Seroprevalence of *Babesia bigemina* in smallholder dairy cattle in Tanzania and associated risk factors

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ABSTRACT

Variations in the seroprevalence of antibody to *Babesia bigemina* infection by farm and animal level risk factors were investigated for 2 contrasting regions of Tanga and Iringa in Tanzania. Tanga is situated in the eastern part of the country and has typical tropical coast climate while Iringa is situated in the Southern Highlands and has a tropical highland climate. Two hundred farms from each region were selected using simple random sampling procedure and visited once between January 1999 and April 1999. Blood samples were collected from 1329 smallholder dairy animals on selected farms for harvesting serum which was subsequently used for serodiagnosis of *B. bigemina* using an indirect enzyme linked immuno-sorbent assay (ELISA). Of the 1329 sera samples screened, 34.9 % were positive for *B. bigemina.* The prevalence was higher in Iringa Region [43 %, 95 % confidence intervals (CI) = 39.5–47.3] than in Tanga Region (27 %, CI = 23.6–30.5). Using a logistic binomial regression model as an analytical method for predicting the likelihood of animal seropositivity, we found (in both regions) that the risk of positive reaction varied with the animal's age, history of grazing and geographical location. Seroprevalence increased with age ($\beta = 0.01$ and 0.01 per year of age, $P < 0.005$ in Tanga and Iringa, respectively). Animals located in Lushoto and Iringa urban district were associated with increased risk of seropositivity [Odds ratio (OR) = 4.24, $P = 0.001$, for Lushoto, and OR = 1.81, $P = 0.040$, for Iringa Urban, respectively). Animals grazed 3 months prior to sampling had higher odds for seropositivity than zero/semi-grazed, despite farmer-reported high frequency of tick control (OR = 2.71, $P = 0.0087$, for Tanga, and OR = 4.53, $P = 0.001$, for Iringa). Our study suggests that even though herd sizes are small, *B. bigemina* infection is widespread in many smallholder dairy farms and endemic stability with respect to this disease has not yet been attained, but the observed levels are sufficiently high to ensure that clinical disease would be a risk.

Key words: *Babesia bigemina,* crossbred dairy cattle, risk factors, seroprevalence, smallholder dairying, Tanzania.

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INTRODUCTION

Babesiosis is an important tick-borne disease that is widespread in tropical and subtropical countries[4,12,20,41]. Bovine babesiosis, caused by intra-erythrocytic parasites, *Babesia bigemina* and *Babesia bovis,* is transmitted by ticks of the genus *Boophilus*[24,40]. *Babesia bovis* is transmitted only by *B. microplus* whereas *B. bigemina* is transmitted by both *B. microplus* and *B. decolaratus*[3,29,41]. The disease is known to cause considerable economic losses indirectly in the form of reduced weight gains and milk production or directly through mortalities and veterinary costs. Unpublished annual animal health reports in Tanzania, obtained from passive-derived data, indicate that tick-borne diseases (TBDs) accounted for 71.4 % of all reported cattle mortality for the period 1981–1993, with theileriosis, anaplasmosis, heart water and babesiosis being responsible for 43.5 %, 16.5 %, 6.3 % and 5.1 %, respectively[14]. These data do not include calf (less than 1 year old) mortality in the indigenous population, which is estimated to vary between 25 % in female calves and 35 % in male calves, 75 % of which is attributed to TBDs[31]. Information on the babesiosis (due to either *B. bigemina* or *B. bovis*) in smallholder dairy sector in the 2 study regions remains largely unknown.

Serological studies conducted in the traditional cattle sector (free-ranging Tanzanian short horn zebu) in Tanzania show that babesiosis due to *B. bigemina* is common, widespread and occurs in an endemic stable state[17]. Little work has been done in the smallholder dairy sector.

Successful management of Babesiosis in smallholder dairy farms depends on increased knowledge of the interactions between the parasites, the vector, specific climate and the ruminant host. Little is known about the epidemiology of *B. bigemina* in smallholder dairy farming systems, and the interaction of management factors and parasitism, is poorly understood[5,13,22].

The present serological study was designed: 1) to demonstrate and establish the prevalence of antibodies to *B. bigemina* in apparently clinically health dairy cattle; 2) to identify farm- and animal-level risk factors for *B.bigemina* infection; 3) to quantify and explore the relationship between these risk factors and the seroprevalance of *B. bigemina* in Tanzanian smallholder dairy cattle.

