hosts of Amblyomma hebraeum and Amblyomma marmoreum (Acari: Ixodidae). *Onderstepoort J Vet Res., 54,* 397-403.

Horak, I. G., Golezardy, H., & Uys, A. C. (2007). Ticks associated with the three largest wild ruminant species in southern Africa. *Onderstepoort Journal of Veterinary Research, 74,* 231-242.

Horak, I. G., Nyangiwe, N., De Mattos, C. & Neves, L. (2009). Species composition and geographic distribution of ticks infesting cattle, goats and dogs in a temperate and in a subtropical region of southeast Africa. *Onderstepoort J.Vet.Res., 76,* 263-276.

Horak, I. G., Heyne, H., Halajian, A., Booysen, S., & Smit, W. J. (2017). Parasites of domestic and wild animals in South Africa. L. Ixodid ticks infesting horses and donkeys. *Onderstepoort Journal of Veterinary Research, 84,* 1-6.

Ibrahim, N., Berhanu, T., Deressa, B., & Tolosa, T. (2011). Survey of prevalence of helminth parasites of donkeys in and around Hawassa town, Southern Ethiopia. *Global veterinaria, 6,* 223-227.

Jansen, B.C. & Neitz, W.O., 1956, 'The experimental transmission of *Theileria ovis* by *Rhipicephalus evertsi*', *Onderstepoort Journal of Veterinary Research* 27, 3–6.

Jani, G. R., Raval, K. S., & Patel, R. P. (1997). Ethnomedicine for ectoparasites in buffaloes: a clinical study on mange dermatitis. In (pp. 46-53).

Jongejan, F. & Uilenberg, G. (2004). The global importance of ticks. *Parasitology, 129 Suppl,* S3-14.

Jongejan, F. & Uilenberg, G. (2018). Vectors: Ticks. *Infectious Diseases of Livestock.*

Kumar, S., Kumar, R., & Sugimoto, C. (2009). A perspective on Theileria equi infections in donkeys. *Japanese Journal of Veterinary Research, 56,* 171-180.

Kyari, F., Benisheikh, A. A. G., Ngoshe, I. Y., Kayeri, B., Dawa, R., Awana, A. U. et al. (2019). Prevalence of tick species infesting donkeys in Borno state, Nigeria.

Laus, F., Spaterna, A., Faillace, V., Veronesi, F., Ravagnan, S., Berib+®, F. et al. (2015). Clinical investigation on Theileria equi and Babesia caballi infections in Italian donkeys. *BMC veterinary research, 11,* 100.

Lawrence, J. A., Perry, B. D., & Williamson, S. M. (2004). East Coast fever. *Infectious diseases of livestock, Volume One,* 448-467.

Madder, M., Thys, E., Achi, L., Toure, A., & De, D. R. (2011). Rhipicephalus (Boophilus) microplus: a most successful invasive tick species in West-Africa. *Exp.Appl.Acarol., 53,* 139-145.

Menks, K. C. (2014). Species composition of ticks on donkeys and their possible role in transmission of diseases in the Mnisi Area (Mpumalanga Province), South Africa. Master's thesis. Universiteit Utrecht. Available at: http://dspace.library.uu.nl:8080/handle/1874/292177

Ministério de Administração Estatal (2005a). Perfil do Distrito de Boane. Available at: *http://www.portaldogoverno.gov.mz/por/content/download/2965/23877/version/%E2%80%A6/Boane.pdf,* 1-54. Accessed March 03, 2019.

Ministério de Administração Estatal (2005b). Perfil do Distrito de Marracuene. Available at: *http://www.portaldogoverno.gov.mz/por/content/download/2966/23882/version/1/file/Marracuene.pdf,* 1-51. Accessed March 03, 2019.

Ministério de Administração Estatal (2005c). Perfil do Distrito de Matutuíne. Available at: *http://portaldogoverno.gov.mz/por/content/download/2964/23872/%E2%80%A6/Matutuine.pdf,* 1-59. Accessed March 03, 2019.

Ministério de Administração Estatal (2005d). Perfil do Distrito de Moamba. Available at: *https://www.portaldogoverno.gov.mz/por/content/download/2962/23862/%E2%80%A6/1/%E2%80%A6/Moamba.pdf,* 1-66.Accessed March 03, 2019.

Mohamed, A. A. & Yagoub, I. A. (1990). Outbreaks of babesiosis in domestic livestock in the eastern region of the Sudan. *Tropical animal health and production, 22,* 123-125.

Mubai, B. A. (2014). Os serviços de extensão agrária pública ao pequeno agricultor familiar

do distrito de Boane-Moçambique. Master's Thesis. Universidade Estadual de Maringá. Available at: http://repositorio.uem.br:8080/jspui/handle/1/2840. Accessed April 06, 2019.

Nabetse, E. (2015). *Evaluation of the haematological profile of donkeys (Equus asinus) in Maputo City and Province.* Hounours thesis, University Eduardo Mondlane, Maputo.

NEMUS África (2017). *Avaliação do Impacto Ambiental do Projecto de Expansão da Capacidade do Terminal Graneleiro da Matola/STEMA* (Rep. No. Parte 2 - Relatório principal). Mozambique. Available at: http://www.nemus.pt/wp-content/uploads/2017/12/t16071_Parte2_REIA_STEMA_Matola_vfinal.pdf Accessed April 03, 2019.

