

Diversity of ticks (Acari: Ixodidae) infesting cheetahs (*Acinoyx jubatus*) at three breeding centres in South Africa and activity patterns of questing ticks

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Abstract

Ticks were collected from 191 cheetahs at three breeding centres in North West and Limpopo Provinces, South Africa. *Haemaphysalis elliptica*, a common tick of large felids, was the most abundant species collected, while *Amblyomma hebraeum* and *Rhipicephalus simus* occurred in lower numbers. In addition to these three species, drag-sampling of the vegetation revealed the presence of *Amblyomma marmoreum*, *Rhipicephalus* (B.) *decoloratus* and *Rhipicephalus zambeziensis*. The presence of free-ranging antelopes, murid rodents and tortoises at the breeding centres probably contributed to the availability of immature tick stages on the vegetation. Diurnal and seasonal questing patterns of ixodid ticks were investigated at monthly intervals at the largest cheetah-breeding centre. Questing ticks were most abundant on the vegetation during the warm summer months. Most questing *H. elliptica* larvae and nymphs were collected from the vegetation in the early morning and late afternoon and fewest during the middle of the day.

Keywords: cheetah, *Haemaphysalis elliptica*, *Rhipicephalus simus*, *Amblyomma hebraeum*, South Africa, questing ticks

Introduction

Classified as “vulnerable” on the *IUCN Red List of Threatened Species* (Durrant et al. 2015) cheetahs (*Acinonyx jubatus*) are bred at various centres in South Africa and elsewhere (Van Dyk, 1991). The animals are confined to enclosures, albeit relatively large ones, which could favour the increase of parasite infestations. Ticks, known vectors of piroplasms and various other pathogens, are of particular importance. At one breeding centre, 64.9% of 97

cheetahs sampled were positive for piroplasms, i.e. *Babesia felis*, *B. leo* or *B. lengau* (Bosman et al., 2007; Bosman et al., 2010). No studies had been conducted on the tick populations occurring at breeding centres.

Tick-control methods at breeding centres, based on empirical experience, includes rotational use of enclosures, selective burning of the vegetation as well as application of acaricides (Verdoorn, 1998). To apply acaricides, the cheetah is lured into a crush. Generally, a pyrethroid-based acaricide (e.g. Flumethrin[®], Bayer Animal Health) is applied by hand-spray. Acaricides are applied at six-weekly intervals during summer and at three-monthly intervals during winter. Rodent control by means of baits containing an anticoagulant (e.g., Racumin[®], Bayer (Pty) Ltd) is aimed primarily at *Rattus rattus* (Verdoorn, 1998), but may affect indigenous murid rodents and reduce availability of hosts for immature stages of ticks infesting cheetahs. Details of control measures are given later.

Studying tick diversity and activity patterns may reveal insights into the ecology of the various tick species. Various factors such as the complexity of a tick's life cycle, the number of stages, the environment and the survival of its free-living stages can enhance its ability to disperse. The characteristics of the hosts contribute to the interactions between parasites and hosts and to the dispersal pattern of parasites (McCoy et al., 2003).

Climate and vegetation are generally accepted as the major factors that determine the geographic distribution ranges of ixodid ticks and also serve as possible predictors of tick diversity within a region (Cumming, 2002). Seasonal variation in terms of the number of questing ticks can be the consequence of their developmental pattern

(Randolph, 2002). Considering the microhabitat selection of ixodid ticks (achieved by responses to environmental cues such as gravity, light and humidity), many species habitually ascend vegetation to heights favourable for contact with their preferred hosts in the early morning and again in the evening (Mehlhorn, 2008; Rechav, 1979). Therefore, the time of day may affect microhabitat selection.

The various stages in the life cycle of ixodid ticks may be most active at different times of the day. In temperate climates, ticks are active throughout the day and year, provided that the environmental temperature is above that of the uncoordinated activity threshold temperature (a temperature below which a tick can no longer coordinate its host-seeking activity) (Rechav, 1979). More important is the activity threshold temperature at which all the tick's activity will cease, thus defining the point at which the termination of tick-host contact occurs (Clark, 1995; Vail and Smith, 1998).

Various studies have been conducted on seasonal activity patterns of ixodid ticks in South Africa (Gallivan et al., 2011; Horak et al., 2011; Nyangiwe et al., 2011; Spickett et al., 2011). Observations on tick activity are mostly based on drag-sampling (Petney and Horak, 1987). Fluctuations in the numbers of each stage are associated with specific microclimatic conditions (Cumming, 2002; Londt and Whitehead, 1972). The differences in tick population density in various seasons of the year may be an indication of the capacity of species to survive limiting environmental conditions. All stages of ixodid ticks can survive for extended periods away from their hosts. Atmospheric humidity is a crucial environmental factor influencing this ability (Knülle, 1966).

Ixodid ticks are remarkably responsive to temperature and relative humidity (Knülle and Rudolph, 1982). Several species have distinct activity patterns induced by these variables and by photoperiod, whereby the most vulnerable stages will only be active or even present during the most favourable time of the year (Fourie and Horak, 1994; Robertson, 1981; Short and Norval, 1981). In various surveys, Rechav (1979) demonstrated the correlation of questing ability with relative humidity. Unlike nymphs and adults, the proportion of the larval population on the grass was higher as the humidity increased. Regarding marked daily differences in temperature and humidity, there may be times of the day when few or no ticks are active on the vegetation (Madden and Madden, 2005; Punyua et al., 1984).

The present study was conducted to determine the diversity of tick species infesting cheetahs at three breeding centres. The seasonal abundance of questing ticks on the vegetation and the diurnal activity pattern of ticks were also studied at one of these centres. At least 19 ixodid tick species have been recorded from cheetahs in South Africa, Namibia and Kenya (Table 1). Theiler (1962) reported cheetahs also being hosts of *Amblyomma lepidum*, *Amblyomma variegatum* and *Rhipicephalus capensis*, but no localities were given. Many of the listed species may have been incidental infestations, with no significant biological implication.

Table 1

List of ixodid ticks recovered from cheetahs in South Africa (SA), Namibia (N) and Kenya (K)

Species	SA	N	K	Reference
<i>Amblyomma hebraeum</i>	+			Baker et al. 1970; Horak et al. 2000, 2010
<i>Amblyomma marmoreum</i>	+			Horak et al. 2000
<i>Haemaphysalis elliptica</i>	+			Horak et al. 2000, 2010
<i>Haemaphysalis leachi</i>			+	Walker 1974
<i>Haemaphysalis zumpti</i>	+			Horak et al. 2000
<i>Hyalomma truncatum</i>	+			Baker et al. 1970
<i>Hyalomma turanicum</i>	+			Horak et al. 2000
<i>Rhipicephor bicornis</i>		+		Horak et al. 2010
<i>Rhipicephor nuttali</i>	+			Horak et al. 2010
<i>Rhipicephalus appendiculatus</i>	+			Baker et al 1970; Horak et al. 2000
<i>Rhipicephalus armatus</i>			+	Walker 1974
<i>Rhipicephalus compositus</i>			+	Walker 1974
<i>Rhipicephalus (B.) decoloratus</i>	+			Horak et al. 2000
<i>Rhipicephalus evertsi evertsi</i>	+			Horak et al. 2000
<i>Rhipicephalus gertrudae</i>		+		Horak et al. 2010
<i>Rhipicephalus maculatus</i>	+			Baker et al. 1970
<i>Rhipicephalus sanguineus group</i> ¹			+	Walker 1974
<i>Rhipicephalus simus</i>	+	+	+	Baker et al. 1970; Horak et al. 2000, 2010; Walker 1974
<i>Rhipicephalus turanicus</i>	+			Horak et al. 2000
<i>Rhipicephalus zambeziensis</i>	+			Horak et al. 2000

¹Possibly *Rhipicephalus turanicus*

Materials and methods

This study was conducted during 2008 and 2010/2011 at three cheetah-breeding centres in South Africa: (a) Ann van Dyk Cheetah Centre–De Wildt/Brits (AVDCC–DWB) (S25°40', E27°55'; altitude 1211 m) situated north west of Pretoria in North West Province; (b) Ann van Dyk Cheetah Centre–De Wildt/Shingwedzi (AVDCC–DWS) (S24°40', E28°2'; altitude 1500 m) located 56 km north west of Bela-Bela, Limpopo Province; and (c) Hoedspruit Endangered Species Centre (HESC) (S24°30', E31°02'; altitude 515 m) situated 32 km south east of Hoedspruit, Limpopo Province. These centres lie within semi-arid rainfall zones with an inland subtropical climate. The vegetation at AVDCC–DWB is Marikana thornveld, at

AVDCC-DWS it is central sandy bushveld, while that at HESC is lowveld mixed woodland (Mucina and Rutherford, 2006).

