In search of the *Rhipicephalus* (*Boophilus*) *microplus* in the western-central regions of the Eastern Cape Province, South Africa

M. Yawa[a], N. Nyangiwe[b,*], C.T. Kadzere[b], V. Muchenje[a], T.C Mpendulo[a], M.C. Marufu[c]

[a] Department of Livestock and Pasture Science, University of Fort Hare, P. Bag X1314, Alice 5700, South Africa

[b]Döhne Agricultural Development Institute, Private Bag X15, Stutterheim 4930, South Africa

[c]Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort 0110, South Africa

*corresponding author: email: nyangiwe1@gmail.com; telephone: +27 43 683 5424; fax: +27 436832890

Abstract

The southern cattle tick, *Rhipicephalus (Boophilus) microplus*, is an economically important tick that parasitises cattle and is found on other host species if they graze with cattle. The *R.* (*B.*) *microplus* is a highly adapted tick species prevalent in tropical and subtropical regions of the world. In Africa, it has expanded its range and was reported to supersede the native tick, *R.* (*B.*) *decoloratus.* The objective of the study was to determine the distribution of *R.* (*B.*) *microplus* under different ecological zones in the western-central regions of Eastern Cape Province. Engorged adult blue ticks were collected monthly from 360 randomly selected cattle and free living ticks from six replicate drags of the vegetation over a period of 1 year at Bedford Dry Grassland (BDG), Kowie Thicket (KT) and Bhisho Thornveld (BT). A special attention was paid to the lower perineum, neck, dewlap and ventral body parts which are the preferred sites for blue ticks during sampling. In this study, 9 species of ticks which grouped under 5 genera were identified. The identified species of ticks were *Amblyomma hebraeum*, *Haemaphysalis elliptica*, *Hyalomma rufipes*, *Ixodes pilosus*, *R.* (*B.*) *decoloratus*, *R. appendiculatus*, *R. evertsi evertsi*, *R. follis* and *R. simus.* Only adult *R.* (*B.*) *decoloratus* (n = 8090) ticks were collected from cattle between April 2016 and March 2017. A total of 4382 females and 3708 males of *R.* (*B.*) *decoloratus* were recovered during the survey. Of the ticks (n = 2885) collected from the vegetation, *R.* (*B.*) *decoloratus* was the most abundant species with a relative prevalence of 58.16%, followed by *R. appendiculatus* (18.37%) and *R. evertsi*

evertsi (16.90%). Least abundant ticks were *H. rufipes* (2.98%), *A. hebraeum* (2.46%), *H. elliptica* (0.38%), *R. follis* (0.34%), *I. pilosus* (0.24%) and *R. simus* (0.17%). The distribution of *R.* (*B.*) *decoloratus* ticks differ significantly (*P*<0.05) among the vegetation types. Significantly more (*P*<0.05) engorged *R.* (*B.*) *decoloratus* were collected in KT during summer season (1.39±0.063 females and 1.30±0.063 males) compared to other vegetation types. The *R.* (*B.*) *decoloratus* larvae were significantly higher (*P*<0.05) in BT (20.56±1.154) and KT (18.50±1.154) vegetation types during the spring season. *R.* (*B.*) *microplus* was not found in the present study, signifying that it is not yet established in western-central regions of the Eastern Cape Province and as such, continuous monitoring would be advisable.

Key words: Cattle, Eastern Cape Province, *Rhipicephalus* (*Boophilus*) *decoloratus*, *Rhipicephalus* (*Boophilus*) *microplus*, season

