International Journal for Parasitology: Parasites and Wildlife 2 (2013) 1–9

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

# Tick infestation patterns in free ranging African buffalo (*Syncercus caffer*): Effects of host innate immunity and niche segregation among tick species ☆

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ARTICLE INFO

*Article history:*
Received 21 September 2012
Received in revised from 30 October 2012
Accepted 1 November 2012

*Keywords:*
*Amblyomma hebraeum*
Co-infestation
Immunity
Host traits
*Rhipicephalus evertsi evertsi*

ABSTRACT

Ticks are of vast importance to livestock health, and contribute to conflicts between wildlife conservation and agricultural interests; but factors driving tick infestation patterns on wild hosts are not well understood. We studied tick infestation patterns on free-ranging African buffalo (*Syncercus caffer*), asking (i) is there evidence for niche segregation among tick species?; and (ii) how do host characteristics affect variation in tick abundance among hosts? We identified ticks and estimated tick burdens on 134 adult female buffalo from two herds at Kruger National Park, South Africa. To assess niche segregation, we evaluated attachment site preferences and tested for correlations between abundances of different tick species. To investigate which host factors may drive variability in tick abundance, we measured age, body condition, reproductive and immune status in all hosts, and examined their effects on tick burdens.

Two tick species were abundant on buffalo, *Amblyomma hebraeum* and *Rhipicephalus evertsi evertsi*. *A. hebraeum* were found primarily in the inguinal and axillary regions; *R. e. evertsi* attached exclusively in the perianal area. Abundances of *A. hebraeum* and *R. e. evertsi* on the host were unrelated. These results suggest spatial niche segregation between *A. hebraeum* and *R. e. evertsi* on the buffalo. Buffalo with stronger innate immunity, and younger buffalo, had fewer ticks. Buffalo with low body condition scores, and pregnant buffalo, had higher tick burdens, but these effects varied between the two herds we sampled.

This study is one of the first to link ectoparasite abundance patterns and immunity in a free-ranging mammalian host population. Based on independent abundances of *A. hebraeum* and *R. e. evertsi* on individual buffalo, we would expect no association between the diseases these ticks transmit. Longitudinal studies linking environmental variability with host immunity are needed to understand tick infestation patterns and the dynamics of tick-borne diseases in wildlife.



## Introduction

Ticks are of considerable importance to wildlife and livestock health due to their role as vectors of an impressive array of infectious agents, as well as direct injury caused by piercing the host's skin (Allen, 1994). Widely distributed infectious agents such as *Ehrlichia ruminantium* (causative agent of heartwater, Allsopp, 2010), *Babesia bigemina* (causative agent of bovine babesiosis/African redwater, Bock et al., 2004; Suarez and Noh, 2011), *Anaplasma marginale* (causative agent of bovine anaplasmosis, Kocan et al., 2010) and *Theileria parva* strains (causing Corridor Disease in South Africa, East Coast Fever in East Africa, and January Disease in Zimbabwe, Bishop et al., 2004) are examples of important tick-borne pathogens causing clinical disease in livestock. These pathogens are often carried by wildlife species, and some also cause zoonotic disease in humans. As such, ticks and the pathogens they transmit are particularly relevant at the wildlife/livestock/human interface (Smith and Parker, 2010).

Despite this, few studies have focused on quantifying the abundance and co-infestation patterns of different tick species on wildlife hosts. When parasites co-occur on a host they may compete for space or resources, facilitate one another, or avoid one another by segregating to occupy different ecological niches (Rohde, 1979; Chilton et al., 1992; Simkova et al., 2002; O'Callaghan et al., 2006). Niche segregation has been observed in both internal parasites (O'Callaghan et al., 2006) and ectoparasites (Spickett et al., 1991; Simkova et al., 2002). Mechanisms of niche segregation in



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http://dx.doi.org/10.1016/j.ijppaw.2012.11.002

parasites include specialization of mouthparts to take advantage of specific attachment sites (Simkova et al., 2002), utilizing different food sources (mites vs. ticks), or temporal separation (winter emergence vs. spring emergence; Mullen et al., 2009). Niche segregation reduces competition over evolutionary time and may allow multiple tick species to coexist on the host. As such, niche segregation may increase the risk of concurrent exposure to pathogens vectored by different tick species. By contrast, current competition among tick species would be expected to reduce vector co-occurrence on individual hosts, leading to disparate infection profiles for the diseases they transmit. Understanding tick co-infestation patterns can thus lead to a better understanding of tick-associated morbidity as well as the epidemiology of tick-borne diseases.

