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# Seroprevalence of *Anaplasma marginale* and *Babesia bigemina* infections and associated risk factors in Machakos County, Kenya

F. D. Wesonga[1] · J. M. Gachohi[2] · P. M. Kitala[3] · J. M. Gathuma[3] · M. J. Njenga[3]

**Abstract** *Anaplasma marginale* and *Babesia bigemina* are important tick-borne pathogens of cattle. A cross-sectional survey was undertaken to determine the seroprevalence of *A. marginale* and *B. bigemina* infections and identify associated risk factors on traditional smallholder farms in Machakos County, Kenya. A total of 421 cattle from 127 farms from four divisions in the county were sampled and visited between September and November 2007. The farms were selected by a proportional allocation approach based on the number of farms in the four divisions previously selected by stratified random sampling method. Information on animal and individual farm management variables was obtained using standardized questionnaires. Prevalence of serum antibodies due to *A. marginale* and *B. bigemina* pathogens was determined using the enzyme-linked immunosorbent assay (ELISA) technique. The relationship between the seropositivity and associated risk factors was assessed by multivariable analyses using standard logistic regression models. The overall estimation (and their 95% confidence intervals) of *A. marginale* and *B. bigemina* seropositivity at the animal level was 53.4% (48.5%, 58.2%) and 40.6% (35.8%, 45.4%), respectively. Two variables, "animal age" and "administrative division," were significantly associated with the *A. marginale* seroresponse. Three variables, "animal age" "grazing system" and "administrative division" were significantly associated with the *B. bigemina* seroresponse. These findings suggest possible indicators of existence of endemic instability for the two infections. The study identifies characterization of environmental suitability for the vectors and how they interact with grazing systems to cause the infections as an area for further studies, for improved understanding of the infections and in designing disease control programs.

**Keywords** *Anaplasma marginale* · *Babesia bigemina* · Seroprevalence · Machakos · Kenya

✉ J. M. Gachohi
mwangigachohi@gmail.com

F. D. Wesonga
fwesonga@yahoo.com

[1] Kenya Agricultural Research Institute (KARI), Muguga South, P.O. Box 32, Kikuyu, Kenya

[2] School of Public Health, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya

[3] Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya

## Introduction

Bovine anaplasmosis is caused by the intra-erythrocytic rickettsia *Anaplasma marginale* and *Anaplasma centrale* in Kenya (de Vos et al. 2004; Ngeranwa et al. 2008). Although *Rhipicephalus* (*Boophilus*) *decoloratus* (*R. decoloratus*) ticks are incriminated as the main vectors for anaplasmosis, mechanical transmission by bloodsucking flies or by fomites (for instance, hypodermic needles) contaminated with blood from infected cattle also occurs (Dreher et al. 2005; Kocan et al. 2010). Bovine babesiosis, on the other hand, is caused by the hemoprotozoa *Babesia bovis* and *B. bigemina*. The main species of *Babesia* that infect cattle in Kenya is *B. bigemina* (Bock et al. 2004; Wesonga et al. 2010). The main tick vector for *B. bigemina* is *R. decoloratus* (Okon et al. 2011). The important distinction between transmission of *Anaplasma* and *Babesia* organisms is that the protozoans are obligate parasites of the vector ticks because they have their essential reproductive phase in the gut of the ticks, followed by their essential transovarian transmission through

the eggs to the larvae of the ticks. Other important tick-borne diseases in Kenya include East Coast fever (ECF) vectored by the three-host tick, *Rhipicephalus appendiculatus*, and Heartwater vectored by the three-host tick, *Amblyomma variegatum*.

Worldwide, anaplasmosis and babesiosis constitute the most widely distributed tick-borne disease (TBD) complex (Minjauw and McLeod 2003). The dynamics of these infections are dependent on factors such as vector population which in turn depends on spatio-temporal habitat suitability patterns (Gachohi et al. 2010), transmission capability of the vectors, host susceptibility (Kocan et al. 2010), and grazing management practices (Gachohi et al. 2012). These infections are responsible for significant economic losses for the livestock industry particularly in the tropical sub-Saharan Africa (Minjauw and McLeod 2003).