Introduction

Ticks belonging to the *Rhipicephalus* (*Boophilus*) spp. are one–host ticks which take about three weeks to complete the parasitic portion of their life cycles on their hosts from unfed larvae to engorged female. Two *R.* (*Boophilus*) species are present in South Africa, namely the endemic *R.* (*B.*) *decoloratus*, and the Asiatic intruder, *R.* (*B.*) *microplus* which was introduced into South Africa with cattle imported from Madagascar after the rinderpest epidemic in 1896 (Nyangiwe et al., 2013; Theiler, 1962; Tønnesen et al., 2004). However, with the introduction of the invasive *R.* (*B.*) *microplus* in the African continent and changes in climate over recent years, there are indications that *R.* (*B.*) *decoloratus* has been subject to competition (Horak et al., 2009; Horak et al., 2015; Nyangiwe et al., 2013; Nyangiwe et al., 2017; Olwoch et al., 2007; Tønnesen et al., 2004). Whereas *R.* (*B.*) *decoloratus* can only transmit *Babesia bigemina*, the causative organism of African redwater, *R.* (*B*) *microplus* is a competent vector of both *Babesia bovis*, the causative organism of the more virulent Asiatic redwater, and of *B. bigemina* (Madder et al., 2007). Likewise, both *R.* (*B.*) *decoloratus* and *R.* (*B.*) *microplus* can transmit *Anaplasma marginale*, the causative organism of anaplasmosis or gall sickness in cattle (Horak et al., 2015). These diseases are responsible for considerable losses in susceptible cattle, and represent a hindrance to livestock farming in tropical and subtropical countries (Madder et al., 2007). Recently, the alarming spread of *R.* (*B.*) *microplus* was reported in West Africa (Adakal et al., 2013; De Clercq et al., 2012; Madder et al., 2007) while the displacement of native tick, *R.* (*B.*) *decoloratus* by *R.* (*B.*) *microplus* was observed in South Africa (Horak et al., 2009; Nyangiwe et al., 2013; Tønnesen et al., 2004). With the range expansion of *R.* (*B.*)

microplus in South Africa, the study seeks to determine the extent of *R.* (*B.*) *microplus* under different ecological zones in the western-central regions of Eastern Cape Province of South Africa.

Materials and methods

Description of study sites and experimental animals

Tick collection was conducted at three agro-ecological zones in 1) Adelaide Research Station representing Bedford Dry Grassland (BDG), 2) Honeydale Research Farm representing Bhisho Thornveld (BT) and Bathurst Research Station representing Kowie Thicket (KT) in the Eastern Cape Province.

Adelaide Research Station is situated at 26°18′E longitude and 32°42′S latitude, and is at 740 m above the sea level. The vegetation is classified as BDG and characterized by medium height grasslands that are interspersed by *Acacia karroo* woodlands. The area contains dwarf shrubby component of karroid origin in South Western parts. The annual minimum rainfall is 350 mm with a maximum of 550 mm. Temperature varies among seasons, and range from 29–32°C to 4–6 °C. The vegetation of BDG is characterised by graminoids such as *Aristida congesta, Cynodon dactylon, Digitaria eriantha, Eragrostis curvula, Heteropogon contortus, Panicum maximum, Sporobolus fimbriatus* and *Themeda trianda*. Succulent herbs, low shrubs and small trees like *Acacia karoo* are also occuring (Mucina et al., 2006).

Honeydale Research Farm is situated in one of the Eastern Cape Universities, Fort Hare, in Alice. It is located at 32°8′E longitude and 26°85′S latitude, with an altitude of 500 m above sea level, and has average annual rainfall of 480 mm and the maximum and minimum temperatures ranges between 18–37°C to 3–13°C. In BT, the vegetation is dominated by trees such *Maytenus polyacantha, Scutia myrtina,* and *Acacia karroo.* The most common grass species are *C. dactylon, D. eriantha, E. plana, H. contortus, Hyparrhenia hirta, S. africanus* and *T. triandra* (Mucina et al., 2006).

Bathurst Research Station is located at 33°30′S latitude and 26°49′E longitude, lies at 708 m above sea level. Its annual rainfall is 624 mm, while the temperature ranges between 13–29°C and 1–12 °C. The vegetation of the area is KT which is characterized by tall-grown thickets. It is dominated by succulent trees especially *Euphorbia* and *Aloe* species. There are also small trees such as *A. karoo, Schotia afra, Schotia latifolia* and tall shrubs like *Coddia rudis, Ehretia rigida, Grewia occidentalis* and *Scutia myrtina* (Mucina et al., 2006).

The study animals were randomly selected and were all older than 12 months and included both sexes, and the sampling was done before cattle were dipped.