Spatial and temporal abundance patterns of ticks have been investigated in the context of environmental factors, such as climatic variables (e.g. Jackson et al., 1996; Olwoch et al., 2007; Randolph, 2008, Estrada-Pena, 2009; Gilbert, 2010), fire (e.g. Davidson et al., 1994; Horak et al., 2006a; Padgett et al., 2009), habitat type and configuration (Ostfeld et al., 1995; Fyumagwa et al., 2007; Thamm et al., 2009). In livestock species, host factors including genetic and immunological traits that confer varying degrees of resistance to tick infestation have been of great interest (O'Neill et al., 2010; Berman, 2011; Carvalho et al., 2011; Neto et al., 2011). Comparisons of tick burdens among African ungulate species have indicated that host body size and habitat preference affect tick abundance, with larger species and browsers more heavily infested with ticks than smaller species and grazers (Olubayo et al., 1993; Gallivan and Horak, 1997). Resistance to ticks has been invoked to explain low tick burdens (e.g. blue wildebeest: Horak et al., 1983), or failure of some tick species to develop on wildlife species (e.g. *Rhipicephalus (Boophilus) decoloratus* on African buffalo: Horak et al., 2006b). behavioral: traits such as grooming (Mooring et al., 2004) and aggregation of conspecifics (Monello and Gompper, 2010) may modify tick burdens, and individual traits such as sex, developmental stage, and reproductive status have been shown to affect tick burdens in several wildlife species (e.g. Cape ground squirrel: Hillegass et al., 2008; bison: Mooring and Samuel, 1998a; impala: Mooring et al., 1996; Mooring and Hart, 1997; mice and chipmunks: Shaw et al., 2003). Seasonal reproductive activities can carry a cost in terms of increased ectoparasite abundances in both males and females (Main et al., 1981; Drew and Samuel, 1985; Sharifi et al., 2008). In contrast to ecological and demographic host characteristics, the connections between host immune parameters and ectoparasite infestation have rarely been explored in wildlife species (but see Oliver et al., 2009 for an example of MHC diversity affecting tick burdens in water voles). Tick feeding induces a complex array of host immune responses involving cellular and serological components of innate immunity (e.g. complement, neutrophils, basophils, eosinophils), and T-cell, as well as B-cell mediated adaptive immune responses (reviewed in Brossard and Wikel, 2004). Associations between any of these immune mediators and tick burdens have rarely been explored in wild mammalian hosts (but see Pfaeffle et al., 2009 on tick infestations and hematology in the European hedgehog).

In southern Africa, many of the tick species and tick-borne pathogens affecting livestock and humans are shared with African buffalo (Norval and Horak, 2004). Individual buffalo typically harbor multiple species of tick, with 11 tick species described from buffalo in South Africa (Horak et al., 2007). However, co-infestation patterns of different tick species on buffalo have not been investigated, and host factors driving variability in tick abundance among buffalo are unknown. We investigated tick co-infestation patterns in 134 female African buffalo at Kruger National Park (KNP), South Africa, focusing on two main questions: (i) what are the patterns of coinfestation among different tick species and stages: is there evidence for niche separation among tick species coinfesting buffalo?; and (ii) how do host characteristics, including age, body condition, reproductive status, and simple measures of innate and adaptive immunity, affect tick intensities?

## Material and methods

### *Study population*

We sampled buffalo from two herds in the southern section of Kruger National Park (KNP), the Lower Sabie (LS) herd and the Crocodile Bridge (CB) herd. Each of our study herds comprises approximately 800–1000 buffalo. KNP is located within the Mpumalanga province of South Africa and covers 18,989 total square kilometers, with a total buffalo population of approximately 30,000 individuals (Cross et al., 2009). Young adult female buffalo were captured via chemical immobilization by helicopter during two separate capture periods from June 23 to July 5, 2008 (LS herd) and October 1 to 8, 2008 (CB herd). These captures were conducted to initiate a 4 year longitudinal study on disease ecology in the buffalo. Animals were immobilized with a combination of etorphine hydrochloride (M99) and ketamine and reversed with diprenorphine (M5050) following data collection. Captures were performed by South African National Parks (SANParks) veterinarians and game capture staff, and all procedures approved by Oregon State University (ACUP# 3267), University of Montana (AUP#: 027-05VEDBS-082205), and SANParks Institutional Care and Use Committees. Two hundred buffalo were captured in total (100 per herd); of these, 166 animals were sampled for ticks. Thirty-four animals were omitted due to time constraints during captures.

### *Specimen collection*

Ticks were collected from three body areas on each buffalo: axilla, inguinal, and perianal. These areas were selected due to the high density of tick attachment associated with these regions in cattle (Barnard et al., 1989), which we confirmed also to be the case in buffalo during preliminary visual surveys. Ears were taken into consideration initially during preliminary surveys, but few ticks were found and so this site was not included in our sampling protocol. Ticks were collected at random by both manual removal and the use of hemostats; adults, nymphs and larvae were included in the collections. At least four adult ticks were collected from each region per host animal, and immature stages where possible. Buffalo were sampled systematically in July to determine tick species composition, and sporadically in October to confirm species identification for October samples. Specimens were preserved in 70% ethanol and shipped to the US Department of Agriculture's National Veterinary Services Laboratories (NVSL) in Ames, Iowa, for identification. A total of 990 ticks were submitted in July and 54 ticks in October.

### *Estimating tick abundance*

Initial detailed surveys on immobilized buffalo were used to identify body areas of high tick attachment. Based on these initial surveys, we selected inguinal, axillar and perianal regions as focal areas for tick abundance estimation. To enable estimation of tick abundances on a large sample size of hosts, given the constraint of limited immobilization time, we then used tick counts from photographs of these areas to estimate tick abundance on each host. Similar methods using focal host body areas to estimate individual tick burdens have been used previously in other ungulate species (e.g. roe deer, Kiffner et al., 2010; cattle, L'Hostis et al., 1994; impala, Matthee et al., 1997; sheep, Ogore et al., 1999; sheep, goats, mountain reedbuck, Fourie and Vanzyl, 1991). Digital photographs were taken of the axillary, inguinal, and perianal re-

gions on each host animal before any specimens were removed. Tick sexes, species, and stages were visually identified and counted by photograph, by a single observer (KA). For adult ticks, we were able to distinguish the two dominant species (*Amblyomma hebraeum* and *R. e. evertsi* – see results) from photos, and we were able to sex adult *Amblyomma* ticks. Sex determination by photograph was not possible for *Rhipicephalus* spp., so all photographed *Rhipicephalus* spp. were grouped together and all photographed immature stages were analyzed together under the category "immatures" for analysis.