AVDCC-DWB was chosen as the principal study locality because of its proximity to Onderstepoort, large cheetah population and heavy tick burdens as detected during a pilot study. This centre, the oldest in South Africa, was established in 1970 in collaboration with the National Zoological Gardens of South Africa (Van Dyk, 1991). Breeding females are housed individually in enclosures of *ca.* 1 ha. The vegetation in the enclosures is heavily trampled and is also regularly mowed. African wild dogs (*Lycaon pictus*) and brown hyaenas (*Parahyaena brunnea*) are also kept at this centre, but their enclosures are not near those of the breeding cheetah females.

Tick infestation on cheetahs

At all three centres cheetahs are chemically restrained for examination at least once a year. Due to time constraints while cheetahs were restrained, full-body collections were not feasible; adult ticks (mostly engorged) only were collected from four attachment sites: 10 x 10 cm patches on the ventral and dorsal aspect of the neck, on the shoulder and on the perineum/tail. Collections were performed at AVDCC-DWB during June-August (winter) and October-November (early summer) 2008, at AVDCC-DWS in June 2008 (winter) and at HESC in July (winter) and November (early summer) 2008.

Questing ticks on the vegetation

Ticks questing for hosts from the vegetation were collected by using a drag-sampling device capable of collecting all stages of ixodid ticks, particularly larvae (Petney and Horak, 1987; Sonenshine et al., 1966). The device consists of ten flannel strips, each 100 cm long and 10 cm wide, attached to a 120 cm-long wooden spar by means of Velcro[®] tape. A 9-cm-long steel rod is sewn into the end of each strip to keep it on the vegetation during dragging, even on windy days. A twine harness is attached to each end of the spar so that the device can be dragged behind the operator (Spickett et al., 1991). Collections were performed at AVDCC–DWB during June–August (winter) and October–November (early summer) 2008, at AVDCC–DWS in June 2008 (winter) and at HESC in July (winter) and November (early summer) 2008.

At monthly intervals, five cheetah enclosures at AVDCC–DWB were randomly chosen. The flannel sampling device was dragged over the vegetation for a distance of 50 m (Zimmerman and Garris, 1985). Drag-sampling was done on sunny mornings. Five separate drags were made. After each 50 m drag, the flannel strips were inspected and all ticks present on the flannels were removed using forceps and placed in labelled glass vials containing 70% ethanol. They were transported to the ectoparasitology laboratory at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, for identification and counting using a stereoscopic microscope. The monthly counts represented the total number of ticks collected from the five drag-samples performed during that month. The immature stages of ticks questing for hosts from the

vegetation were identified under a stereoscopic microscopy at a magnification set at 40x or 50x.

In a second study at AVDCC–DWB, the hourly fluctuation in the numbers of questing ticks on the vegetation was monitored. Single drag-samples were collected at hourly intervals from 08h00 to 17h00, care being taken not to sample the same area more than once. The atmospheric temperature in the shade at ground level (measured with a maximum/minimum thermometer) as well as the time at which each drag was done was recorded throughout the day. The ticks collected were identified and counted as described above.

Tick-infestation of small mammals

In an extension of the study, small mammals were trapped in Sherman live-traps at AVDCC–DWB at two-monthly intervals from July 2010 to May 2011 and in two sessions (November 2010 and May 2011) at HESC. At AVDCC–DWB a census line of 40 traps, 10 m apart, was set against the western fence of a series of occupied cheetah enclosures. The control line was set against the eastern fence of a series of unoccupied enclosures, separated from the census line by a strip 20 m wide between the two rows of enclosures. Traps were baited with a mixture of rolled oats, peanut butter, a dash of sunflower seed oil and cane syrup. Traps were set for three consecutive nights, generating 120 trapping nights per line per trapping session. Traps were checked in the morning and re-baited in the late afternoon. At the request of the AVDCC-DWB management, traps were closed during the day to avoid

catching yellow-footed squirrels (*Paraxerus cepapi*). Diurnally active rodents were therefore not trapped. A similar lay-out pattern for trap-lines was used at HESC.

Animals trapped in the census line were collected for further processing, while those trapped in the control line were marked (a spot of red Aerolac spray paint on the back) and released. Individuals re-trapped during any particular trapping session were discounted. Taxonomic identification was based on Bronner et al. (2003) and Skinner and Chimimba (2005). Rodents were placed individually in labelled cloth bags and euthanized at the processing laboratory by intraperitoneal injection of 1 ml pentobarbitone (Eutha-naze®, Bayer Animal Health Division). Carcasses were individually placed in labelled clear plastic bags and soaked overnight in a suspension (4 ml per litre of water) of a tick-detaching agent (Amitix®, Schering-Plough Animal Health Division) (Horak et al., 1986).

The following morning the carcass was thoroughly washed and the skin scrubbed with steel-bristle brushes. Washings and scrubbing were collected in bottles. Bottles were processed individually. The contents were slowly poured into a steel-mesh sieve (apertures of 150 µm) and washed with a strong jet of water. The contents of the sieve were transferred into a square Perspex tray and examined under a stereoscopic microscope for collection of ticks (Horak et al., 1992). The ticks collected from each carcass were placed in a labelled glass vial containing 70% ethanol as preservative prior to being examined under a stereoscopic microscope for identification (as described above).

Results

Tick infestation of cheetahs

Adult ticks were collected from 100 cheetahs during winter and from 91 cheetahs during early summer (Tables 2 & 3). At all breeding centres, *Haemaphysalis elliptica* was the most abundant species collected at each occasion, while much lower numbers of *Amblyomma hebraeum* and *Rhipicephalus simus* were collected. *Amblyomma hebraeum* was collected at HESC in both winter and early summer, but only in summer at AVDCC–DWB. *Rhipicephalus simus* was collected at AVDCC–DWB only, and only in summer.