Tick collection and identification

A total of 360 cattle were used to collect ticks from all different agro-ecological zones. At each locality, 10 animals were randomly selected monthly and ticks were collected while the cattle are restrained in a crush pen. Both engorged and free-living ticks were collected monthly across different seasons from April 2016 till March 2017. Ticks were collected mainly from one side of the animal and attention was paid to the predilection sites of blue ticks and the ears, neck and dewlap, abdomen, feet, tail and perianal region of each animal were carefully examined. As the survey was aimed at determining the extent of *R.* (*B.*) *microplus* ticks and not their intensity of infestation, none of the collections that were made from cattle were intended to be complete. At all sampling sites, adult ticks were collected from cattle while drag-sampling which favours the collection of questing ixodid larvae was used to collect ticks from the vegetation. Adult ticks were identified at the University of Fort Hare (Animal Science Laboratory) and non-parasitic ticks were transported to Döhne Agricultural Development Institute in Stutterheim, Eastern Cape Province for subsequent identification by an experienced acarologist. Ticks were identified at genus and species level using a standard stereomicroscope. A manual guide of tick identification by Walker et al. (2003) was used to identify adult tick species. The larvae of multi-host species were identified using the descriptions by Arthur (1973, 1975) and Walker et al. (2000) whereas the larvae of *R.* (*B.*) *decoloratus* were identified using the descriptions of Gothe (1967). Moreover, for larval identification, reference specimens from the University of Pretoria tick museum were used (thanks to Prof Horak) and an experienced acarologist identified the immature ticks from drags (Nyangiwe et al., 2011, 2013).

The drag sampling method is described in detail in Nyangiwe et al. (2013). But at each site, six replicate drags of 100 m, approximately 50 m apart, were performed at monthly intervals. After each drag, all ticks on the flannel strips were removed using forceps and stored in 70% ethanol in a labelled glass vials for later identification and counting. Drags were not done over dew-laden grass early in the morning or over grass after rain, as this wet the flannel strips and decreased their efficacy. Therefore, drag sampling was done during sunny days between 10h00 and 16h00 to avoid poor tick collections.

Data analysis

The data for tick counts was transformed using the formula y = log 10 (x+1) to check for normality and stabilize the variances before applying ANOVA. The data for questing and engorged ticks was analysed using Statistical Analysis System version 9.1 (SAS 2003). Interaction between vegetation types and season on tick counts was determined using the generalised linear model procedures for repeated measures of SAS (2003). Statistical significance was tested at 95% level with all results with $P<0.05$ considered to be statistically significant. Mean separation of the least square means were performed using PDIFF procedure. The prevalence for tick species and frequencies were determined using PROC FREQ of SAS (2003).

Results

Of the total 10975 ticks collected, 8090 ticks were collected from cattle while 2885 were free-living ticks from the vegetation. The ticks collected belong to 5 different genera, namely *Ambylomma, Haemaphysalis, Hyalomma, Ixodes* and *Rhipicephalus* including the subgenus *Boophilus* (Table 1). From the vegetation, *R.* (*B.*) *decoloratus* was the most abundant species with a relative prevalence of 58.16%, followed by *R. appendiculatus* (18.37%) and *R. evertsi evertsi* (16.90%). Least abundant ticks were *Hyalomma rufipes* (2.98%), *Amblyomma hebraeum* (2.46%), *Haemaphysalis elliptica* (0.38%), *R. follis* (0.34%), *Ixodis pilosus* (0.24%) and *R. simus* (0.17%) (Table 1). Apart from larvae and adult ticks collected, 338 nymphs of *R. appendiculatus* were collected from the vegetation.

The study focused on *R.* (*Boophilus*) spp. and only *R.* (*B.*) *decoloratus* was found during the 12- month study period. No *R.* (*B.*) *microplus* was found in the study area (Table 1 and Table 2). There was high count (20.56±1.154) of *R.* (*B.*) *decoloratus* larvae on vegetation during the spring season than summer in BT followed by KT agro-ecological zone (18.50±1.154) (Table 3). Significantly more ($P<0.05$) engorged *R.* (*B.*) *decoloratus* were collected from cattle in KT during the summer season (1.39±0.063 females and 1.30±0.063 males) compared to other agro-ecological zones. However, the distribution of *R.* (*B.*) *decoloratus* ticks differ significantly ($P<0.05$) among the agro-ecological zones (Table 3).