Assessment of epidemiological patterns of bovine anaplasmosis and babesiosis in a given area allows in the understanding of the infection dynamics. The epidemiological situation of the infections can be classified as three types: endemic stability, endemic instability, and disease-free areas (Norval et al. 1992). This classification covers difficult and controversial topics. There is no quantitative classification on the basis of seroprevalence for these infections. We adopt the classification assumed for *Theileria* spp. infections (>70%, <30%, and 0% endemic stability endemic instability and disease-free areas, respectively) (Norval et al. 1992). This classification has been used in designing of tick-borne disease control strategies (Norval et al. 1992; Gachohi et al. 2010, 2012).

Studies on the epidemiological patterns of bovine anaplasmosis and babesiosis in Kenya are scarce (Gachohi et al. 2010; Wesonga et al. 2010). Considering the significant livelihood benefits that are derived from cattle in Kenya, epidemiological studies are crucial in the study of the distribution and associated factors of tick-borne infections in specified livestock populations to apply the knowledge in the control of the infections. This is particularly due to the multiplicity of diverse risks of the infections to cattle. Moreover, the infections are likely to become important as *Theileria parva* infection gets increasingly controlled by vaccination (Gachohi et al. 2012) leading to farmers relaxing the control of ticks.

The present serological study was conducted in Machakos County in Kenya and designed to (1) determine the prevalence of antibodies to *A. marginale* and *B. bigemina* infections in cattle raised in a traditional mixed crop-livestock system, and (2) to identify farm- and animal-level risk factors associated with each infection. Similar to the study of Wesonga et al. (2015), this study was carried out as part of a broader study aimed at identifying constraints to livestock production in Machakos County and ultimately recommend appropriate interventions for the constraints (Wesonga et al. 2010).

## Materials and methods

This study used samples that were collected and analyzed for *Theileria parva* seropositivity and published (Wesonga et al. 2015). Therefore, details in the materials and methods in the paper (Wesonga et al. 2015) also apply for this study. Field visits were carried out between September and November 2007.

## Study site, study animals, study design, and animal selection

The cross-sectional study was undertaken in four divisions in Machakos County, namely, Athi River, Kangundo, Matungulu, and Ndithini. The study area description and location is provided in details in Wesonga et al. (2015). Similarly, details on the study animals, study design, and animal selection are provided in the same manuscript (Wesonga et al. 2015).

## Data and biological sample collection

Farm-level data was collected using a standard questionnaire as stated in Wesonga et al. (2015). Animal-level variables included age, sex, and breed. Farm-level variables information included tick occurrence on animals and control, grazing management, and herd size. Investigation on tick control variable was expected to reveal the importance and differences of tick control as a disease control intervention across the study area. Grazing system has previously been proven to affect exposure of cattle to ticks leading to large differences in tick infestation levels and corresponding infection risk of tick-borne infections (Gitau et al. 1999). Herd size was assumed to be influenced by grazing system strategies and to influence tick control strategies based on farm-level economics (Gachohi et al. 2012). In this study, the only area level variable was division.

The samples collected and assessed for the levels of antibodies to *T. parva* (Wesonga et al. 2015) were used for the current study. Enzyme-linked immunosorbent assays (ELISAs) were used to detect antibodies to *A. marginale* and *B. bigemina* infections using the 19-kDa recombinant antigen and the 200-kDa antigen, respectively (Katende et al. 1998). The test's sensitivity and specificity for *A. marginale* and *B. bigemina* are (90%, 90%) and (97%, 98%), respectively (Katende et al. 1998). Samples were tested in duplicates. Each ELISA test plate included predetermined positive and negative control sera. Optical density (OD) readings from the reference highly positive control sera were used to compute the percent positivity (PP) for the test sera, expressed as PP = (OD of test serum/OD of strong positive) × 100. For ease of interpretation and comparison, animals were classified as

seropositive if the PP was ≥15% (Katende et al. 1998) for each infection.