Table 2

Adult tick load¹ of infested cheetahs at three breeding centres (winter)

Tick species	AVDCC – de Wildt			AVDCC – Shingwedzi			Hoedspruit ESC		
	No of ticks	No of cheetahs infested	Mean tick load / cheetah	No of ticks	No of cheetahs infested	Mean tick load / cheetah	No of ticks	No of cheetahs infested	Mean tick load/ cheetah
<i>Amblyomma hebraeum</i>	-	-	-	-	-	-	19	12	1.6
<i>Haemaphysalis elliptica</i>	928	57	16.3	206	17	12.1	91	19	4.8
TOTAL	928	57	16.3	206	17	12.1	110	26 ²	4.2

¹Ticks collected from four 10x10 cm patches / cheetah

²Some cheetahs were infested with both tick species

Table 3

Adult tick load¹ of infested cheetahs at two breeding centres (summer)

Tick species	AVDCC – de Wildt			Hoedspruit ESC		
	No of ticks	No of cheetahs infested	Mean tick load / cheetah	No of ticks	No of cheetahs infested	Mean tick load / cheetah
<i>Amblyomma hebraeum</i>	30	15	2.0	44	6	7.3
<i>Haemaphysalis elliptica</i>	1238	56	22.1	712	23	31.0
<i>Rhipicephalus simus</i>	102	18	5.7	-	-	-
TOTAL	1370	62 ²	22.1	756	29	26.1

¹Ticks collected from four 10x10 cm patches / cheetah

²Some cheetahs were infested with multiple tick species

Questing ticks on the vegetation

The species and numbers of questing ixodid ticks collected from the vegetation at the three localities are summarised in Table 4. Six tick species belonging to three genera were collected: *Amblyomma hebraeum*, *Amblyomma marmoreum*, *Haemaphysalis elliptica*, *Rhipicephalus (B.) decoloratus*, *Rhipicephalus simus* and *Rhipicephalus zambeziensis*, with *H. elliptica* being the most abundant species at all localities. Larvae predominated in all collections. Of the 12353 specimens collected at AVDCC–DWB, 86.8% were *H. elliptica*, 10.2% *A. hebraeum*, and 2.9% *R. simus*. Larvae (91.2%) and nymphs (8.4%) made up the bulk of the collections; the only adults collected (0.4%), were *H. elliptica*.

Table 4

Diversity and numbers of all ixodid ticks collected by drag-sampling the vegetation at three cheetah-breeding centres (AVDCC = Ann van Dyk Cheetah Centre; DWB = De Wildt/Brits; DWS = De Wildt/Shingwedzi; HESC = Hoedspruit Endangered Species Centre; LL = larvae; NN = nymphs; ♂ and ♀ = adults)

Tick species	AVDCC-DWB ¹				AVDCC-DWS ²				HESC ³			
	LL	NN	♂	♀	LL	NN	♂	♀	LL	NN	♂	♀
<i>Amblyomma hebraeum</i>	1170	96	-	-	-	-	-	-	93	28	5	10
<i>Amblyomma marmoreum</i>	-	-	-	-	37	-	-	-	-	-	-	-
<i>Haemaphysalis elliptica</i>	9743	931	25	25	228	34	13	2	2042	235	15	6
<i>Rhipicephalus (B.) decoloratus</i>	-	-	-	-	-	-	-	-	1002	-	-	-
<i>Rhipicephalus simus</i>	356	7	-	-	-	-	-	-	-	-	-	-
<i>Rhipicephalus zambeziensis</i>	-	-	-	-	88	-	-	-	96	42	-	-

¹Sampled monthly (March 2008 to February 2009; July 2010 to May 2011); ²Sampled once (June 2008);

³Sampled twice (July and November 2008)

Mean monthly abundance (with standard deviation) of all questing ticks collected from the vegetation at AVDCC–DWB is shown in Fig. 1. Most *H. elliptica* larvae were collected during the summer months (December to March) and fewest during mid-winter (July) (Fig. 2). Most *H. elliptica* nymphs and adults were present from November to February (Fig. 2). The other two species collected, *A. hebraeum* and *R. simus*, occurred in much lower numbers and were most abundant during the warmer months of the year (Fig. 3).

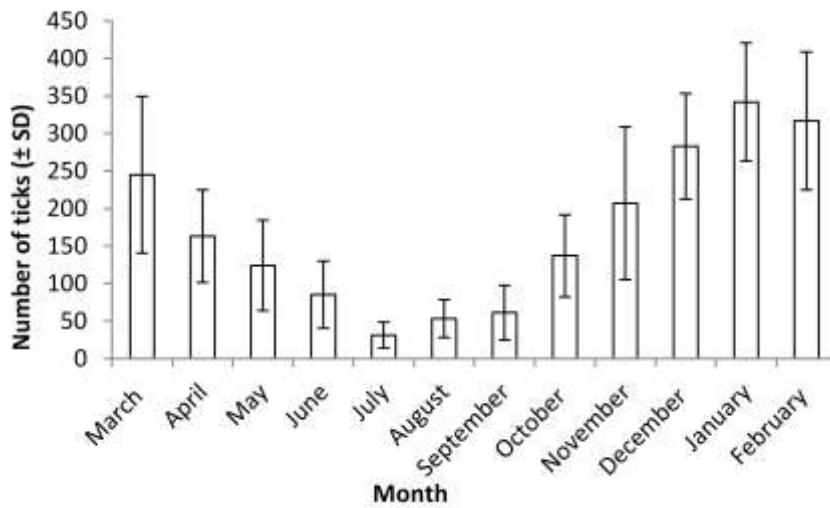


Fig. 1. Mean monthly abundance (with standard deviation) of all stages of development of questing ixodid ticks collected by drag-sampling from the vegetation at Ann Van Dyk Cheetah Centre–De Wildt/Brits (March 2008 to February 2009)

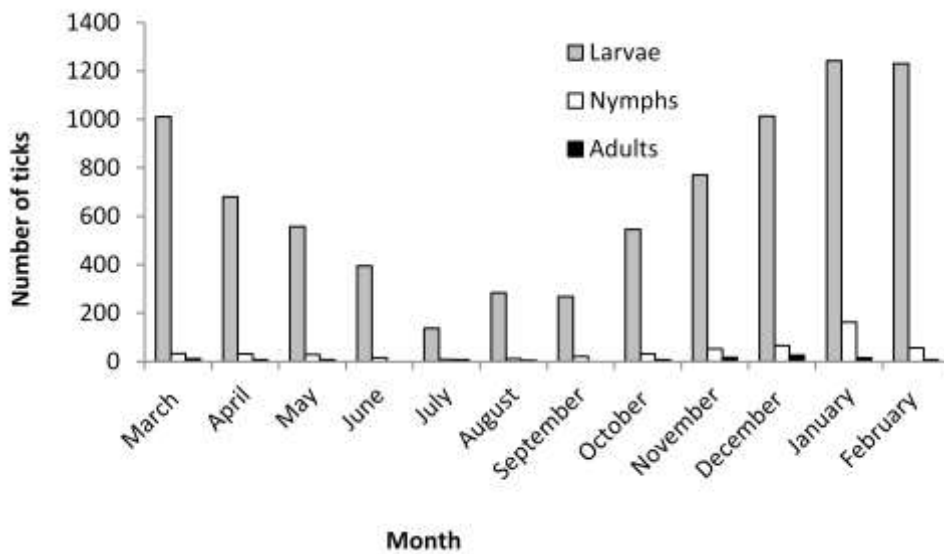


Fig. 2. Monthly total numbers of *Haemaphysalis elliptica* larvae, nymphs and adults collected by drag-sampling the vegetation at the Ann van Dyk Cheetah Centre–De Wildt/Brits (March 2008 to February 2009)

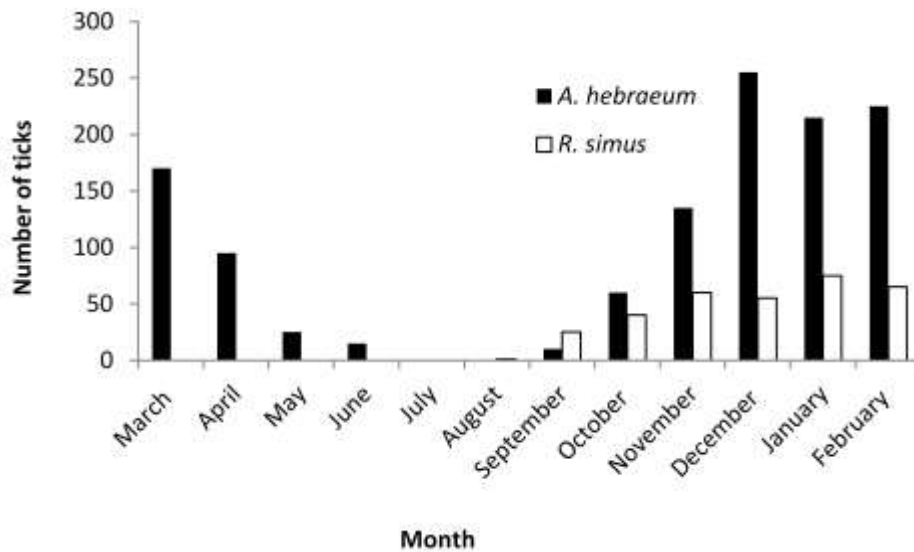


Fig. 3. Seasonal abundance of *Amblyomma hebraeum* and *Rhipicephalus simus* collected by drag-sampling vegetation at the Ann van Dyk Cheetah Centre–De Wildt/Brits (March 2008 to February 2009)

The numbers of larvae and nymphs of *A. hebraeum*, *H. elliptica* and *R. simus* collected at hourly intervals during the day in June and December 2008, respectively, at AVDCC–DWB are shown in Figs 4, 5, 6 and 7. Very few ticks were collected during the warmest hours of the day, compared to the early-morning and late-afternoon hours.