Discussion

In the present study, the identified species of ticks were *A. hebraeum, H. elliptica, H. rufipes, I. pilosus, R.* (*B.*) *decoloratus, R. appendiculatus, R. evertsi evertsi, R. follis* and *R. simus.* The *R.* (*B.*) *decoloratus* was the most abundant species (58.16%) in the study area. Similar results had been reported in Free State Province, where the indigenous tick was more abundant (53.19%), and this was also observed in Limpopo Province of South Africa (Hlatshwayo et al., 2001; Schroder and Reilly, 2013). These findings are also in agreement with Horak et al. (2009), Katiyatiya et al. (2014), Marufu et al. (2011) and Muchenje et al. (2008) who found *R.* (*B*) *decolaratus* from cattle as the commonest and most wide spread ticks in savanna regions of the Eastern Cape Province. Adult *R.* (*B.*) *decoloratus* were largely observed in KT during the summer season compared to BDG and BT vegetation. Therefore, this study, confirms that seasonal variation has an influence on ticks distribution. In addition, Marufu et al. (2013) found similar results of engorged *R.* (*B.*) *decoloratus* from open and tall grassland regions in the Eastern Cape Province during warm seasons. The present findings are also in line with Wolde and Mohamed (2014) who found the highest prevalence of *R.* (*B*) *decolaratus* in most areas of Ethiopia. Similar findings were also observed in Cameroon, where *R.* (*B.*) *decoloratus* was the most abundant species (62.2%) collected monthly from 60 animals during a 12 month study period (Awa et al., 2015).

The current study further reports high count (20.56±1.154) of *R.* (*B.*) *decoloratus* larvae during the spring season than summer in BT followed by KT region (18.50±1.154). For one host-ticks, such as *R.* (*B.*) *decoloratus,* low winter temperatures synchronise egg development and hatching, so that large numbers of larvae are present on the vegetation during spring season (Norval and Horak, 2004). The development of the free-living stages is temperature dependent and the threshold for development is approximately 10 °C (Horak et al., 2011; Spickett and Heyne, 1990). Both KT and BT have the average temperatures of between 12 °C – 13 °C for winter season which is slightly above the threshold temperature for development. These findings are in line with the findings of Nyangiwe et al. (2011) who reported larvae abundance during spring in False Thornveld and Döhne Sourveld in the Eastern Cape Province. From the vegetation, *R. appendiculatus* and *R. evertsi evertsi* were also collected at large numbers. Similarly, Horak et al. (2009) collected large numbers of larvae *R. evertsi evertsi* and *R. appendicultus* from drag-samples taken at 72 dip-tanks in the eastern region of the Eastern Cape Province. Less commonly collected species in the study area were *H. rufipes, A. hebraeum, H. elliptica, R. follis, I. pilosus* and *R. simus* in order of abundance. This is in agreement with Nyangiwe et al. (2011) who reported similar observation in camps grazed by Nguni or Bonsmara breed at Döhne Sourveld, Eastern Cape Province.

The tick under investigation, *R.* (*B.*) *microplus* was not found from cattle and on the vegetation during the study period. Recently reports confirmed that *R.* (*B.*) *microplus* is expanding its range and was displacing the indigenous tick, *R.* (*B.*) *decoloratus* in Africa. The rapid spread of *R.* (*B.*) *microplus* in South Africa (Horak et al., 2009; Nyangiwe et al., 2013; Nyangiwe et al., 2017; Tønnesen et al., 2004), Tanzania (Lynen et al., 2008), Zimbabwe (Smeenk et al., 2000; Sungirai et al., 2015) and West Africa (Adakal et al., 2013; Madder et al., 2007, 2012) is an indication of such example. The *R.* (*B.*) *microplus* is a highly adaptable ectoparasite that has become established in nearly all tropical and subtropical regions of the world where cattle production occurs (Bram et al., 2002; Busch et al., 2014). The invasive pantropical blue tick, *R.* (*B.*) *microplus,* also represents more of a threat to cattle farming than *R.* (*B.*) *decoloratus* because it transmits both *Babesia bovis* and *B. bigemina* in cattle, whereas *R.* (*B.*) *decoloratus* transmits only *B. bigemina*, which is less pathogenic (Madder et al., 2012).

Conclusions

The results of this survey indicates that *R.* (*B.*) *decoloratus* is widespread throughout the study areas. Therefore ecological preferences of ticks need to be considered when developing effective control strategies against ticks. Although *R. (B.) microplus* was not found in this study, therefore it could be key to investigate factors that promote its absence in the study area.

Conflict of Interest

The authors declare that they have no conflict of interest with regards to the entirety of this work.