MATERIALS AND METHODS

Description of study area

The study sites have been described in detail elsewhere[26]. Briefly, the study was carried out in 2 regions of Tanzania: the coastal Tanga Region (lying between longitude 38° and 39°E and latitude 4° and 6°S) and the inland highland Iringa Region (lying between longitude 35° and 36°E and latitude 7° and 8°S). The study took place in 2 of the 6 administrative districts in Iringa Region (Iringa urban and Iringa rural, now Kilolo), and 5 of the 8 administrative district and subdistricts of Tanga Region. The latter comprised Amani, Muheza, Maramba, Korogwe, Lushoto, Pangani, Tanga Urban and Tanga rural. These districts were included as farm-level (fixed effects) in the statistical analyses described below.

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Study animals, study design and farm selection

Study animals comprised crosses of *Bos taurus* cattle (mainly Ayrshire, Simmental, Friesian and Jersey) with the *Bos indicus* breed, the indigenous Tanzania short horn zebu (TSHZ) or Boran or Sahiwal. The level of *taurine* blood genes inheritance varied from 50 % for F1; 62.5 % for F2 and equal or above 75 % for F3. Farms in both study regions were estimated to have an average of 3–4 dairy cattle of any age and sex so a sample size of 200 farms in each study region was considered necessary to provide between 600 and 800 animals required for the study. A sample size of farms and animals was estimated using Epi-Info version 6.04b (CDC, Atlanta, USA) in order to provide 80 % power, with a confidence of $\alpha = 0.05$, to estimate disease prevalence and detect associations between dependent and independent variables[7,9]. Two hundred farms in each study region were randomly selected from a sampling frame of 3001 and 500 in Tanga and in Iringa, respectively, using the databases of the Tanga and Iringa Dairy Development Projects. Farms recorded to have more than 10 animals were excluded from the selection process because farms of this size are not considered as 'smallholder' farms[38] although a small number of selected farms had more than 10 cattle by the time sampling began.

Data collection

Data were collected from farms by 2 separate teams of researchers, 1 in each region (ESS for Tanga and EDK for Iringa). Animal sampling and data collection were carried out between January and April 1999. One person in each region collected farm- and animal-level data using a structured questionnaire, which was administered on all selected farms on a single visit. The information collected concerned farm and animal events that occurred during 1998, including tick control practices, feeding methods and feed types, cattle movements on and off the farm, grazing and housing practices. The responses to many of these questions were investigated as explanatory variables in analysis of seroconversion to *B. bigemina.* These included variables that could be considered as farm-level variables including 'farm class' (whether the farm was located in an urban, rural or peri-urban situation) as defined by Swai *et al.*[37], acaricide application frequency (coded as low: an interval of greater than 2 weeks between treatments, moderate: an interval of 1–2 weeks, and intensive: an interval of less than 1 week), acaricide application method (*e.g.* handspray, brush, pour-on, diptank or spray race), frequency of contact with extension officers (rare, moderate or intensive) and farmer attendance at a Dairy Development Project training course.

Animal-level variables included age (transformed into age-centre to normalise and ease analysis), sex, breed, level of *taurine* blood genes inheritance (F1, F2, F3), source of animals (home-bred or brought-in), source of brought-in animals (charity gift, dairy development project credit agreement, or cash purchase), and whether or not the animal had been zero-grazed or allowed to graze at pasture in the 3 months prior to the onset of the sampling period (October to December 1998).

Collection and laboratory analysis of serum

During the visit to each farm, blood was collected from each animal into a 10 mℓ plain vacutainer tubes (Becton Dickinson Vacutainer Systems, UK) by jugular venipuncture. Labelling of tubes was carried out and verified before drawing the blood from the animals. After collection, blood samples were stored in iceboxes until they could be refrigerated (usually within 2–6 h). Upon arrival from field or the next day, the sera were separated by centrifugation at 3000 × *g* for 20 min and divided into 3 aliquots of 1–3 mℓ and stored at local laboratories (–20 °C) prior to dispatch in refrigerated containers to Sokoine University of Agriculture (SUA) for storage and on ward dispatch to the International Livestock Research Institute (ILRI, Nairobi, Kenya) for serological assays. Sera were subjected to indirect enzyme linked immuno sorbent assay (ELISA) to evaluate the level of antibodies to *B. bigemina* as described[23]. Results were expressed as percentage positivity (PP) values of optical densities[43], relative to those of a strong positive control serum. Test sera were assayed in duplicate and controls in triplicate. For ease of interpretation and comparison with other studies, animals were classified as seropositive if the PP was 15 %.