## Ethical approval

Ethical approval for this study was obtained from the then Kenya Agricultural Research Institute–Veterinary Research Center (KARI-VRC), Institutional Animal Care and Use Committee (IACUC) (Approval KARI-VRC/IACUC008/03062006). Verbal consent was obtained from cattle owners at the time of the farm visit.

## Data management and analysis

Data on seropositivity (positive or negative) to *A. marginale* and *B. bigemina* infections and questionnaire data were entered into the Microsoft Access database (Microsoft Corporation, USA) and exported to STATA (StataCorp 2007, version 10) for statistical analyses. The association between the risk variables from the questionnaire data and *A. marginale* and *B. bigemina* seropositivity was initially assessed with the risk variables independently under univariable analyses using a logistic regression model with a level of significance set at $P \leq 0.1$. A standard multivariable logistic regression model for each infection was then constructed by extending the univariable models to include the significant variables using backward variable selection process based on Wald's test statistics ($P < 0.05$). Building of the multivariable model also included evaluation for confounding in the form of estimate changes >30% in risk variables (Dohoo et al. 2003).

## Results

### Farm and animal characteristics

All smallholder farms approached agreed to participate in the study. Briefly, as highlighted in Wesonga et al. (2015), the characteristics of the 421 cattle sampled in terms of animal, farm, and area level are summarized in Table 1. Majority (74.1%) of the selected cattle were of indigenous zebu breed (Table 1). Most of the cattle sampled (46.3%) were adult cattle. Female cattle constituted the majority (70.1%) of the sampled animals. Most of the cattle (67.9%) were reared under the free-grazing system while the rest were reared under the semi-zero and zero-grazing systems. Regular tick control was applied on 58.2% of the sampled cattle. Majority (45.1%) of the sampled animals were from Kangundo Division (Table 1).

**Table 1** Characteristics of the 421 selected cattle in terms of animal-, farm-, and area-level variables

| Variable | Levels | Frequency | % |
|---|---|---|---|
| Animal variables | | | |
| Breed | Indigenous[a] | 312 | 74.1 |
| | Mixed[b] | 109 | 25.9 |
| Age | Calf[c] | 92 | 21.8 |
| | Yearling[d] | 134 | 31.9 |
| | Adult[e] | 195 | 46.3 |
| Sex | Male | 126 | 29.9 |
| | Female | 295 | 70.1 |
| Farm variables | | | |
| Tick control[f] | Regular | 245 | 58.2 |
| | Irregular | 176 | 41.8 |
| Grazing system[g] | Free grazing | 286 | 67.9 |
| | Semi-zero grazing | 49 | 11.6 |
| | Zero grazing | 86 | 20.5 |
| Area variable | | | |
| Division | Kangundo | 190 | 45.1 |
| | Masinga | 97 | 23.0 |
| | Matungulu | 74 | 17.6 |
| | Athi River | 60 | 14.3 |

Source: Wesonga et al. 2015

[a] Indigenous: zebu (*Bos indicus*) cattle breed

[b] Mixed: crosses between exotic (*Bos taurus*) cattle breed and zebu cattle

[c] Calf: 5 and 12 months old

[d] Yearling: 13 and 24 months old

[e] Adult: >24 months of age;

[f] Tick control: regular—tick control applied at 1–2-week interval; irregular—tick control applied only at times of high tick challenge

[g] Grazing system: free grazing—cattle were routinely grazed on pastures outside the designed farms; semi-free grazing—cattle mostly confined on farms but occasionally grazed outside the designed farms; zero grazing—cattle are confined in an enclosed, shaded area where they are provided with fodder and water

### Seroprevalence and univariable analyses of factors associated with the seroprevalence of *A. marginale* and *B. bigemina* infections

The overall individual animal-level seroprevalence of *A. marginale* antibodies was 53.4% ($n = 225$). The highest seroprevalence for *A. marginale* was estimated in Athi River Division (75.0%, $n = 45$) (Table 2). The seroprevalence in order of increasing magnitude was Kangundo, Matungulu, and Ndithini being 39.5% ($n = 75$), 55.4% ($n = 41$), and 66.0% ($n = 64$), respectively (Table 2).