In the axillary and inguinal regions, tick counts were done on photos from one side of each animal – whichever side could be exposed sufficiently during immobilizations to allow representative photography. These areas were exposed by raising the upper limb of the laterally recumbent buffalo. For the perianal region, a photograph of the entire area was taken for each animal, and all photographed ticks counted. The overall tick abundance for each subject animal was then estimated as $2 \times$ (inguinal count + axillary count) + perianal count. Photos that were blurry, dark, or taken from too far away were discarded from the data set. Tick abundances were thus estimated for 134 buffalo.

### *Host characteristics*

For each immobilized buffalo we collected information on age, body condition, pregnancy, lactation status, innate and adaptive immunity. Descriptive statistics for these host traits are summarized in Table 1. Age in buffalo was determined by evaluating incisor eruption in animals that were aged 2–5, and based on body size and horn development in animals that were younger than 2. Animals that were 6+ were aged by wear of incisor one (Jolles, 2007). Body condition was determined by palpating and visually examining main areas of body fat storage on buffalo (spine, ribs, hips, and tail base). Condition scores for each body area were assigned on a scale of 1–5 (5 being the best possible condition), and averaged across all body areas to obtain an overall condition score for each animal, and this index is significantly correlated with kidney fat index in buffalo (Ezenwa et al., 2009). Pregnancy status was determined by rectal palpation while lactation was assessed by milking all four teats. Innate immunity was assessed using a previously described bacterial killing assay (BKA; Beechler et al., 2012), which measures bactericidal activity of whole blood against a standard laboratory strain of *Escherichia coli* (ATCC 8739). Bacterial killing in blood is mediated by serological (primarily complement) and cytological (chiefly neutrophils, macrophages) effectors of the innate immune system. The BKA thus gives an integrative and easily interpreted index of innate immunity, where more bacterial killing indicates greater innate immune activity. To assess adaptive immunity, we measured the interferon-gamma (IFNγ) response to *in vitro* challenge with pokeweed mitogen, following previously described protocols (Jolles et al., 2008; Ezenwa et al., 2010), but with the addition of pokeweed (Sigma–Aldrich, product #L9379) as an immune stimulant. IFNγ is a cytokine released by T-helper lymphocytes (type 1) upon antigenic or mitogenic (e.g. pokeweed) stimulation, and acts as a primary mediator of macrophage activation (Abbas et al., 1996). We quantified T-lymphocyte responsiveness as the proportionate change in IFNγ titers, ln([IFNγ (pokeweed)]/[ IFNγ (control)]), where IFNγ (pokeweed) is the concentration of IFNγ measured in samples stimulated with pokeweed, and IFNγ (control) is the concentration of IFNγ measured in control samples, representing circulating IFNγ levels.

**Table 1**
Host traits of buffalo captured in July (Lower Sabie herd) and October (Crocodile Bridge herd) 2008. Buffalo captured in October were in poorer condition (*t*-test, $t = 16.5$, $p < 0.0001$), had more ticks (Mann–Whitney *U* test, $Z = 10.2$, $p < 0.0001$), and were less likely to be lactating ($\chi^2 = 8.9$, $p < 0.01$), than buffalo captured in July.

| *N* | July capture/ LS herd 84 | October capture/ CB herd 58 |
|---|---|---|
| Ticks: median [range] | 131 [36–339] | 1282 [663–1321] |
| Age: median [range] | 2 [1–15] | 4 [2–14] |
| Pregnant:% [number] | 11.9% [10] | 10.3% [6] |
| Lactating:% [number] | 17.9% [15] | 1.7% [1] |
| Condition: average ± SE | 4.28 ± 0.05 | 2.81 ± 0.08 |
| BKA:% average ± SE | 0.56 ± 0.03 | 0.57 ± 0.04 |
| Mitogen response: average ± SE | 1.65 ± 0.09 | 1.96 ± 0.07 |

### *Statistical analyses*

Tick infestation prevalence was calculated as the number of buffalo infested with a given tick species divided by the total number of buffalo examined. The distribution of ticks among buffalo was not normal for either of our sampling periods, but was normalized for data analysis by log-transforming the data. All analyses evaluating effects of host traits on tick burdens were therefore performed using log-transformed tick burden data as the response variable. We assessed the effects of host characteristics (e.g. age, pregnancy, lactation, body condition, BKA, pokeweed response, herd/capture period) on tick burdens using generalized linear models (GLZ), with a normal distribution for the response variable and a log link function. Our model included all variables as main effects, as well as interactions of all predictor variables, except lactation, with herd/capture period, since tick burdens differed dramatically between capture periods/herds (Table 1). We omitted a herd/capture period $\times$ lactation interaction, because only one animal was lactating in the October/CB sample. Variables were evaluated based on statistical significance (at $\alpha = 0.05$). We examined co-infestation patterns among the various tick species, sexes and stages using simple linear regressions. We used chi-square tests to test for differences in attachment site preferences between tick species and stages.

## Results

### *Tick species and prevalences*

*R. e. evertsi* and *A. hebraeum* were identified in all life stages on sampled buffalo for both sampling periods. *Rhipicephalus appendiculatus* was identified in all life stages in October, and all life stages except larvae were identified in July. For ticks submitted to the NVSL, *R. appendiculatus* was found in the lowest numbers, with only 2 adults of 134 total *Rhipicephalus* adults submitted. Our adult tick counts from photographs indicated that all buffalo were infested with *A. hebraeum*, while prevalence of *Rhipicephalus* was also high (85.5% in LS herd, 97.1% in CB herd). *Amblyomma* were numerically dominant, with 79% (SE = 1.4) of a host's adult tick burden composed of *Amblyomma*.