Norval, R. A. I. & Horak, I. G. (2004). Vectors: ticks. *Infectious Diseases of Livestock, 1,* 3-42.

Oudman, L. (2004). *Donkeys for traction and tillage*. (2nd ed.) Wageningen: Agromisa/CTA.

Potgieter, F.T. (1981) Tick transmission of anaplasmosis in South Africa. In G.B. Whitehead & J.E. Gibson (eds.), *Tick biology and control. Proceedings of an international conference*, Rhodes University, Grahamstown, South Africa, January 27–29, 1981, pp. 53–56.

Porter, G. (2002). Intermediate means of transport: a review paper with special reference to Ghana. Available at: https://dro.dur.ac.uk/14145/1/14145.pdf?DDD5+dan1rep. Accessed October 07, 2020.

Rikhotso, B. O., Stoltsz, W. H., Bryson, N. R., & Sommerville, J. E. M. (2005). The impact of 2 dipping systems on endemic stability to bovine babesiosis and anaplasmosis in cattle in 4 communally grazed areas in Limpopo Province, South Africa. *Journal of the South African Veterinary Association, 76,* 217-223.

Rothschild, C. M. (2013). Equine piroplasmosis. *Journal of Equine Veterinary Science, 33,* 497-508.

Saul, C., Siefert, L., & Opuda-Asibo, J. (1997). Disease and health problems of donkeys: a case study from eastern Uganda. *International Animal Traction Network for Eastern and Southern Africa (ATNESA) workshop, Conference proceedings,* 5-9. Available at: https://www.atnesa.org/donkeys/Donkeys2-Saul.pdf. Accessed: October 07, 2020.

Scoles, G. A. & Ueti, M. W. (2015). Vector ecology of equine piroplasmosis. *Annual review of entomology, 60,* 561-580.

Starkey, P. (2001). Local transport solutions-people, paradoxes and progress: lessons arising from the spread of intermediate means of transport. *Sub-Saharan Africa Transport*

Policy Program working paper series (SSATP), 56. Available at: https://openknowledge.worldbank.org/handle/10986/17694 Accessed December 30, 2018.

Starkey, P., Ellis, S., Hine, J., & Ternell, A. (2002). *Improving rural mobility: options for developing motorized and nonmotorized transport in rural areas*. The World Bank Technical Paper, nº 525, Washington DC. Available at: https://openknowledge.worldbank.org/handle/10986/15230 Accessed December 30, 2018.

Starkey, P. & Starkey, M. (2000). Regional and world trends in donkey populations. *The Animal Traction Network for Eastern and Southern Africa (ATNESA).,* 1-21.

Tefera, M., Worku, A., Tolosa, T., & Bitew, M. (2011). Prevalence and risk factors for donkey babesiosis in and around Debre Zeit, Central Ethiopia. *Vet.Res, 4,* 56-60.

The National Statistics Institute (2011). The Census of Agriculture and Livestock (2009-2010) - Definitive results: Mozambique. *The National Statistics Institute*.

Thrusfield, M. (2007). *Veterinary Epidemiology*. (3 ed.) Oxford, UK: Blackwell Publishing company.

Tonnesen, M. H., Penzhorn, B. L., Bryson, N. R., Stoltsz, W. H., & Masibigiri, T. (2004). Displacement of Boophilus decoloratus by *Boophilus microplus* in the Soutpansberg region, Limpopo Province, South Africa. *Exp Appl Acarol, 32,* 199-208.

Walker, A. R., Bouattour, A., Camicas, J. L., Estrada-Pena, A., Horak, I. G., Latif, A. A. et al. (2003). *Ticks of domestic animals in Africa: a guide to identification of species*. Bioscience reports Edinburgh.

Walker, J. B. (1991). A review of the ixodid ticks (Acari, Ixodidae) occurring in southern Africa. *The Onderstepoort journal of veterinary research, 58,* 81.

Supplementary information

Table S1 Information regarding study area and number of donkeys screened

District	Administrative post	Village	Latitude	Longitude	Date of Collection	No. donkeys screened
Marracuene	Nhongonhana	-	-25.637734	32.67402	16/04/2015	7
		-	-25.880614	32.59601	17/04/2015	3
	Bobole	-	-25.607194	32.67186	17/04/2015	3
	3 Bairro	Missawize	-25.91939	32.58326	16/04/2015	12
Matutuine	Catuane	Mahau	-26.491965	32.22403	28/04/2015	8
		Ndlala	-26.805426	32.18221	28/04/2015	16
Boane	Boane	Munguene	-26.110981	32.28664	22/04/2015	17
	Sonte	-	-25806617	32348500	17/02/2016	
Moamba	Mulotane	Tetene	-25.806617	32.3485	17/02/2016	5
	Ressano Garcia	Chanculo	-25.533333	32.1	13/07/2017	7
	Sonte	-	-25.805145	32.34325	01/03/2016	1
Matola	Machava	Trevo	-5.92312691	32.5084	18/02/2016	2
		Trevo	-5.92312691	32.5084	18/02/2016	7