Univariable analysis of seropositivity to *A. marginale* infection returned breed and age of the animal, the grazing system adopted, and administrative division as significant factors ($P \leq 0.1$). Indigenous animals had a higher risk of exposure to *A. marginale* infection relative to animals of mixed breeds

**Table 2** Univariable analysis ($P \leq 0.1$) for exposure to *A. marginale* infection in cattle in Machakos County

| Variable | Levels | *A. marginale* seropositivity | | *b* | 95% CI | *P* value |
|---|---|---|---|---|---|---|
| | | *n* | *%* | | | |
| **Animal variables** | | | | | | |
| Breed | Indigenous | 177 | 56.7 | 0 | – | 0.022 |
| | Mixed | 48 | 44.0 | −0.51 | (−0.95, −0.07) | |
| Age | Calf | 29 | 31.5 | 0 | – | 0.000 |
| | Yearling | 62 | 46.3 | 0.62 | (0.07, 1.18) | |
| | Adult | 134 | 68.7 | 1.56 | (1.03, 2.10) | |
| Sex | Female | 155 | 52.5 | 0 | – | 0.570 |
| | Male | 70 | 55.6 | 0.12 | (−0.29, 0.54) | |
| **Farm variables** | | | | | | |
| Tick control | Regular | 132 | 53.9 | 0 | – | 0.833 |
| | Irregular | 93 | 52.8 | −0.04 | (−0.42, 0.34) | |
| Grazing system | Free grazing | 164 | 57.3 | 0 | | 0.052 |
| | Semi-zero grazing | 24 | 49.0 | −0.33 | (−0.94, 0.27) | |
| | Zero grazing | 37 | 43.0 | −0.57 | (−1.06, −0.08) | |
| **Area variable** | | | | | | |
| Division | Athi | 45 | 75.0 | 0 | – | 0.000 |
| | Ndithini | 64 | 66.0 | −0.43 | (−1.15, 0.28) | |
| | Kangundo | 75 | 39.5 | −1.52 | (−2.17, −0.87) | |
| | Matungulu | 41 | 55.4 | −0.88 | (−1.62, −0.13) | |
| **Interactions** | | | | | | |
| Division × breed | | | | −0.15 | (−0.27, −0.04) | 0.006 |
| Division × age | | | | 0.08 | (0.02, 0.14) | 0.004 |
| Division × tick control | | | | −0.08 | (−0.19, 0.02) | 0.129 |
| Division × grazing system | | | | −0.10 | (−0.18, −0.03) | 0.003 |

(Table 2). Both yearlings and adults had a higher risk of exposure to *A. marginale* infection relative to calves (Table 2). Semi-zero and zero-grazed animals had a lesser risk of exposure to *A. marginale* infection relative to free-grazed animals (Table 2). Animals from Athi River Division had the highest risk of exposure to *A. marginale* infection (Table 2). Interaction terms between administrative division and animal breed, age, and grazing system were significant for *A. marginale* infection (Table 2).

The overall individual animal-level seroprevalence for *B. bigemina* was 40.6% ($n = 171$). The highest seroprevalence for *B. bigemina* was estimated in Matungulu Division (58.1%, $n = 43$) (Table 3). The seroprevalence in order of increasing magnitude was Kangundo, Ndithini, and Athi River divisions being 31.1% ($n = 59$), 39.2% ($n = 38$), and 51.7% ($n = 31$), respectively. On the other hand, univariable analysis of seropositivity to *B. bigemina* infection returned age of the animal, the grazing system adopted, and division of origin as significant factors ($P \leq 0.1$). Similar to *A. marginale* infection analyses, both yearlings and adults had a higher risk of exposure to *B. bigemina* infection relative to calves (Table 3). Similar to *A. marginale* infection analyses, semi-zero and zero-grazed

animals had a lesser risk of exposure to *B. bigemina* infection relative to free-grazed animals (Table 3). Animals from Matungulu Division had the highest risk of exposure to *B. bigemina* infection (Table 3). Interactions between administrative division and animal breed, tick control, and grazing system were significant for *A. marginale* infection (Table 3).