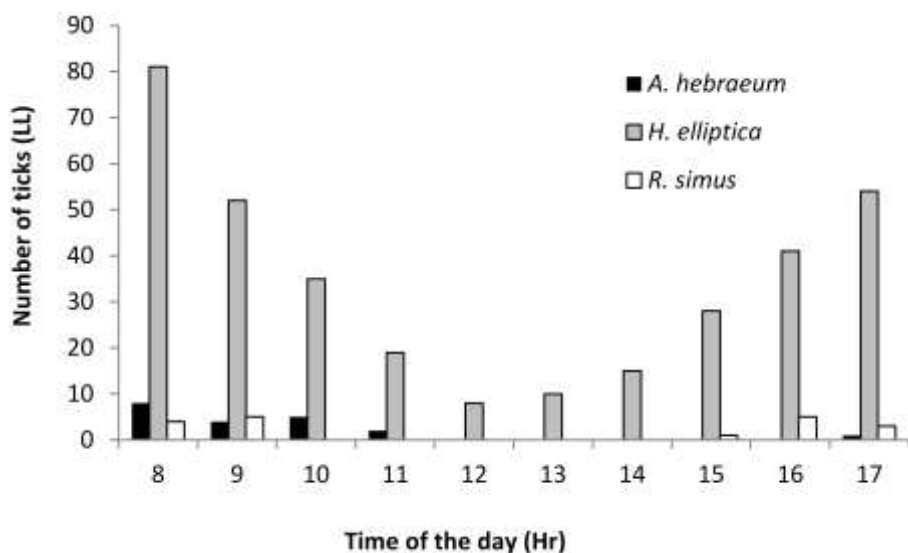


Fig. 4. *Amblyomma hebraeum*, *Haemaphysalis elliptica* and *Rhipicephalus simus* larvae collected at hourly intervals from vegetation at the Ann van Dyk Cheetah Centre–De Wildt/Brits in winter (June 2008)

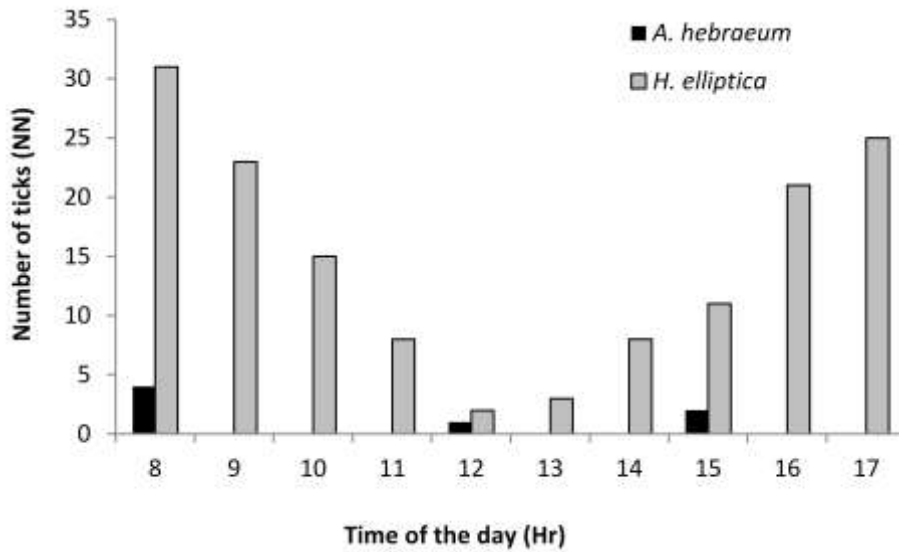


Fig. 5. *Amblyomma hebraeum* and *Haemaphysalis elliptica* nymphs collected at hourly intervals from vegetation at the Ann van Dyk Cheetah Centre–De Wildt/Brits in winter (June 2008) (No *Rhipicephalus simus* nymphs were recovered.)

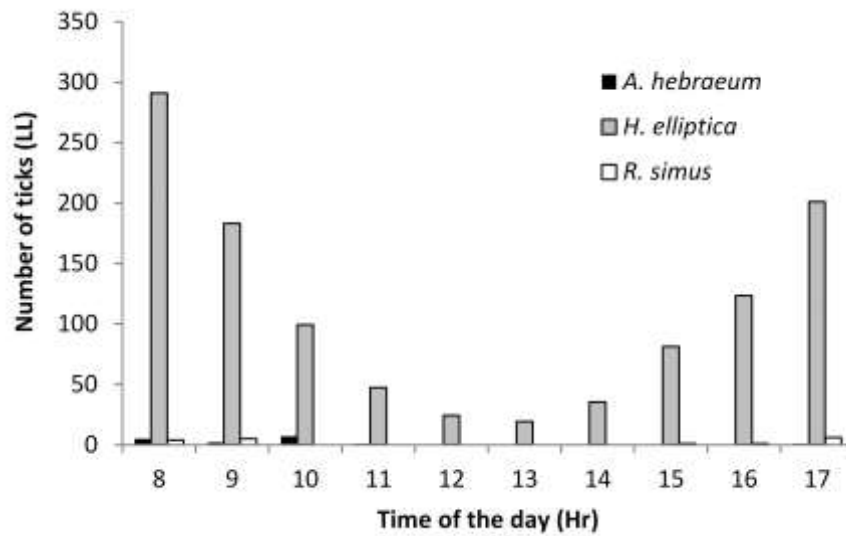


Fig. 6. *Amblyomma hebraeum*, *Haemaphysalis elliptica* and *Rhipicephalus simus* larvae collected at hourly intervals from vegetation at the Ann van Dyk Cheetah Centre–De Wildt/Brits in summer (December 2008)

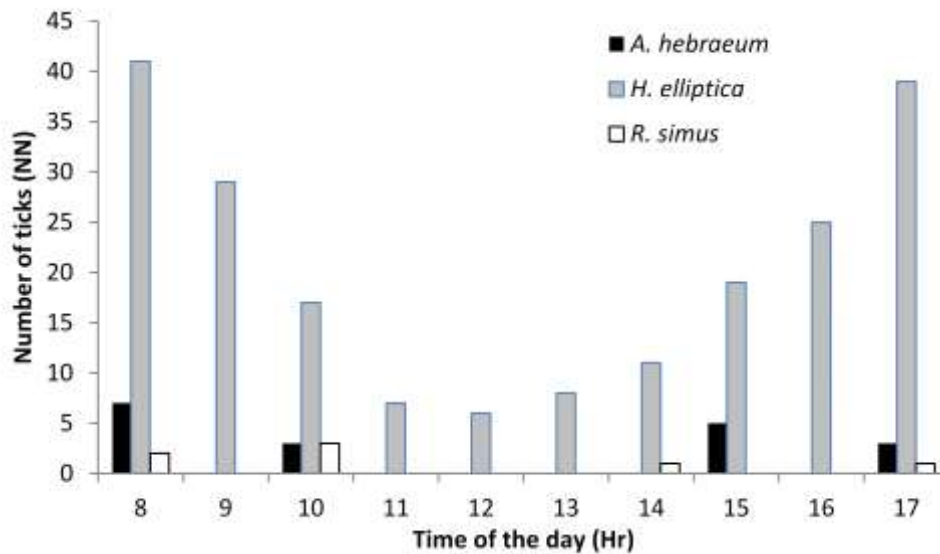


Fig. 7. *Amblyomma hebraeum*, *Haemaphysalis elliptica* and *Rhipicephalus simus* nymphs collected at hourly intervals from vegetation at the Ann van Dyk Cheetah Centre–De Wildt/Brits in summer (December 2008)

The rainfall at AVDCC–DWB varied considerably from month to month during the study period (Fig. 8). No rainfall was recorded during the winter months June–August 2008; the highest rainfall was recorded in February 2009. The temperature in the shade at ground level in December 2008 varied between 19.9°C and 32.7°C (Fig. 9) on the day of drag-sampling and the average relative humidity was 35%. As the temperature rose, the number of questing larvae and nymphs declined (Figs. 6 & 7).