Statistical analysis

The unit of analysis was the individual animal. Data files of questionnaires and laboratory results were prepared in Epi-Info version 6.04b (Epi-info, 1996). The serology results (positive or negative) were the outcome variable in mixed effects logistic regression analyses performed in EGRET for Windows, version 2.0 (Cytel software Corporation, 1999). The animal- and farm-level variables described above served as explanatory variables. Associations between explanatory variables and the outcome were investigated singly and in multivariable models. The differences in *B. bigemina* antibody prevalences were compared across farm and animal-level explanatory variables and confidence limits for binomial proportion generated[32]. In multivariable models, backwards and forwards substitution and elimination were performed to find the most parsimonious model from which no explanatory variables could be removed without significantly affecting model deviance. Cross-tabulations and correlations were performed on explanatory variables to identify highly associated variables that could not be incorporated in the same multivariable models. In all models, the farm ID number was considered a random effect[16] to account for clustering of animals by farm. The level of significance was $P < 0.05$ throughout. Separate statistical analyses were performed for the data from the 2 regions because previous studies have indicated that parasite ecology and epidemiology may be very different in the 2 regions[35,36].

RESULTS

Farm response rate

All selected 200 farms from each of Tanga and Iringa regions were visited during the period of January to April 1999. A voluntary participatory rate of 100 % was thus achieved. In Tanga, a total of 697 animals kept on 185 farms (92.5 % of the sample) were examined and sampled. Fifteen farms (7.5 %) had no animals during the survey period. In Iringa, a total of 698 animals from 195 farms (97.5 % of the sample) was examined and sampled. Three farms (1.5 %) had no animals and animals on 2 farms (1 %) could not be sampled, as owners could not be traced. Overall, the mean (±SE) herd size was 3.5 ± 2.4 (range, 0–13) animals. The number of animals examined per herd ranged from 1 to 13 animals. The age range of animals examined varied from 1 day to 13 years. The characteristics of the sample of cattle in each region are detailed in Table 1.

Serum antibody prevalence of *B. bigemina*

Results were available from 666 of the 697 animals sampled in Tanga, and 663 of the 698 animals sampled in Iringa. The missing results are due to the loss of labels during transportation to laboratories. Of the 1329 serum samples screened in both Tanga and Iringa, 465 were positive, leading to an overall antibody prevalence of 34.9 % (95 % CI = 32.3–37.5). The mean antibody prevalence was 43 % (CI = 39.5–47.3) in Iringa and 27 % (CI = 23.6–30.5) in Tanga (Table 2).

Table 1: **Proportions of cattle in each category of each variable investigated during the study (Iringa, *n* = 698;Tanga, *n* = 697; U = urban, R = rural).**

Variable	Categories	No. of animals (%)	
		Iringa	Tanga
Animal-level variables			
Sex	Male	182 (26)	146 (21)
	Female	516 (74)	551 (79)
Source of animal	Homebred	406 (58)	436 (63)
	Brought-in	292 (41)	261 (37)
Filial generation	F1	350 (50)	217 (31)
	F2	347 (49)	459 (66)
	F3	1 (0.1)	21 (3)
Breed codes	Ayrshire cross	403 (58)	169 (24)
	Friesian cross	305 (44)	604 (86)
	Jersey cross	0	12 (2)
	Simmental cross	1 (0.1)	5 (1)
	Sahiwal cross	0	12 (2)
	TSHZ cross	150 (22)	541 (77)
	Boran cross	549 (78)	121 (17)
Age	<3 years	440 (63)	396 (57)
	3 to <6 years	165 (24)	214 (31)
	>6 years	93 (13)	87 (12)
Grazing history in last 3 months of 1998	Zero-grazing	423 (61)	628 (90)
	Semi-/free-grazing	275 (39)	69 (10)
Farm-level variables			
Farm classification	Peri-urban	109 (16)	117 (17)
	Urban	391 (56)	318 (46)
	Rural	198 (28)	262 (37)
Tick control	Yes	662 (95)	656 (94)
	No	36 (5)	41 (6)
Acaricide application methods	Dipping	5 (1)	43 (6)
	Hand-spraying	456 (65)	387 (56)
	Hand-dressing	95 (13)	34 (5)
	Pour on	60 (9)	159 (23)
	Brush	78 (11)	74 (11)
	Ethnoveterinary	4 (1)	0
Acaricide application frequency	Intensive	509 (73)	395 (57)
	Moderate	87 (12)	267 (38)
	Rare	102 (15)	35 (5)
Farmer attended training course	Yes	210 (30)	424(60.8)
	No	488 (70)	273 (39.2)
Frequency of extension officer contact	Rare	15 (2)	6 (1)
	Moderate	596 (85)	659 (95)
	Intensive	87 (13)	32 (5)
District (Iringa)	Iringa Urban	461 (66)	N/A
	Iringa Rural (Kilolo)	237 (34)	N/A
District (Tanga)	N/A	Tanga U	190 (27.2)
	N/A	Tanga R	151 (21.6)
	N/A	Muheza	76 (10.9)
	N/A	Amani	79 (11.4)
	N/A	Maramba	36 (5.3)
	N/A	Pangani	24 (3.4)
	N/A	Korogwe	60 (8.6)
	N/A	Lushoto	81 (11.6)

The mean antibody prevalences (with 95 % confidence limits for the binomial proportion) for *B. bigemina* by district/or subdistrict are shown in Fig. 1.