### *Co-infestation patterns*

The numbers of male and female *Amblyomma* were tightly correlated (LS: $r = .57$, $t = 6.21$, $p < .001$, $N = 84$; CB: $r = .83$, $t = 11.42$, $p < .001$, $N = 59$); Fig. 1a and b), but the sex ratio of adult *Amblyomma* showed a strong male bias (LS: male:female = 7.74, SD = .10; CB: 3.2, SD = .41). The number of immatures found on each host was also positively correlated with the number of adult *Amblyomma* (LS: $r = .33$, $t = 3.14$, $p = .0024$, $N = 84$; CB: $r = .39$, $t = 3.07$, $p = .0033$, $N = 56$); Fig. 1c and d). *Rhipicephalus* numbers

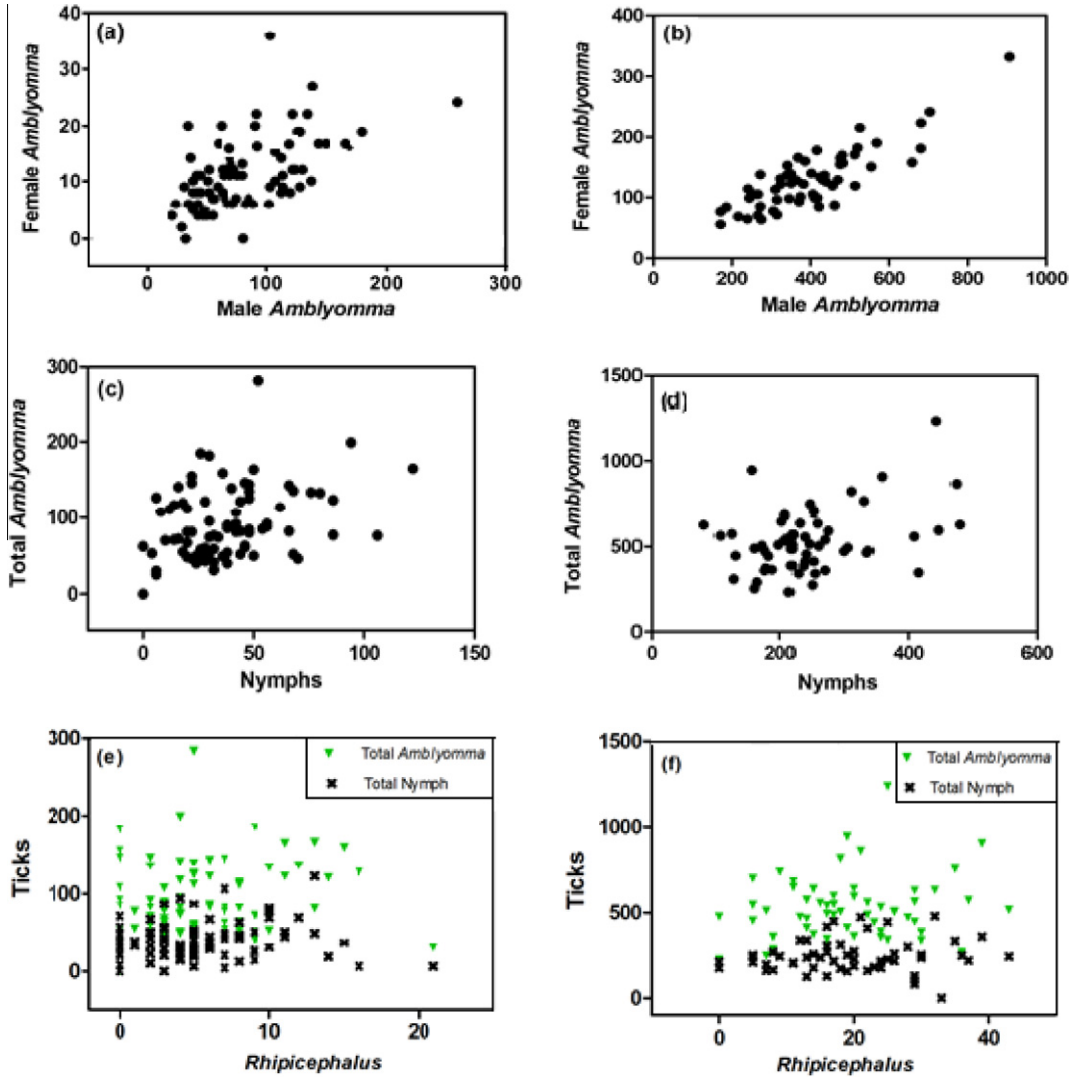


**Fig. 1.** Coinfection patterns between male and female adult *Amblyomma hebraeum*, immature ticks and *Rhipicephalus* spp. on buffalo hosts. Panels on the left show data for buffalo captured in July 2008 (Lower Sabie herd); panels on the right show patterns for buffalo captured in October 2008 (Crocodile Bridge herd). "Total *Amblyomma*" in panels c and d refers to the sum of male and female *Amblyomma* observed on each buffalo.

did not correlate to adult *Amblyomma* (LS/July: $r = .17$, $t = 1.60$, $p = .114$, $N = 84$; CB/October: $r = .21$, $t = 1.641$, $p = .106$, $N = 59$; Fig. 1e and f) or immature stages (correlation; LS/July: $r = .10$, $t = .934$, $p = .353$, $N = 84$; CB/October: $r = .158$, $t = 1.179$, $p = .244$, $N = 56$); Fig. 1e and f).