Acknowledgements

The Department of Rural Development and Agrarian Reform is acknowledged for allowing us to use its facilities and animals for the success of this study. We further acknowledge National Research Foundation (Grant number: 102941) for providing the funding that empowered us to complete this study.

References

Adakal, H., Biguezoton, A., Zoungrana, S., Courtin, F., De Clercq, E.M., Madder, M., 2013. Alarming spread of the Asian cattle tick *Rhipicephalus microplus* in West Africa- another three countries are affected: Burkina Faso, Mali and Tsogo. Exp. Appl. Acarol. 61, 383-386.

Arthur, D.R., 1973. Scanning electron microscope studies on the morphology of immature stages of *Ambylomma hebraeum*, Koch 1844. J. Entomol. Soc. S. Afr. 36, 63–85.

Arthur, D.R., 1975. The larvae of some ixodid ticks (Acarina) from the Eastern Cape Province of South Africa. B. Entomol. Res. 65, 405–421.

Awa, D.N., Adakal, H., Luogbou, N.D.D., Wachong, K.H., Leinyuy, I., Achukwi, M.D., 2015. Cattle ticks in Cameroon: Is *Rhipicephalus* (*Boophilus*) *microplus*absent in Cameroon and the Central African region? Ticks Tick Borne Dis. 6, 117–122.

Bram, R.A., George, J.L., Reichard, R.E., Tabachnick, W.J., 2002. Threat of foreign arthropod-borne pathogens to livestock in the United States. J. Med. Entomol. 39, 405-416.

Bush, J.D., Stone, N.E., Nottingham, R., Araya-Anchetta, A., Lewis, J., Hochhalter, C., Giles, J.R., Gruendike, J., Freeman, J., Buckmeier, G., Bodine, D., Duhaime, R., Miller, R. J., Davey, R.B., Olafson, P.U., Scoles, G.A., Wagner D.M., 2014.Widespread movement of invasive cattle fever ticks (*Rhipicephalus microplus*) in southern Texas leads to shared local infestations on cattle and deer. Parasites Vectors 7, 188-203.

De Clercq, E.M., Vanwambeke, S.O., Sungirai, M., Adehan, S., Lokossou, R., Madder, M., 2012. Geographic distribution of the invasive cattle tick *Rhipicephalus microplus*, a country-wide survey in Benin. Exp. Appl. Acarol. 58, 441–452.

Gothe, R., 1967. Ticks in the South African Zoological Survey Collection. Part XII. The genera *Boophilus* Curtice, 1891 and *Margaropus* Karsch, 1879. Ondertepoort J. Vet. Res. 34, 81-107.

Hlatshwayo, M., Mbati, P.A., Dipeolu, O.O., 2001. Seasonal abundance of adult ixodid ticks infesting cattle belonging to resource-limited farmers in the north-eastern Free State Province of South Africa. Ondertepoort J. Vet. Res. 69, 1–6.

Horak, I.G., Jordaan, A.J., Nel, P.J. Van Heerden, J., Heyne, H., Van Dalen, E.M., 2015. Distribution of endemic and introduced tick species in Free State Province South Africa. J. S. Afr. Vet. Assoc. 1255, 1–9.

Horak, I.G., Nyangiwe, N., De Matos, C., Neves, L., 2009. Species composition and geographic distribution of ticks infesting cattle, goats and dogs in a temperate and a subtropical coastal region of south-eastern Africa, Ondertepoort J. Vet. Res. 76, 263–278.

Katiyatiya, C.L.F., Muchenje, V., Mushunje, A., 2014. Farmers' Perceptions and Knowledge of Cattle Adaptation to Heat Stress and Tick Resistance in the Eastern Cape, South Africa. Asian-Australas J. Anim. Sci. 27, 1663–1670.

Lynen, G., Zeman, P., Bakuname, C., Di Giulio, G., Mtui, P., Sanka, P., Jongejan, F., 2008. Shifts in the distributional changes of *Boophilus* ticks, in Tanzania: evidence that a parapatric boundary between *Boophilus microplus* and *Boophilus decoloratus* follows climate gradients. Exp. Appl. Acarol. 44, 147–164.

Madder, M., Thys, E., Geysen, D., Baudoux, C., Horak, I., 2007. *Boophilus microplus* ticks found in West Africa. Exp. Appl. Acarol. 43, 233–234.