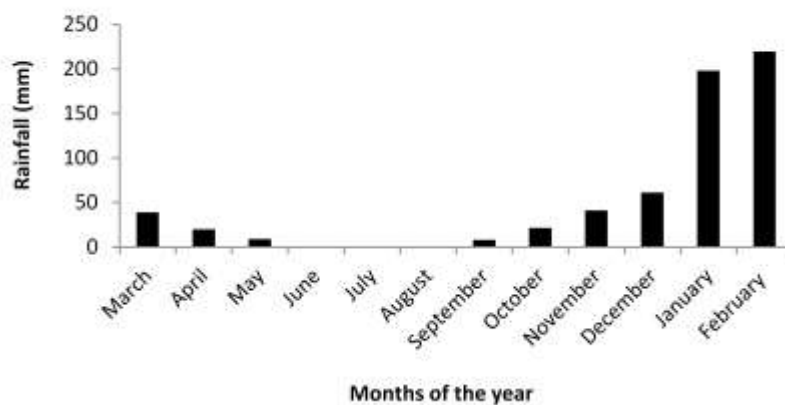


Fig. 8. Monthly rainfall at the Ann van Dyk Cheetah Centre–De Wildt/Brits (March 2008 to February 2009)

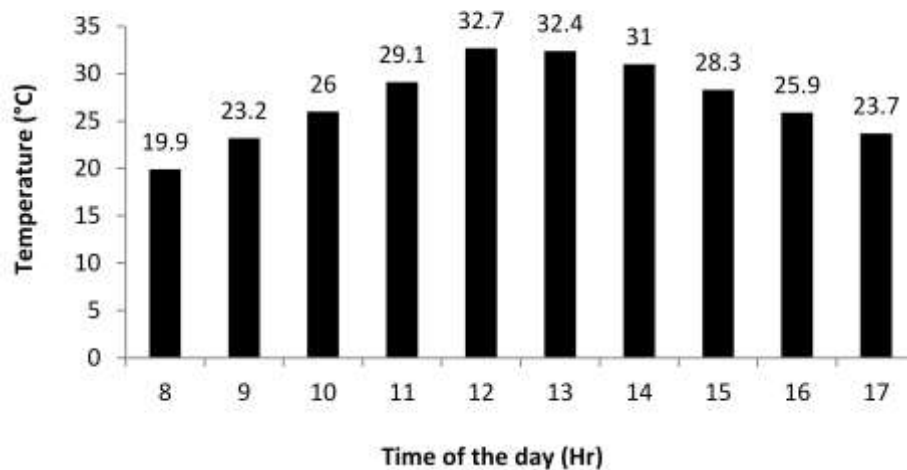


Fig. 9. Atmospheric temperature at hourly intervals at the Ann van Dyk Cheetah Centre–De Wildt/Brits (December 2008)

Table 5

Murid rodents trapped in the census and control lines at Ann van Dyk Cheetah Centre–De Wildt/Brits (AVDCC–DWB) and Hoedspruit Endangered Species Centre (HESC) during 2010 and 2011

Species	AVDCC-DWB		HESC	
	Census	Control	Census	Control
<i>Aethomys</i> sp.	8	7	-	-
<i>Graphiurus murinus</i>	1	1	-	-
<i>Mastomys</i> sp.	8	18	1	-
<i>Mus minutoides</i>	-	1	-	1
<i>Saccostomys campestris</i>	-	-	4	3
<i>Tatera leucogaster</i>	-	-	-	1
Total catch	17	27	5	7

Tick-infestation of small mammals

Trapping success of small mammals was very low. In addition to a single shrew (*Crocidura hirta*) trapped in the control line at HESC, 55 rodents were trapped: 44 during 1440 trap-nights at AVDCC–DWB and 12 during 480 trap-nights at HESC (Table 5). Larvae and nymphs of four ixodid tick species were recovered from 14 mice trapped at AVDCC–DWB (Table 6).

Haemaphysalis elliptica was the only species recovered from mice at HESC: *Mastomys* sp. (n=1) yielded 8 larvae, while *Saccostomys campestris* (n=4) yielded 86 larvae and 5 nymphs.

Table 6

Ticks recovered from murid rodents collected at Ann van Dyk Cheetah Centre–De Wildt/Brits during 2010 and 2011 (LL=larvae; NN=nymphs)

Host	<i>Haemaphysalis elliptica</i>		<i>Rhipicephalus (B.) decoloratus</i>		<i>Rhipicephalus simus</i>		<i>Rhipicephalus zambeziensis</i>	
	LL	NN	LL	NN	LL	NN	LL	NN
<i>Aethomys</i> sp (n=5)	227	27	-	-	22	17	-	-
<i>Graphiurus murinus</i> (n=1)	19	-	-	-	1	-	-	-
<i>Mastomys</i> sp. (n=8)	28	7	2	-	-	1	2	-
Subtotal	274	34	2	-	23	18	2	-
Total	308		2		41		2	

Discussion

We adopted a holistic approach to the identification of the immature stages of the ticks that were collected from the vegetation and from murid rodents. This approach can be criticised because it does not focus solely on the morphological features of taxa, but it does eliminate a number of species of which the immature stages would be difficult to identify to species level.

The larvae and nymphs of *A. hebraeum* were identified using descriptions provided by Arthur (1973) and Voltzit and Keirans (2003). They are characterised by their long and slender palps, broadly oval bodies, orange coloured scutum, which is long and distinctly tapers to its posterior margin, and slightly concave posterolateral margins. The eyes are flat

and they are associated with distinct small patches of dark grey or brown. There are a few medium-sized punctations on the anterior surface of the scutum of the nymph. The only adults of an *Amblyomma* species collected from the cheetahs at AVDCC–DWB were those of *A. hebraeum*.

The larvae of *A. marmoreum* were identified using the descriptions of Arthur (1975) and Voltzit and Keirans (2003). Their palps are shorter and more robust than those of *A. hebraeum*, their bodies nearly circular and their festoons clearly visible. The scutum is a light to dark yellow in colour, it is shallow and broad, and its posterolateral and posterior margin are slightly convex. The eyes are flat and there are distinct small patches of dark grey or brown associated with them. The collection of *A. marmoreum* larvae can only be ascribed to the presence of a tortoise or tortoises, most probably a leopard tortoise (*Stigmochelys pardalis*) at the breeding centre.

The larvae and nymphs of *H. elliptica* are difficult to differentiate from those of other species in the *H. (Rhipistoma)* group. The descriptions by Apanaskevich et al. (2007) were used as an aid to their identification. All the adult ticks of this taxon collected from cheetahs at the breeding centres were identified as *H. (Rhipistoma) elliptica*. Hence we thought it unlikely that the larvae and nymphs of a species other than *H. elliptica* would be present in such numbers on the vegetation.