### *Niche segregation*

Tick species and stages showed very distinct distributions among host body areas. Adult *Amblyomma* were found in decreasing order of abundance in the following regions – inguinal, axillary, and perianal regions (Fig. 2a and b). Adult *Rhipicephalus* were found exclusively in the perianal area (Fig. 2a and b). Immature ticks were found in relatively equal intensities between the inguinal and axillar regions, and rarely in the perianal area (Fig. 2a and b), suggesting that most immatures counted from photographs may have been *Amblyomma*. There was no difference in attachment site preference between male and female *Amblyomma* (LS herd: $\chi^2$ test, $\chi^2 = 1.325$, $p = .5156$; CB herd: $\chi^2$ test, $\chi^2 = 5.481$, $p = .0645$), but adult *Amblyomma* differed significantly from immatures (LS herd: $\chi^2$ test, $\chi^2 = 21.600$, $p = <.001$; CB herd: $\chi^2$ test, $\chi^2 = 80.262$, $p = <.0001$) and from adult *Rhipicephalus* (LS herd: $\chi^2$ test, $\chi^2 = 30.455$, $p = <.0001$; CB herd: $\chi^2$ test, $\chi^2 = 138.046$, $p = <.0001$), as did immatures and *Rhipicephalus* (LS herd: $\chi^2$ test, $\chi^2 = 39.132$, $p = <.0001$; CB herd: $\chi^2$ test, $\chi^2 = 203.117$, $p = <.0001$). Adults of *Amblyomma* and *Rhipicephalus* only co-occurred in the perianal region. However, attachment densities of the two species in the perianal region were uncorrelated (correlation; LS herd: $r = .07$, $t = .64$, $p = .53$, $n = 80$; CB herd: $r = .17$, $t = 1.44$, $p = .153$, $n = 70$).

Attachment site preference did not vary by herd for any of the ticks (female *Amblyomma*: $\chi^2$ test LS herd vs. CB herd, $\chi^2 = 2.162$, $p = .3393$; male *Amblyomma*: $\chi^2$ test LS herd vs. CB herd, $\chi^2 = 3.731$, $p = .1548$; immatures: $\chi^2$ test LS herd vs. CB herd, $\chi^2 = 2.0984$, $p = .3502$; 100% of *Rhipicephalus* species located in the perianal area in both LS herd and CB herd).

### *Effects of host traits on tick infestation*

Animals sampled in October (CB herd) had more ticks than those sampled in July (LS herd); buffalo with weaker innate immunity, and older buffalo, had more ticks than younger animals and those with stronger innate immunity (Table 2). Animals in poorer body condition had more ticks in the October capture period/CB

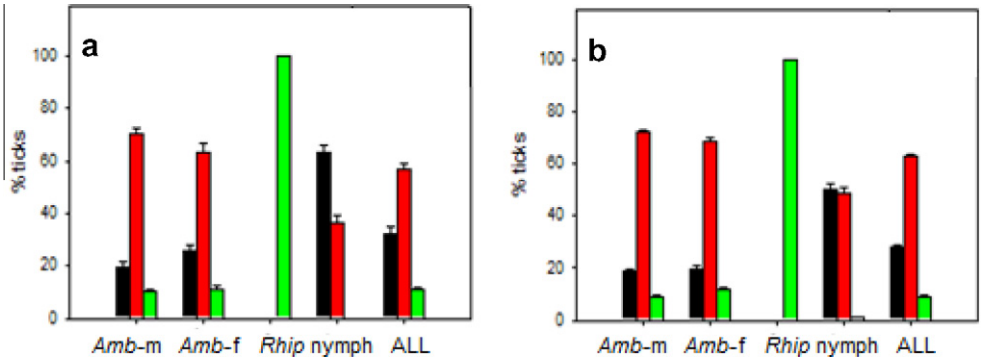


**Fig. 2.** Attachment site preferences of male *Amblyomma hebraeum* (*Amb*-m), female *A. hebraeum* (*Amb*-f), *Rhipicephalus* spp. (*Rhip*), immature ticks, and all species and stages pooled. Panel a shows data from animals caught in July 2008 (Lower Sabie herd), panel b shows data from the October 2008 (Crocodile Bridge herd) capture. Black bars denote the fraction of ticks of each group that were found attached to the axillary area of the buffalo, red bars show the fraction of ticks on the inguinal area, and green bars show the fraction of ticks attached to the perianal area of the host. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Effects of host traits and capture period/herd affiliation on tick burden in African buffalo. Predictors n2shown in red are statistically significant at $\alpha = 0.05$.

| | Estimate ± SE | Wald – Statistic | p |
|---|---|---|---|
| Intercept | 1.805 ± 0.046 | 1548.545 | 0.000000 |
| Capture period/herd (Oct) | −0.233 ± 0.043 | 29.005 | 0.000000 |
| Age | 0.006 ± 0.003 | 5.016 | 0.025115 |
| Condition | −0.007 ± 0.009 | 0.671 | 0.412590 |
| Pregnant (no) | −0.015 ± 0.008 | 3.311 | 0.068820 |
| Milk (no) | −0.010 ± 0.013 | 0.638 | 0.424572 |
| BKA | −0.040 ± 0.015 | 7.164 | 0.007437 |
| Mitogen responsiveness (MR) | −0.003 ± 0.007 | 0.172 | 0.678476 |
| Capture period × age | 0.002 ± 0.002 | 0.565 | 0.452164 |
| Capture period × condition | 0.019 ± 0.009 | 4.423 | 0.035455 |
| Capture period × pregnant | −0.022 ± 0.008 | 8.116 | 0.004387 |
| Capture period × BKA | −0.020 ± 0.015 | 1.663 | 0.197188 |
| Capture period × MR | −0.003 ± 0.008 | 0.129 | 0.719286 |

herd, but not in the July capture period/LS herd, resulting in a significant interaction term between condition and capture period/herd (Table 2). Pregnant animals carried higher tick burdens in the July capture/LS herd, but this effect was not evident in the October capture period/CB herd, resulting in a significant interaction between pregnancy and capture period/herd (Table 2). The effect of innate immunity (BKA) on tick burden was much stronger in the July capture period/LS herd than in the October capture period/CB herd; but the BKA × capture period/herd interaction term was not statistically significant. Lactation and immune responsiveness to mitogen stimulation had no effect on tick burdens.