## Multivariable analysis ($P \leq 0.05$) for exposure to *A. marginale and B. bigemina* infections in cattle in Machakos County

Upon multivariable analyses of the significant variables obtained under univariable analyses for *A. marginale* infection, variables breed of the animal and the grazing system dropped out. Two variables (age and administrative division) remained as being significantly associated with variation in antibody prevalence to *A. marginale* infection ($P < 0.05$) (Table 4). On the other hand, only the variable breed of the animal dropped out upon multivariable analyses of *B. bigemina* infection. This left three factors (age, grazing system, and division) which were significantly associated with variation in antibody prevalence to *B. bigemina* infection ($P < 0.05$) (Table 4).

**Table 3** Univariable analysis ($P \leq 0.1$) for exposure to *B. bigemina* infection in cattle in Machakos County

| Variable | Levels | *B. bigemina* seropositivity | | *b* | 95% CI | *P* value |
|---|---|---|---|---|---|---|
| | | *n* | (%) | | | |
| **Animal variables** | | | | | | |
| Breed | Indigenous | 135 | 43.3 | 0 | – | 0.058 |
| | Mixed | 36 | 33.0 | −0.43 | (−0.89, 0.02) | |
| Age | Calf | 26 | 28.3 | 0 | – | 0.006 |
| | Yearling | 52 | 38.8 | 0.47 | (−0.09, 1.04) | |
| | Adult | 93 | 47.7 | 0.84 | (0.30, 1.37) | |
| Sex | Female | 126 | 42.7 | 0 | – | 0.178 |
| | Male | 45 | 35.7 | −0.29 | (−0.72, 0.13) | |
| **Farm variables** | | | | | | |
| Tick control | Regular | 98 | 40.0 | 0 | – | 0.760 |
| | Irregular | 73 | 41.5 | 0.06 | (−0.33, 0.45) | |
| Grazing system | Free grazing | 138 | 48.3 | 0 | | 0.000 |
| | Semi-zero grazing | 18 | 36.7 | −0.47 | (−1.09, 0.15) | |
| | Zero grazing | 15 | 17.4 | −1.48 | (−2.08, −0.88) | |
| **Area variable** | | | | | | |
| Division | Athi | 31 | 51.7 | 0 | – | 0.000 |
| | Ndithini | 38 | 39.2 | −0.50 | (−1.15, 0.14) | |
| | Kangundo | 59 | 31.1 | −0.86 | (−1.45, −0.27) | |
| | Matungulu | 43 | 58.1 | 0.26 | (−0.42, 0.94) | |
| **Interactions** | | | | | | |
| Division × breed | | | | −0.19 | (−0.31, −0.07) | 0.001 |
| Division × age | | | | −0.00 | (−0.06, 0.05) | 0.882 |
| Division × tick control | | | | −0.10 | (−0.21, 0.01) | 0.071 |
| Division × grazing system | | | | −0.25 | (−0.33, −0.16) | 0.000 |

The risk of exposure to both infections increased with age (Table 4) and was lowest in zero-grazing system and increased through to semi-zero-grazing to free-grazing systems. The risk of exposure to *A. marginale* infection was lowest in the semi-humid Kangundo division and increased through semi-humid to semi-arid Matungulu Division to the semi-arid Ndithini and Athi River divisions. Conversely, the risk of exposure to *B. bigemina* infection did not demonstrate a pattern related to the agro-ecological zoning: the risk was lowest in the semi-humid Kangundo and semi-arid Ndithini divisions and increased in the semi-humid to semi-arid Matungulu and the semi-arid Athi River divisions. None of the interaction terms