Madder, M., Adehan, S., De Deken, R., Adehan, R., Lokossou, R., 2012. Newfoci of *Rhipicephalus microplus* in West Africa. Exp. Appl. Acarol. 56, 385–390.

Mucina, L., Hoare, D.B., Lötter, M.C., du Preez, P.J., Rutherford, M.C., Scott-Shaw, C.R., Bredenkamp, G.J., Powrie, L.W., Scott, L., Camp, K.G.T., Cilliers, S.S., Bezuidenhout, H., Mostert, T.H., Siebert, S.J., Winter, P.J.D., Burrows, J.E., Dobson, L., Ward, R.A., Stalmans, M., Oliver, E.G.H., Siebert, F., Schimidt, E., Kobisi, K., Kose, L., Smit, J.H.L., Goodman, P.S., Ellis, F., Henning, B.J., Venter, C.E., Matthews, W.S., van Rooyen, N., Williamson, S., Hurter, P.J.H., Vlok, J.H.J., Euston-Brown, D.I.W, Palmer, A.R., Lechmere-Oertel, R.G., Proches, S.M., Dold, A.P., Ward, R.A., 2006. Grassland Biome, Savanna biome and Albany Thicket Biome. In: Mucina L, Rutherford MC (eds) The vegetation of South Africa, Lesotho and Swaziland, vol 19. South African National Biodiversity Institute, Pretoria, pp 347–558.

Marufu, M.C., Chimonyo, M., Mans, B.J., Dzama, K., 2013. Cutaneous hypersensitivity responses to *Rhipicephalus* tick larval antigens in pre-sensitized cattle. Ticks Tick Borne Dis. 4, 311–316.

Marufu, M.C., Chimonyo, M., Mapiye, C., Dzama, K., 2011. Tick loads in cattle raised on sweet and sour rangelands in the low-input farming areas of South Africa. Trop. Anim. Health Prod. 43, 307–313.

Muchenje, V., Dzama, K., Chimonyo, M., Raats, J. G., Strydom, P. E, 2008. Tick susceptibility and its effects on growth performance and carcass characteristics on Nguni, Bonsmara and Angus steers raised on natural pasture. Animals. 2, 298–304.

Norval, R.A.I., Horak, I.G., 2004: Vectors: Ticks. In: J. A. W. Coetzer and R. C. Tustin (eds), *Infectious Diseases of Livestock*, 2nd edn., 3-42. Oxford University Press Southern Africa, Cape Town.

Nyangiwe, N., Goni, S., Hervé-Claude, L.P., Ruddat, I., Horak, I.G., 2011. Ticks on pastures and on two breeds of cattle in the Eastern Cape Province, South Africa, Onderstepoort J. Vet. Res., 78, 1–9.

Nyangiwe, N, Harrison, A., Horak, I.G., 2013. Displacement of *Rhipicephalus decoloratus* by *Rhipicephalus* microplus (Acari: Ixodidae) in the Eastern Cape Province, South Africa. Exp. Appl. Acarol. 61, 371–382.

Nyangiwe, N., Horak, I.G., Van der Mescht, L., Sonja, M., 2017. Range expansion of the economically important Asiatic blue tick, *Rhipicephalus microplus,* in South Africa. J S Afr Vet Assoc 88:a1482. https ://doi. org/10.4102/jsava .v88i0 .1482.

Olwoch, J.M., Van Jaarsveld, A.S., Scholtz, C.H., Horak, I.G., 2007. Climate change and the genus *Rhipicephalus* (Acari: Ixodidae) in Africa. Onderstepoort J. Vet. Res. 74, 45–72.

Schroder, B., Reilly, B.K., 2013. Seasonal variations in ixodid tick populations on a commercial game farm in the Limpopo Province, South Africa. African Invertebrates. 54, 491–498.

Smeenk, I., Kelly, P.J., Wray, K., Musuka, G., Trees, A.J., Jongejan, F., 2000. *Babesia bovis* and *B. bigemina* DNA detected in cattle and ticks from Zimbabwe by polymerase chain reaction. J. S. Afr. Vet Assoc. 71, 21-24.

Spickett, A.M., Heyne, H., 1990. The prehatch period and larval survival of *Boophilus decoloratus* (Koch, 1844) (Acarina: Ixodidae) under natural conditions in the Transvaal, South Africa.