## Discussion

### *Tick species detected*

The three tick species identified in this study, *A. hebraeum*, *R. e. evertsi* and *R. appendiculatus,* are ixodid ticks and have previously been observed on buffalo and in KNP (Horak et al., 2007, 2011; Gallivan, 2011; Spickett, 2011). All three species infest a broad range of host species (*A. hebraeum*: Horak et al., 1987; *R. e. evertsi* and *R. appendiculatus*: Walker et al., 2003); and serve as vectors for several important disease agents of wildlife and livestock. *A. hebraeum* transmits *E. ruminantium*, *Theileria mutans* and *T. velifera; R. e. evertsi* vectors *Anaplasma marginale*, *Babesia bigemina*, *B. caballi*, *Eimeria ovina* and *Theileria equi*, and can cause paralysis in sheep, lambs, and calves from components of its saliva; and *R. appendiculatus* acts as vector for *Eimeria bovis*, *Theileria parva* and *T. taurotragi* (Norval and Horak, 2004). Based on previous surveys of ectoparasites on buffalo in similar regions of South Africa, we might have also expected to find *R. (Boophilus) decoloratus*, (Young and van den Heever, 1969), *Amblyomma marmoreum*, *Hyalomma truncatum*, or *Rhipicephalus simus* (Horak et al., 2007) – but these were not encountered in our southern KNP hosts during the July and October sampling periods. We did not enumerate total tick populations on each host. In particular, we expect to have missed most larval and many nymphal ticks because live sampling of ticks biases toward adults (Horak et al., 1995), and some adults preferring attachment sites outside of the body areas we focused on. Consequently, we may have missed ticks that are present on buffalo predominantly in the larval stage, such as *R. decoloratus* – though limited sampling in and near KNP also found no *R. decoloratus* on buffalo (Horak et al., 2006b), despite the fact that the species is common in the park (Horak et al., 2011). In addition, tick species vary tremendously in their seasonal emergence patterns, and as such, any given sample represents an incomplete snapshot in time of the larger tick community present in a habitat. For example, *H. truncatum* and *R. simus* adults are most abundant in summer (December–April), rather than during the dry winter months and spring when we sampled buffalo. The same is true of *R. appendiculatus* adults, which we may also have expected to see in larger numbers, had our sampling periods included the summer months (Norval and Horak, 2004).

### *Co-infestation patterns and Niche segregation*

Our data revealed striking differences in attachment site preferences among tick stages and species, leading to minimal interspecific overlap in body areas utilized on the host. *A. hebraeum* adults and immatures were found abundantly in the axillar and inguinal areas, and least often in the perianal area on buffalo, whereas *R. e. evertsi* adults were restricted to perianal attachment sites. These results are consistent with previous qualitative observations (Walker et al., 2003). We also counted far more males on the host than females for *A. hebraeum.* This is typical for most ixodid species, where males spend more time on the host than females (Horak et al., 2007). Mechanisms underlying this sex bias in attachment duration may include engorged females being detached from the host more easily than males by host grooming behaviors (Horak et al., 2006b). In addition, female ticks leave the host voluntarily once engorged, while males may tend to remain on the host for longer to continue questing for mates. Attachment patterns for *Amblyomma* are male pheromone driven for both female and immature stages (reviewed in Sonenshine, 2006). This mechanism

may explain our observation that numbers of female *A. hebraeum* and immature tick stages strongly correlated with male *Amblyomma* burdens.

Attachment site preferences have been described for a range of tick species and stages on a variety of hosts (e.g. different stages of *Ixodes ricinus* on roe deer, Kiffner et al., 2011; and sheep, Ogden et al., 1998; *Ixodes scapularis* on white-tailed deer, Schmidtmann et al., 1998; *I. rubicundus* on different cattle breeds, Fourie and Horak, 1993; *Rhipicephalus sanguineus* on dogs, Dantas-Torres and Otranto, 2011), though seldom in the context of coinfestation by multiple tick species (but see Fourie and Kok, 1995: *Hyalomma truncatum* and *H. marginatum* on sheep; Vathsala et al., 2008: four tick species on sheep and goats; Jacobs et al., 2001: *R. sanguineus* and *Haemaphysalis leachi* on dogs). Niche segregation of ticks has been primarily studied in terms of environmental conditions such as temperature and humidity, as they relate to tick geographic distributions and potential responses to climate change (e.g. reviews by Daniel et al., 2003; Estrada-Pena, 2009). Niche segregation on the host has been examined in two tick species parasitizing reptiles, *Amblyomma limbatum* and *Aponomma hydrosauri* (Chilton et al., 1992), which attach to different areas on the host. The proximate benefits for this site specificity are unclear. Experimental data show that both species can feed and reproduce at other attachment sites than the preferred areas; however, spatial segregation of parasite species on their hosts may aid in finding mates efficiently. Ultimately the two species do avoid competing directly under this arrangement; perhaps the current status quo represents the "ghost of competition past". We have not conducted experiments in our study system to determine the mechanisms underlying spatial segregation of *A. hebraeum* and *R. e. evertsi*, but their co-infestation patterns suggest that these species may currently not be competing strongly on African buffalo as hosts: Abundances of *A. hebraeum* and *R. e. evertsi* were uncorrelated on the buffalo, and this result was robust to comparing tick burdens in the perianal region only, or including all three body areas considered in this study. Based solely on vector distributions among hosts, we would thus not expect to see positive or negative associations between infectious microparasites vectored by these two species in African buffalo. If such associations do exist, they are likely to have other underlying causes than heterospecific tick aggregations on their buffalo hosts.