The larvae and nymphs of *Rhipicephalus simus* were identified by comparison with the descriptions and scanning electromicrographs of Walker et al. (2000), who placed this species in the *R. simus* group of ticks. They are characterized by narrow, sloping palps, with

a slight space between the inner surface of the second segment of the palps and the lateral margin of the hypostome; the basis capituli is hexagonal, shallow with long, acute, lateral angles, the anterolateral margins of the basis capituli are straight, the posterolateral margins slightly concave and the posterior margin straight, there are no cornua; the scutum is broad and the cervical fields are visible. The only adult ticks of this group collected from the cheetahs were identified as *R. simus*. Here too we were able to eliminate the immature stages that could be confused with those of *R. simus* by referring to their host preferences and geographic distributions. There are six ticks in the *R. simus* group of species of Walker et al. (2000), of which four are present in South Africa. *Rhipicephalus distinctus* is a parasite of rock hyraxes (*Procavia capensis*) and is confined to rocky outcrops. *Rhipicephalus simpsoni* is a tick of cane rats (*Thryonomys swinderianus*) and is seldom collected in South Africa. It is unlikely that cane rats would survive at any cheetah breeding facility. *Rhipicephalus zumpti* is a tick of domestic dogs and bushpigs (*Potamochoerus porcus*) and is confined to the coastal regions of South Africa. The immature stages of ticks in the *Rhipicephalus follis* group of species of Walker et al. (2000) are also morphologically similar in appearance to those of *R. simus*. However, *R. follis*, *R. gertrudae*, *R. lounsburyi*, *R. lunulatus* and *R. neumanni* can all be eliminated because of habitat preference or geographic distribution. Moreover, *R. lounsburyi* and *R. neumanni* as well as *R. tricuspis* can be eliminated on their host preferences. The adults of *R. lounsburyi* and *R. neumanni* prefer domestic and wild ruminants and attach around their hooves, while the adults of *R. tricuspis* prefer steenbok (*Raphicerus campestris*) and spring hares (*Pedetes capensis*) as hosts; the hosts of the immature stages are unknown (Walker et al. 2000).

During the rainy season and warm months of the year, drag-sampling showed an increase in the questing activity of ticks on vegetation, whereas a decline was evident in the dry season and cold months of the year. The intensity of sunshine and rise in the midday temperature had a negative effect on questing activity, however, as few ticks were recovered from the vegetation.

Amblyomma hebraeum occurs from the northern and north-western provinces into KwaZulu-Natal and the Eastern Cape Province of South Africa, as well as into Swaziland (Howell et al., 1978). In the Eastern Cape Province and central Limpopo Province populations of adult *A. hebraeum* reach a peak during the summer months, larvae during autumn and winter and nymphs during winter and spring (Horak, 1982; Knight and Rechav, 1978; Londt et al., 1979; Norval, 1977). However, large numbers of adults have been recovered from eland (*Tragelaphus oryx*) during spring and from giraffes (*Giraffa camelopardalis*) during winter in the Kruger National Park, while large numbers of adults have been collected from buffaloes (*Syncerus caffer*) in north-eastern KwaZulu-Natal in spring (Horak et al., 1983). There appears to be no definite pattern of seasonal abundance for any of the life stages on nyalas (*Tragelaphus angasii*) in north-eastern KwaZulu-Natal (Horak et al., 1995), or on greater kudu (*Tragelaphus strepsiceros*) or impalas (*Aepyceros melampus*) in the Kruger National Park (Horak et al., 1992; Horak et al., 2003). The larvae of *A. hebraeum* quest for their hosts from the vegetation (Horak et al. 2011), whereas the nymphs and adults hunt for their hosts from the soil surface (Bryson et al., 2000). The recovery of a substantial number of nymphs on the drag-samples in the present study is probably due to the shortness of the grass sward. The source of questing larvae and nymphs

of *A. hebraeum* at AVDCC-DWB could be the cheetahs themselves, which harboured a number of adult ticks, or free-ranging impalas at the study site.

Amblyomma marmoreum is widely distributed in South Africa (Horak et al., 2006). Adults have a preference for tortoises, particularly leopard tortoises, while the immature stages may infest a variety of reptiles as well as mammals and birds (Horak et al., 2006). The presence of tortoises and birds at AVDCC-DWS probably accounted for the abundance of *A. marmoreum* at the centre.

Haemaphysalis elliptica, which is widespread in southern Africa, was formerly incorrectly lumped with *H. leachi* (Apanaskevich et al., 2007). Various wild and domestic carnivores are the preferred hosts of the adults of *H. elliptica*, including domestic dogs and cats as well as lions (*Panthera leo*), leopards (*Panthera pardus*) and cheetahs (Apanaskevich et al., 2007; Horak et al., 1987; Horak et al., 2000; Horak and Matthee, 2003; Horak et al., 2010). Several murid rodent species are the preferred hosts of its immature stages (Hoogstraal, 1956; Horak et al., 2005; Petney et al., 2004). Although Jacobs et al. (2004) demonstrated that this tick can complete more than one life cycle annually under laboratory conditions, they doubted whether this would occur in nature. *H. elliptica* is the proven vector of *Babesia rossi*, the main causative organism of canine babesiosis in domestic dogs in South Africa (Lewis et al., 1996). In a long-term survey of questing ticks in the Kruger National Park very few larvae or nymphs on this species were collected from the vegetation (Gallivan et al., 2011). The authors believed that because rodents are preferred hosts of these stages they possibly quest from the soil surface for their rodent hosts and are thus unlikely to attach to the flannel strips. Because of the preponderance of this species at AVDCC-DWB and the

generally short length of the vegetation large numbers were picked by the flannel strips. Excluding the other tick species the present survey indicated that the coolest hours of the day were possibly the prime time during which rodents were infested with the immature stages of *H. elliptica* because of the greater numbers actively questing.

Rhipicephalus simus is extensively distributed throughout southern African (Walker et al., 2000). Among domestic animals, the adult ticks primarily parasitise cattle and dogs (Horak et al., 1987; Walker et al., 2000), but they have also been recovered from many wild animals including felids (Horak et al., 1983; Horak et al., 1987; Horak et al., 2000; Norval and Mason, 1981). The immature stages prefer murid rodents as hosts, some species of which may be burrow-dwelling (Braack et al., 1996; Hoogstraal, 1956; Norval and Mason, 1981). Presence of rodents in the enclosure can have a significant impact on the population of this tick species.

The hosts of *Rhipicephalus zambeziensis* range widely from impala, bushbuck (*Tragelaphus scriptus*), nyala, greater kudu, eland and African buffalo to domestic cattle (Norval et al., 1982; Horak et al., 1983; Horak et al., 1992; Horak et al., 2003; Walker et al., 1983, 2000). All stages may use the same host species in order to complete their life cycle. The distribution of this tick is confined to the North West, Limpopo and Mpumalanga Provinces of South Africa (Horak et al., 1992; Horak et al., 2003; Norval et al., 1982). The presence of impalas, bushbuck, nyalas, kudus and elands provided suitable hosts for *R. zambeziensis* to complete its life cycle at AVDCC–DWS and at HESC.

Conclusions

Our findings indicate that, despite intensive control efforts, various tick species maintain viable populations at the three cheetah-breeding centres. We confirm that *H. elliptica* is the most numerous tick infesting cheetahs. Whether *H. elliptica* is a vector of felid-related piroplasms, e.g. *Babesia lengau*, remains to be determined.