Statistical Analysis System. 2003. Statistical Analysis System Institute Inc. Users Guide, Version 9, Carry, NC, USA.

Sungirai, M., Madder, M., Moyo, D.Z., De Clercq, P., Abatih, E.N., 2015. An update on the ecological distribution of the Ixodidae ticks in Zimbabwe. Exp. Appl. Acarol. 66: 269–280.

Theiler, G., 1962. The Ixodoidea parasites of vertebrates in Africa south of the Sahara (Ethiopian Region). Project S 9958, Report to the Director of Veterinary Services, Onderstepoort. Mimeographed, 260 pp.

T¢nnesen, 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., Pegram, R. G., Preston, P. M., 2003. Ticks of domestic animals in Africa, A guide to identification of species, Bioscience Reports, 157.

Walker, J. B., Keirans, J. E., Horak, I. G., 2000. The genus *Rhipicephalus* (Acari, Ixodidae):a guide to the brown ticks of the world. Cambridge: Cambridge University Press.

Wolde, A., Mohamed, A., 2014. Prevalence of Ixodid ticks on bovine in Soddo Zuria Districts, Wolaita Zone, Ethiopia. Acta Parasitol Glob 5, 188–197.

Table 1 The overall tick species abundance collected from the vegetation in the study area.

	Larvae	
Tick species	**Counts**	**Prevalence (%)**
Amblyomma hebraeum	71	2.46
Haemaphysalis elliptica	11	0.38
Hyalomma rufipes	86	2.98
Rhipicephalus appendiculatus	530	18.37
Rhipicephalus (*Boophilus*) *decoloratus*	1678	58.16
Rhipicephalus evertsi evertsi	487	16.90
Rhipicephalus simus	5	0.17
Rhipicephalus follis	10	0.34
Ixodes pilosus group	7	0.24
Totals	2885	100

Table 2 Prevalence of *Rhipicephalus* (*Boophilus*) *decoloratus* ticks collected from cattle and on the vegetation at three agro-ecological zones in the Eastern Cape Province

	***Rhipicephalus* (*Boophilus*) *decoloratus* ticks**					
	Adult females on cattle		**Adult males on cattle**		**Free-living ticks on vegetation**	
Vegetation type	**Counts**	**Prevalence (%)**	**Counts**	**Prevalence (%)**	**Counts**	**Prevalence (%)**
Dry grassland	1307	29.83	1267	34.17	419	24.97
Thicket	1712	39.07	1515	40.86	537	32.00
Thornveld	1363	31.10	926	24.97	722	43.03
Totals	**4382**	**100**	**3708**	**100**	**1678**	**100**

Table 3 Least square means (±s.e.) for vegetation type and season of engorged *Rhipicephalus* (*Boophilus*) *decoloratus* ticks collected from cattle and of free-living *Rhipicephalus* (*Boophilus*) *decoloratus* ticks collected from vegetation

		***Rhipicephalus* (*Boophilus*) *decoloratus* ticks**		
Vegetation type	**Season**	**Engorged females**	**Engorged males**	**Free-living ticks**
Dry grassland	**Autumn**	0.97±0.063[cd]	0.99±0.063[bc]	6.00±1.154[c]
	Spring	1.08±0.063[bc]	1.08±0.063[b]	12.15±1.154[b]
	Summer	1.07±0.063[bc]	1.17±0.063[ab]	7.78±1.154[c]
	Winter	0.43±0.063[c]	0.36±0.063[c]	2.56±1.154[e]
Thicket	**Autumn**	0.10±0.063[d]	0.76±0.063[d]	7.39±1.154[c]
	Spring	1.15±0.063[b]	1.16±0.063[ab]	18.50±1.154[a]
	Summer	1.39±0.063[a]	1.30±0.063[a]	7.44±1.154[c]
	Winter	0.73±0.063[d]	0.76±0.063[d]	4.06±1.154[de]
Thornveld	**Autumn**	0.73±0.063[d]	0.53±0.063[c]	7.06±1.154[cd]
	Spring	1.06±0.063[bc]	1.08±0.063[b]	20.56±1.154[a]
	Summer	1.19±0.063[b]	0.90±0.063[c]	12.39±1.154[b]
	Winter	0.80±0.063[d]	0.52±0.063[e]	3.67±1.154[e]

[abcde] Means in the same column with different superscripts differ significantly ($P<0.05$).