### *Effects of host traits on tick infestation*

#### *Herd affiliation/sampling period*

Buffalo we sampled in the CB herd in October had much higher tick burdens than those sampled in the LS herd in July. However, because we did not resample the same herds (or ideally, the same individuals) during the two capture periods, we are unable to assign causation of this difference in tick burden unambiguously. Seasonal differences in exposure or susceptibility to questing ticks may play a role; but the two buffalo herds we accessed also have distinct home ranges and might thus experience differences in tick exposure and susceptibility based on the habitat they utilize and resources available to them. All of the tick species reported here show pronounced seasonal abundance patterns (*R. appendiculatus*: Okello-Onen et al., 1999; *A. hebraeum*, *R. e. evertsi*: Horak et al., 1983). Our results are consistent with Horak et al. (1987), who found *A. hebraeum* burdens to be higher during the dry season. They suggest that this may be due to resource restriction leading to reduced grooming by the host. In addition, resource restriction (Lochmiller and Deerenberg, 2000; Alonso-Alvarez and Tella, 2001; Houston et al., 2007; Martin et al., 2008) and photoperiod (Zhou et al., 2002; Prendergast et al., 2008) may also affect immune function and thus susceptibility to parasites. Seasonal variability in exposure and susceptibility to ticks is thus likely to contribute to the observed difference in tick burdens between our July (LS herd) and October (CB herd) samples. In addition, resource availability is strongly habitat dependent in our study system. The CB herd (October sample) experiences extreme and more prolonged food restriction in the dry season than does the LS herd (July sample; Jolles and Ezenwa, unpublished data), evident in our data as poor body condition and virtual absence of lactating females in the CB herd in October. Any effect of season on host susceptibility or exposure to ticks may therefore be mediated by herd affiliation due to differential resource availability for the two herds.

#### *Host age*

Our finding that older buffalo carried higher tick burdens than younger animals is consistent with previous studies in cattle (Okello-Onen et al., 1999), impala (Gallivan et al., 1995), roe deer (Vor et al., 2010), dogs and rabbits (Brown, 1984). This pattern may simply be due to larger size of adult buffalo, offering more habitat for ectoparasites; or grooming by their dam may reduce tick burdens in juveniles (Okello-Onen et al., 1999). Literature on the evolution of parasite-defense grooming in ungulates also suggests a "body size principle", based on the recognition that smaller animals, with a greater surface area-to-mass ratio, may incur higher costs for a given density of tick infestation relative to larger ones (Hart et al., 1992). Accordingly, smaller individuals have been observed to groom at a higher rate than larger conspecifics in impala (*Aepycerus melampus*, Mooring and Hart, 1997), bison (*Bos bison*, Mooring and Samuel, 1998a) and elk (*Cervus elaphus*, Mooring and Samuel, 1998b). Another hypothesis suggests that young animals are more capable of protecting themselves from ticks by innate immunity and cell mediated immunity (Brown, 1984; Okello-Onen et al., 1999), though in our study animals there was no age trend in innate immunity (Beechler et al., 2012).

#### *Host immune status*

Our data revealed a negative association between innate immunity (measured as host blood bacterial killing ability, BKA) and tick abundance, but no association with lymphocyte response to mitogen stimulation, a measure of adaptive immune responsiveness. The long blood meal of ticks (days to weeks) requires that they deregulate host physiological processes such as hemostasis, vasoconstriction, inflammation, pain perception and immunity. As such, several aspects of host immunity are relevant in limiting tick feeding success, including both innate and adaptive immune defenses. Our measure of innate immunity, BKA, is strongly driven by complement and neutrophil activity (Beechler et al., 2012). The complement system is a powerful component of innate immunity; its activation results in production of inflammatory anaphylatoxins, marking ("opsonizing") of incoming microbes and foreign particles for destruction by phagocytic cells, and assembly of a membrane attack complex that disrupts cellular lipid bilayers. Tick saliva contains several proteins that specifically inhibit complement function (reviewed in Schroeder et al., 2009). Complement inhibition is essential for completion of the tick's blood meal; and specificity of complement inhibitor proteins to particular host species correlates with the host range of the tick (Lawrie et al., 1999; Schroeder et al., 2007). Complement inhibition by tick salivary proteins may also facilitate host invasion by tick-borne-pathogens (Schuijt et al., 2011). In addition to complement inhibition, tick salivary proteins suppress inflammatory immune responses by neutralizing chemokines that normally recruit cells of the innate immune system (Deruaz et al., 2008). They also disrupt neutrophil function (Montgomery et al., 2004; Guo et al., 2009), and migration and phagocytosis by macrophages (Bowen et al., 2010; Kramer et al., 2011). These immunomodulatory activities by ticks, targeted to disrupt host innate immune function, highlight the importance of innate immunity, including

complement and granulocyte activity, in resistance to tick infestation. Our observation that hosts with high innate immunocompetence carried fewer ticks reflects the central role of innate immunity in resistance to tick infestation.