**Table 4** Multivariable analyses ($P \leq 0.05$) for exposure to *A. marginale* and *B. bigemina* infections in cattle in Machakos County

| Variable | Level | *A. marginale* infection | | | *B. bigemina* infection | | |
|---|---|---|---|---|---|---|---|
| | | *b* | 95% CI | *P* value | *b* | 95% CI | *P* value |
| Age | Calf | 0 | – | 0.000 | 0 | | 0.0001 |
| | Yearling | 0.83 | (0.21, 1.44) | | 0.58 | (−0.02, 1.20) | |
| | Adult | 1.95 | (1.35, 2.56) | | 1.17 | (0.59, 1.74) | |
| Grazing system | Free grazing | – | – | – | 0 | | 0.000 |
| | Semi-zero grazing | – | – | | −0.87 | (−1.59, −0.16) | |
| | Zero grazing | – | – | | −1.63 | (−2.32, −0.94) | |
| Division | Athi | 0 | – | 0.000 | 0 | | 0.0005 |
| | Ndithini | −0.78 | (−1.57, 0.01) | | −0.67 | (−1.36, 0.00) | |
| | Kangundo | −2.03 | (−2.77, −1.29) | | −0.35 | (−1.03, 0.31) | |
| | Matungulu | −1.10 | (−1.90, −0.29) | | 0.66 | (−0.09, 1.42) | |

turned significant on multivariable analyses in both infections (Table 4).

## Multivariable analysis ($P \le 0.05$) for exposure to *A. marginale* and *B. bigemina* infections in cattle without the variable division

In the analyses, the variable "division" was significantly associated with both *A. marginale* and *B. bigemina* seropositivity. As administrative divisional boundaries cannot be expected to describe ecological and/or environmental factors associated with infections' seropositivity, multivariable models without the variable "division" were built with all the significant univariable variables for each infection to assess whether there were factors masked by this area-level variable (Gachohi et al. 2010). These models are shown in Table 5. For *A. marginale* infection, only grazing system turned significant ($P < 0.05$) (Table 5). In addition, the magnitude of effect of the variable age reduced substantially (compare Tables 4 and 5). Although none of the variables for *B. bigemina* infection turned significant ($P < 0.05$), the magnitude of effect of the variable age reduced marginally (compare Tables 4 and 5). However, the model with variable "division" fitted the data better than the model without division.

## Discussion

This study was the first of its kind to provide a population-structured assessment of serum antibody prevalence of *A. marginale* and *B. bigemina* infections in Machakos County. The findings confirmed exposure of cattle to these two parasites in the county. However, more cattle (53%) were exposed to *A. marginale* relative to *B. bigemina* (41%). Both *A. marginale* and *B. bigemina* parasites are vectored by the same tick (*R. decoloratus*). The distribution of *B. bigemina* in cattle can only closely correspond with the vector ticks, by absolute biological necessity, unless the cattle are moved to new locations where the tick can survive. In this study, the higher *A. marginale* seroprevalence could most likely be due to

the additional mechanical transmission by blood-sucking arthropods and fomites (Potgieter and Stoltsz 2004).

With epidemiologic endemic stability and instability being characterized by high and low prevalence of serum antibodies to TBD infections (>70 and <30%, respectively) (Norval et al. 1992), the overall findings in this study suggest an indication of endemic instability for both *A. marginale* and *B. bigemina* infections. Endemic stability results from a complex interaction of several variables such as the high innate resistance of zebu cattle to TBD infections, ability of zebu cattle to rapidly and effectively develop immunity to TBD infections, suitable ecological factors for the vectors, and regular transmission to the host population, thus, regularly boosting immunity (Norval et al. 1992). Specifically, to achieve endemic stability, a sufficiently high number of infected ticks are necessary on the animals at a young age to ensure exposure to the pathogen before colostral and innate immunity disappears. The high levels of protective immunity, therefore, may reduce the prevalence of babesiosis in older animals. For most tick-borne diseases, this age is around 9 months. Hence, pre-immunity may be of importance in endemic stability for babesiosis where there is "sufficient" tick challenge with infected ticks at an early age. There appears to be no pre-immunity to *Anaplasma* spp. Immune response to anaplasmosis is induced by both humoral and cell-mediated responses. Under endemic stability, the disease is naturally controlled. By being vectored by the same vector tick, the additional transmission dynamics in *A. marginale* infection seems, therefore, to favor the situation to endemic stability.