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References

- Apanaskevich, D.A., Horak, I.G., Camicas, J.L., 2007. Redescription of *Haemaphysalis (Rhipistoma) elliptica* (Koch, 1844), an old taxon of the *Haemaphysalis (Rhipistoma) leachi* group from East and Southern Africa, and of *Haemaphysalis (Rhipistoma) leachi* (Audouin, 1826) (Ixodida, Ixodidae). Onderstepoort J. Vet. Res. 74, 181-208.
- Arthur, D.R., 1973. Scanning electron microscope studies on the morphology of the immature stages of *Amblyomma hebraeum* Koch, 1844. J. Entomol. Soc. Sthn Afr. 36, 63-85.
- Arthur, D.R., 1975. The larvae of some ixodid ticks (Acarina) from the eastern Cape Province of South Africa. Bull. Entomol. Res. 65, 405-421.
- Baker, M.K., Keep, M.E., 1970. Checklist of the ticks found on the larger game animals in the Natal game reserves. Lammergeyer 12, 41-47.
- Bosman, A.-M., Oosthuizen, M.C., Peirce, M.A., Venter, E.H., Penzhorn, B.L., 2010. *Babesia lengau* sp. nov., a novel *Babesia* species in cheetah (*Acinonyx jubatus*, Schreber, 1775) populations in South Africa. J. Clin. Microbiol. 48, 2703-2708; <http://dx.doi:10.1128/JCM.02266-09>.
- Bosman, A.-M., Venter, E.H., Penzhorn, B.L., 2007. Occurrence of *Babesia felis* and *Babesia leo* in domestic cats and various wild felid species in Southern Africa. Vet. Parasitol. 144, 33-38; <http://dx.doi:10.1016/j.vetpar.2006.09.025>.
- Braack, L.E.O., Horak, I.G., Jordaan, L.C., Segerman, J., Louw, J.P., 1996. The comparative host status of red veld rats (*Aethomys chrysophilus*) and bushveld gerbils (*Tatera leucogaster*) for epifaunal arthropods in the southern Kruger National Park, South Africa. Onderstepoort J. Vet. Res. 63, 149-158.

- Bronner, G.N., Hoffmann, M., Taylor, P.J., Chimiba, C.T., Best, P.B., Matthee, C.A., Robinson, T.J., 2003. A revised systematic checklist of the extant mammals of the southern African subregion. *Durban Mus. Novit.* 28, 56-103.
- Bryson, N.R., Horak, I.G., Venter, E.H., Yunker, C.E., 2000. Collection of free-living nymphs and adults of *Amblyomma hebraeum* (Acari: Ixodidae) with pheromone/carbon dioxide traps at 5 different ecological sites in heartwater endemic regions of South Africa. *Exp. Appl. Acarol.* 24, 971-982.
- Clark, D.D., 1995. Lower temperature limits for activity of several ixodid ticks (Acari: Ixodidae): effects of body size and rate of temperature change. *J. Med. Entomol.* 32, 449-452.
- Cumming, G.S., 2002. Comparing climate and vegetation as limiting factors for species ranges of African ticks. *Ecology* 83, 255-268; <http://doi.org/10.2307/2680136>.
- Durant, S., Mitchell, N., Ipavec, A., Groom, R., 2015. *Acinonyx jubatus*. *The IUCN Red List of Threatened Species 2015*: e.T219A50649567; <http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T219A50649567.en>. Downloaded on 09 December 2015.
- Fourie, L.J., Horak, I.G., 1994. The life cycle of *Ixodes rubicundus* (Acari: Ixodidae) and its adaptation to a hot, dry environment. *Exp. Appl. Acarol.* 18, 23-35.
- Gallivan, G., Spickett, A., Heyne, H., Spickett, A.N., Horak, I.G., 2011. The dynamics of questing ticks collected for 164 consecutive months off the vegetation of two landscape zones in the Kruger National Park (1988-2002). Part III. The less commonly collected species. *Onderstepoort J. Vet. Res.* 78, 1-9.
- Hoogstraal, H., 1956. African Ixodidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary reviews of the genera *Boophilus*, *Margaropus*

and *Hylomma*). Department of the Navy Bureau of Medicine and Surgery. Washington DC.

Horak, I.G., 1982. Parasites of domestic and wild animals in South Africa. XV. The seasonal prevalence of ectoparasites on impala and cattle in the Northern Transvaal. Onderstepoort J. Vet. Res. 49, 85-93.

Horak, I.G., Boomker, J., Flamand, J.R.B., 1995. Parasites of domestic and wild animals in South Africa. XXXIV. Arthropod parasites of nyalas in north-eastern KwaZulu-Natal. Onderstepoort J. Vet. Res. 62, 171-179.

Horak, I.G., Boomker, J., Spickett, A.M., de Vos, V., 1992. Parasites of domestic and wild animals in South Africa. XXX. Ectoparasites of kudu in the eastern Transvaal Lowveld and the Eastern Cape Province. Onderstepoort J. Vet. Res. 59, 259-273,

Horak, I.G., Braack, L.E.O., Fourie, L.J., Walker, J.B., 2000. Parasites of domestic and wild animals in South Africa. XXXVIII. Ixodid ticks collected from 23 wild carnivore species. Onderstepoort J. Vet. Res. 67, 239-250.

Horak, I.G., Fourie, L.J., Braack, L.E.O., 2005. Small mammals as hosts of immature ticks. Onderstepoort J. Vet. Res. 72, 255-261.

Horak, I.G., Gallivan, G.J., Braack, L.E.O., Boomker, J., de Vos, V., 2003. Parasites of domestic and wild animals in South Africa. XLI. Arthropod parasites of impalas (*Aepyceros melampus*) in the Kruger National Park. Onderstepoort J. Vet. Res. 70, 131-163.

Horak, I.G., Gallivan, G.J., Spickett, A.M., 2011. The dynamics of questing ticks collected for 164 consecutive months off the vegetation of two landscape zones in the Kruger National Park (1988-2002). Part I. Total ticks, *Amblyomma hebraeum* and *Rhipicephalus decoloratus*. Onderstepoort J. Vet. Res., 78, 8-17; doi: 10.4102/ojvr.v78i1.32

- Horak, I.G., Heyne, H., Donkin, E.F., 2010. Parasites of domestic and wild animals in South Africa. XLVIII. Ticks (Acari: Ixodidae) infesting domestic cats and wild felids in Southern Africa. *Onderstepoort J. Vet. Res.* 77, 1-7.
- Horak, I.G., Jacot Guillarmond, A., Moolman, L.C., de Vos, V., 1987. Parasites of domestic and wild animals in South Africa. XXII. Ixodid ticks on domestic dogs and on wild carnivores. *Onderstepoort J. Vet. Res.* 54, 573-580.
- Horak, I.G., Matthee, S., 2003. Parasites of domestic and wild animals in South Africa. XLIII. Ixodid ticks of domestic dogs and cats in the Western Cape Province. *Onderstepoort J. Vet. Res.* 70, 87-195.
- Horak, I.G., McKay, I.J., Heyne, H., Spickett, A.M., 2006. Hosts, seasonality and geographic distribution of the South African tortoise tick, *Amblyomma marmoreum*. *Onderstepoort J. Vet. Res.* 73, 13-25.
- Horak, I.G., Potgieter, F.T., Walker, J.B., de Vos, V., Boomker, J., 1983. The ixodid tick burdens of various large ruminant species in South African nature reserves. *Onderstepoort J. Vet. Res.* 50, 221-228.
- Horak, I.G., Sheppey, K., Knight, M.M., Beuthin, C.L., 1986. Parasites of domestic and wild animals in South Africa. XXI. Arthropod parasites of vaal ribbok, bontebok and scrub hares in the western Cape province. *Onderstepoort J. Vet. Res.* 53, 187-197.
- Howell, C.J., Walker, J.B., Nevill, E.M., 1978. Ticks, mites and insects infesting domestic animals in South Africa. *Science Bulletin*, 393. Department of Agricultural Technical Services, Pretoria.
- Jacobs, P.A.H., Fourie, L.J., Horak, I.G., 2004. A laboratory comparison of the life cycle of the dog ticks *Haemaphysalis leachi* and *Rhipicephalus sanguineus*. *Onderstepoort J. Vet. Res.* 71, 15-28.