We used an *in vitro* lymphocyte stimulation assay as a measure of adaptive immune responsiveness in our study animals. The assay quantifies the ability of lymphocytes in whole blood to proliferate and produce cytokine in response to stimulation with a novel antigen (pokeweed). T cell activity mediates inflammation, which helps limit tick feeding success (Brossard and Wikel, 2004) and is disrupted specifically by tick immunomodulation (Garg et al., 2006). Immunomodulation by ticks also tends to bias host immunity to a type 2 response (Schoeler and Wikel, 2001), suppressing pro-inflammatory signaling cascades and type 1 immune responses (Mosmann et al., 1986; Abbas et al., 1996). As such, we expected higher tick burdens to be associated with suppressed type 1 responses such as IFNγ production following mitogen stimulation. There are several reasons why we might not have detected this pattern. First, our mitogen response assay measured output of IFNγ, a flagship cytokine of type 1 immunity, which primarily mediates immunity to intracellular pathogens. IFNγ is also a component of pro-inflammatory signaling pathways, but it has not been linked directly with immune responses to ticks. There are a number of cytokines that are more central to pro-inflammatory signaling in the context of parasitism by ticks, such as tumor necrosis factor-α (TNFα), or interleukin-2 (IL-2), which promotes localized itching responses leading to active removal of ticks by the host (Murphy et al., 2008). Targeting our T cell immune measure to one of these cytokines that are more directly involved in the immune response to ticks might have yielded a more detectable association between tick abundance lymphocyte responsiveness. (The choice of IFNγ was driven by this study forming part of a larger project examining immunity to bovine tuberculosis in the buffalo, for which IFNγ is a key mediator.) Second, while pro-inflammatory immune pathways play a role in host responses to ticks, it is not clear that they are always helpful in limiting tick infestation (Piper et al., 2009). Finally, interactions between ticks and the host immune system occur in the host's skin. Some of the relevant cytokine patterns may be detectable only locally, and not systemically (Brossard and Wikel, 2004). Our assay used blood collected by jugular venipuncture and thus could only detect systemically expressed immune patterns. An adaptive immune assay targeted at cutaneous immune responses near tick attachment sites may prove more informative in this context.

*Host reproductive status*

Pregnant animals carried more ticks than non-pregnant animals in our July/LS sample. Other studies have shown that pregnant animals downregulate their immune response, as a result of trade-offs among these energetically expensive life history functions (Adamo et al., 2001; Friedl and Edler, 2005; French et al., 2007) or to protect the growing fetus from maternal immune defenses (Tizard, 2004). However, it is as yet unclear why we should see an effect of pregnancy on tick burdens in our July/LS sample, but not in October/CB. With most births occurring between December and April in KNP buffalo (Ryan et al., 2007), pregnancies during the October capture period would have been more advanced than during the July capture period. It is possible that female buffalo only suffer increased susceptibility to ticks during early gestation.

*Host body condition*

In the October/CB sample, buffalo with higher tick burdens had lower body condition scores. We cannot be certain whether poor body condition was causing increased susceptibility to ticks, or whether ticks were causing their hosts to lose condition. However, to date, we have found no evidence for an effect of body condition on innate (Beechler et al., 2012) immunity, and only weak evidence, restricted to bovine tuberculosis-positive animals, for an effect of body condition on adaptive immunity (Ezenwa et al., 2010) in buffalo. On the other hand, studies in cattle indicate that heavy infestations with the tick species reported here can cause significant weight loss in the host (Walker et al., 2003). It stands to reason that we might see such effects in the buffalo sampled at the end of the dry season and from the herd subsisting in poorer habitat (October sample/Crocodile Bridge herd). We would not expect to detect an effect of ticks on host body condition in the July/LS herd sample, as body condition scores in this group of buffalo were almost uniformly high (Table 1): these animals were experiencing resource conditions of abundance, and were maintaining fine body condition irrespective of variation in ectoparasite load.

*Conclusion*

This study is one of the first to reveal a significant negative association between tick abundance and innate immunity in a free-ranging mammalian host population. Buffalo with higher innate immunocompetence were less infested with ticks in our study population, and this association was robust to controlling for the effects of host age, body condition, and reproductive status. Our data also indicated strong niche segregation between the two most abundant tick species observed on the buffalo, *A. hebraeum* and *R. e. evertsi*. This resulted in independent abundance patterns for the two tick species on their buffalo hosts. Taken together, these findings point to a dominant role for host immunity, relative to direct parasite interactions, in driving patterns of ectoparasite infestation in this system. These insights have important implications for the dynamics of tick-borne microparasitic infections, and should encourage further research aimed at understanding variability in immunity and parasite infestation among wild hosts. Longitudinal studies allowing environmental and seasonal effects on immunity and parasite infestation to be distinguished are urgently needed.

## Acknowledgements

We are grateful to Dr. James Mertins of the US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories for identifying tick specimens, providing reference materials, and constructive suggestions on early drafts of the manuscript. This project was made possible through the support of SANParks, in particular the Veterinary Wildlife Services Department, led by Dr. Markus Hofmeyr. Veterinarian Dr. Peter Buss and the SANP Veterinary Wildlife Services staff provided the expertise and person-power to conduct the buffalo captures. We would like to thank field technicians Robert Spaan and Jo Calldo, and veterinary student Austin Bell, for their assistance with data collection. Veterinarians Dr. Danny Govender and Dr. Kristie Thompson helped with all aspects of sample and data collection. This research was funded by NSF awards #0723928 and #0723918 to Principle Investigators (V. Ezenwa and A. Jolles, respectively). Kadie Anderson was funded by support from the Biomedical Research Department at Oregon State University.

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