However, when the finer spatial scale was considered, both Athi River and Ndithini divisions could be considered as endemic stable or near endemic stable for *A. marginale* infection. Athi River and Ndithini divisions are situated in the lower agro-ecological zones. Previous studies have reported higher risk of *A. marginale* infection in lower elevation zones (Rubaire-Akiiki et al. 2004; Gachohi et al. 2010). This finding partly suggests heterogeneity in vector tick and other blood-sucking arthropods' suitability habitats resulting most likely from varying ecological and climatic variability across the county.

**Table 5** Multivariable analyses ($P \le 0.05$) for exposure to *A. marginale* and *B. bigemina* infections in cattle without the variable division in Machakos County

| Variable | Level | *A. marginale* infection | | | *B. bigemina* infection | | |
|---|---|---|---|---|---|---|---|
| | | b | 95% CI | P value | b | 95% CI | P value |
| Age | Calf | 0 | – | 0.000 | 0 | | 0.0009 |
| | Yearling | 0.61 | (0.04, 1.17) | | 0.47 | (−0.11,1.05) | |
| | Adult | 1.66 | (1.11, 2.20) | | 0.99 | (0.44,1.55) | |
| Grazing system | Free grazing | – | – | 0.006 | 0 | | 0.000 |
| | Semi-zero grazing | −0.53 | (−1.18,0.11) | | −0.56 | (−1.20,0.07) | |
| | Zero grazing | −0.79 | (−1.31, −0.27) | | −1.60 | (−2.22, −0.98) | |

The situation seemed different for *B. bigemina* infection. Differences in seroprevalence in *B. bigemina* infection were not as large as for *A. marginale* infection across administrative divisions. Although *B. bigemina* infection was significantly associated with administrative division, suggesting likely ecological and climatic variability in the vector (*R. decoloratus*), there was no clear pattern that corresponded to the agro-ecological zoning. Distribution of *B. bigemina* infection is known to closely follow the distribution of *R. decoloratus* (de Vos et al. 2004). It is well established that both the transmission dynamics of *Theileria* and *Babesia* spp. protozoa are tightly constrained by the ecological conditions favorable for their different vector ticks, and for human factors such as acaricide use and vaccination. Previous studies have reported higher presence of *R. decoloratus* and correspondingly higher *B. bigemina* seroprevalence in higher elevation zones compared to the lower zones (Gitau et al. 1997; Rubaire-Akiiki et al. 2004). Studies are needed to establish whether there is a dynamic relationship between *R. decoloratus* distribution and socio-demographic and ecological processes as suggested for *T. parva* (Gachohi et al. 2012).

In both infections, there was a clear gradient of age effects where the risk of exposure increased with age. Increasing age has been frequently reported to be associated with increased TBDs seroprevalence majorly because age is a proxy for exposure time (Gachohi et al. 2012). This has an epidemiological implication in the control of TBDs. Given the low turnover in the cattle population dynamics, animals that survive early infection are likely to remain seropositive and immune for life particularly if there is constant infected tick challenge. This is the basis of the infection-and-treatment approach in *T. parva* infection prevention when implemented in calves (Di Giulio et al. 2009). However, although there has been some progress in the development of vaccines for anaplasmosis and babesiosis, no truly effective and safe vaccine is currently commercially available in Kenya. With *T. parva* immunization being intensively rolled out in Kenya (Gachohi et al. 2012), farmers may relax tick control on their animals with the consequent concern that anaplasmosis and babesiosis occurrence and distribution may, therefore, be expanded.