- Knight, M.M., Rechav, Y., 1978. Ticks associated with kudu in the Eastern Cape: preliminary report. *Jl S. Afr. Vet. Assoc.* 49, 343-344.
- Knülle, W., 1966. Equilibrium humidities and survival of some tick larvae. *J. Med. Entomol.* 2, 335-338.
- Knülle, W., Rudolph, D., 1982. Humidity relationships and water balance of ticks. In: F.R. Obenchain, F.R., Galun, R. (Eds.), *Physiology of ticks*. Pergamon, New York, pp. 43-70.
- Lewis, B.D., Penzhorn, B.L., Lopez-Rebollar, L.M., de Waal, D.T., 1996. Isolation of a South African vector-specific strain of *Babesia canis*. *Vet. Parasitol.* 63, 9-16 ; [http://dx.doi:10.1016/0304-4017\(95\)00881-0](http://dx.doi:10.1016/0304-4017(95)00881-0).
- Londt, J.G.H., Horak, I.G., de Villiers, I.L., 1979. Parasites of domestic and wild animals in South Africa. XIII. The seasonal incidence of adult ticks (Acarina: Ixodidae) on cattle in the northern Transvaal. *Onderstepoort J. Vet. Res.* 46, 31-39.
- Londt, J.G.H., Whitehead, G.B., 1972. Ecological studies of larval ticks in South Africa (Acarina: Ixodidae). *Parasitology* 65, 469-490.
- Madden, S.C., Madden, R.C., 2005. Seasonality in diurnal locomotory patterns of adult black-legged ticks (Acari: Ixodidae). *J. Med. Entomol.* 42, 582-588.
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. *Evolution* 57, 288-296.
- Mehlhorn, H., 2008. *Encyclopaedia of parasitology*, third ed. Springer-verlag, Berlin, Heidelberg, New York.
- Mucina, L., Rutherford, M.C., 2006. *Vegetation of South Africa, Lesotho and Swaziland*. National Botanical Institute, Pretoria.

- Norval, R.A.I., 1977. Ecology of the tick *Amblyomma hebraeum* Koch in the Eastern Cape Province of South Africa. I. Distribution and seasonal activity. J. Parasitol. 63, 734-739.
- Norval, R.A.I., Mason, C.A., 1981. The ticks of Zimbabwe. II. The life cycle, distribution and hosts of *Rhipicephalus simus* Koch, 1844. Zimbabwe Vet. J. 12, 2-9.
- Norval, R.A.I., Walker, J.B., Colborne, J., 1982. The ecology of *Rhipicephalus zambeziensis* and *Rhipicephalus appendiculatus* (Acarina, Ixodidae) with particular reference to Zimbabwe. Onderstepoort J. Vet. Res. 49, 181-190.
- Nyangiwe, N., Goni, S., Hervé-Claude, L.P., Ruddat, I., Horak, I.G., 2011. Ticks on pastures and two breeds of cattle in the Eastern Cape Province, South Africa. Onderstepoort J. Vet. Res. 78, 88-96; doi: 10.4102/ojvr.v78i1.320
- Petney, T.N., Horak, I.G., 1987. The effect of dipping on parasitic and free-living populations of *Amblyomma hebraeum* on a farm and on an adjacent nature reserve. Onderstepoort J. Vet. Res. 54, 529-33.
- Petney, T.N., Horak, I.G., Howell, D.J., Meyer, S., 2004. Striped mice, *Rhabdomys pumilio*, and other murid rodents as hosts for immature ixodid ticks in the Eastern Cape Province. Onderstepoort J. Vet. Res. 71, 313-318.
- Punyua, D.K., Newson, R.M., Mutinga, M.J., 1984. Diurnal and seasonal activity of unfed adult *Rhipicephalus appendiculatus* (Acarina: Ixodidae) in relation to some intrinsic and extrinsic factors – I. Insect Sci. Appl. 6, 63-70.
- Randolph, S.E., 2002. Quantitative ecology of ticks as a basis for transmission model of tick-borne pathogens. Vector-Borne Zoonotic Dis. 2, 209-215.
- Rechav, Y., 1979. Migration and dispersal patterns of three African ticks (Acari: Ixodidae) under field conditions. J. Med. Entomol. 16, 150-163.

- Robertson, W.D., 1981. A four year study of the seasonal fluctuations in the occurrence of the blue tick *Boophilus decoloratus* (Koch) in the coastal regions of the eastern Cape. In: Whitehead, G.B., Gibson, J.E. (Eds.), Tick Biology and Control. Proc. Int. Conf., 27–29 January 1981, Rhodes University, Grahamstown, South Africa, pp. 199-204.
- Short, N.J., Norval, R.A.I., 1981. The seasonal activity of *Rhipicephalus appendiculatus* Neumann 1901 (Acarina: Ixodidae) in the Highveld of Zimbabwe/Rhodesia. J. Parasitol. 67, 77-84.
- Skinner, J.D., Chimimba, C.T., 2005. Mammals of the Southern African Subregion, third ed. Cambridge University Press, Cape Town.
- Sonenshine, D.E., Atwood, E.L., Lamb, J.T., 1966. The ecology of ticks transmitting Rocky Mountain spotted fever in a study area in Virginia. Ann. Entomol. Soc. Am. 59, 1234-1262; <http://dx.doi.org/10.1093/aesa/59.6.1234>.
- Spickett, A.M., Gallivan, G.J., Horak, I.G., 2011. The dynamics of questing ticks collected for 164 consecutive months in off the vegetation of two landscape zones in the Kruger National Park (1988-2002). Part II. *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*. Onderstepoort J. Vet. Res. 78, 18-26; <http://dx.doi:10.4102/ojvr.v78i1.233>
- Spickett, A.M., Horak, I.G., Braack, L.E.O., van Niekerk, A., 1991. Drag-sampling of free-living ixodid ticks in the Kruger National Park. Onderstepoort J. Vet. Res. 58, 27-32.
- Theiler, G., 1962. The Ixodoidea Parasites of Vertebrates in Africa South of the Sahara (Project S.9958). Report to the Director of Veterinary Services, Onderstepoort.
- Vail, S., Smith, G., 1998. Air temperature and relative humidity effects on behavioral activity of blacklegged tick (Acari: Ixodidae) nymphs in New Jersey. J. Med. Entomol. 35, 1025-1028.

- Van Dyk, A., 1991. The Cheetahs of De Wildt. Struik, Cape Town.
- Verdoorn, G.H., 1998. Controlling ectoparasites in a cheetah breeding facility. In: Penzhorn, B.L. (Ed.), Proceedings of a Symposium on Cheetahs as Game Ranch Animals. Wildlife Group of the South African Veterinary Association, Onderstepoort, pp. 50-53.
- Voltzit, O.V., Keirans, J.E., 2003. A review of African *Amblyomma* species (Acari, Ixodida, Ixodidae). *Acarina* 11, 135-214.
- Walker, J.B., 1974. The ixodid ticks of Kenya. Commonwealth Institute of Entomology, London.
- Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. Academic Press, Cambridge.
- Walker, J.B., Norval, R.A.I., Corwin, M.D., 1981. *Rhipicephalus zambeziensis* sp. nov., a new tick from Eastern and Southern Africa, together with a redescription of *Rhipicephalus appendiculatus* Neuman, 1901 (Acarina: Ixodidae). *Onderstepoort J. Vet. Res.* 48, 87-104.
- Zimmerman, R.H., Garris, G.I., 1985. Sampling efficiency of three dragging techniques for the collection of nonparasitic *Boophilus microplus* (Acari: Ixodidae) larvae in Puerto Rico. *J. Econ. Entomol.* 78, 627-631; <http://dx.doi.org/10.1093/jee/78.3.627>.