Grazing system as a variable was associated with *A. marginale* and *B. bigemina* in different dimensions despite being vectored by the same tick species. Controlling for age and administrative division, grazing system was not a significant factor under multivariable analyses for *A. marginale*. However, controlling for age alone, grazing system was a significant factor under multivariable analyses for *A. marginale*. On the other hand, controlling for age and administrative division, grazing system was a significant factor under multivariable analyses for *B. bigemina*. These analyses suggested confounding effects of division and age. Similar findings have been reported previously (Gachohi et al. 2010; Wesonga et al. 2015). This analysis highlights the importance

of analyzing variables while controlling for others. In this study, further evidence was demonstrated when a number of interaction terms were significant in the univariable analyses but not significant controlling for other factors. However, these findings also need to be interpreted with caution bearing in mind that this was a cross-sectional study. A single cross-sectional study can only give an indicator of the significant variable based on their prevalence. Over time, the magnitude of the effect of a given variable on the occurrence of a given infection may alter simply because the prevalence of its causal complements in various causal mechanisms may also change. For instance, seroprevalence of TBD infections can vary quite substantially with climatic conditions, vector tick abundance, and distribution (Gitau et al. 1999; Rubaire-Akiiki et al. 2006). Other factors that may influence TBD transmission and prevalence include host population size and density, habitat modification, vector control programs, and the social environment (Olwoch et al. 2008). For these reasons, findings obtained from cross-sectional studies are preliminary and need to be confirmed in structured longitudinal studies.

The grazing system effect on TBD pathogen seropositivity emanates from the nature of exposure from the vectors. Thus, this ranges from unrestricted tick exposure in free-grazing systems through restricted exposure in mixed grazing, where animals are alternately open grazed and stall fed, to no exposure where cattle are kept under confinement under zero-grazing system (Gitau et al. 1999). Our findings were consistent with this phenomenon basically due to these differential exposures. For instance, animals raised under open grazing management are frequently exposed to infected ticks and, thus, are more likely to develop circulating antibodies to the relevant pathogen. However, whereas grazing system was not significant controlling for administrative division for *A. marginale* infection, the converse was true for *B. bigemina*. Although this finding was unexpected, a possible explanation was the effect of the additional mechanical transmission by blood-sucking arthropods and fomites for *A. marginale* infection. However, it is difficult to attribute the seropositivity to either biological or mechanical transmission.

In addition, the grazing system has been reported to interact with the area variables including administrative areas and agro-ecological zones in TBD epidemiology (Gachohi et al. 2012). In this study, as indicated in Wesonga et al. (2015), cattle from Kangundo Division (an area with semi-humid to semi-arid agro-ecological zones) had the lowest *A. marginale* infection risk, while cattle from Athi River Division (an area with semiarid to arid agro-ecological zones) had the highest *A. marginale* infection risk. This was attributed to other several observations: most farmers from Kangundo Division kept exotic and crossbreeds of cattle reared under zero-grazing system that restricts tick exposure on cattle. These observations were converse for Athi River Division where farmers kept

indigenous breeds of cattle which are perceived to be resistant to ticks and tick-borne diseases. Moreover, the majority of farmers in Athi River Division reared cattle under the free-grazing system, thereby increasing the risk of exposure to the pathogens. As mentioned earlier on in this paper, longitudinal studies are needed to characterize risk both in spatial and temporal dimensions.

Tick control practices/regime was not a significant factor in this study despite over 50% of cattle reported to be on regular control. Our field hitherto observations during the many of the surveys involving tick control are that although the tick control may be regular, the effectiveness of the tick control is in doubt (personal observation—first author). For instance, the use of the hand pump, which is the predominant method used, is not reliable where issues like acaricide mixing (whether correctly diluted) and if the predilection sites for ticks are targeted, if the right quantity of acaricide is sprayed, etc.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

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