The prevalence amongst study animals investigated was higher in Iringa Region than in Tanga. An age-specific sero-prevalence rate for *B. bigemina* is shown in Fig. 2.

Factors influencing serum antibody prevalence

In both regions, 3 factors were significantly associated with variation in antibody prevalence to *B. bigemina* in the multivariable model: history of grazing, age and geographical location of the animal ($P < 0.05$) (Tables 3, 4).

Animals in Lushoto were 4 times more likely to seroconvert than animals in other districts or subdistricts. Animals in Iringa

Table 2: **Prevalence** (±95 % CI) **of cattle seropositive for *Babesia bigemina* in Tanga and Iringa – adjusted for farm effects.**

Region	Number tested	Number positive	Seroprevalance (%)	95 % CI	
				Lower	Upper
Tanga	666	180	27	23.6	30.5
Iringa	663	285	43	39.5	47.3

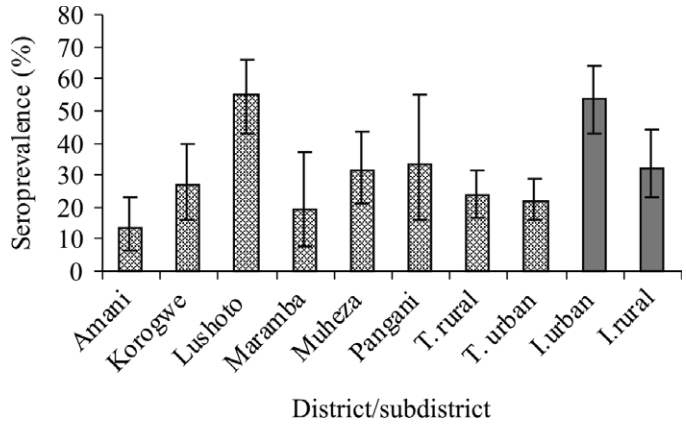

Fig. 1: **Seroprevalence profile** (±95 % CI) **of *Babesia bigemina* by district/subdistrict in Tanga** (stippled) **and Iringa** (grey) **– adjusted for farm effects.**

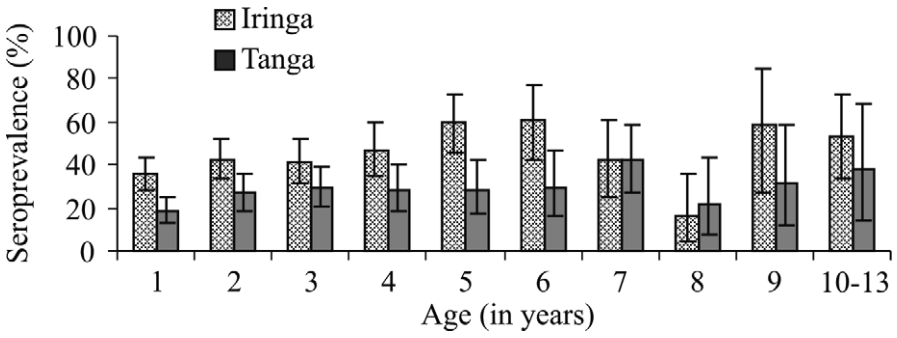

Fig. 2: **Age seroprevalence profile** (±95 % CI) **of *Babesia bigemina* in Tanga** (grey) **and Iringa** (stippled) **– adjusted for farm effects.**

urban district were twice as likely to have seroconverted to *B.bigemina* than those in Iringa rural district ($P = 0.040$). Grazing significantly increased the likelihood that animal was seropositive (Tanga OR = 2.71, Iringa OR = 4.53) (Fig. 3). The likelihood that animals were seropositive increased significantly with age (Tanga coefficient = 0.012, SE = 0.004, $P = 0.0017$; Iringa coefficient = 0.01, SE = 0.005, $P = 0.008$). None of the other investigated farm- and animal-level factors were associated with differences in prevalence values.

DISCUSSION

In this study, there was evidence that exposure of cattle to *B. bigemina* was widespread in the study regions. Furthermore, there was evidence of geographical variation in the distribution of pathogens between the 2 regions. This means there must be geographical variations in either the density of tick vectors or the prevalence of *B. bigemina* in host-seeking ticks or both. Geographical variation was widest, ranging from 13 to 55 % in Tanga Region while the seroprevalence was more uniform and generally higher in Iringa Region. Clinical babesiosis has not been reported to be a major problem in the 2 regions[33,38]. However, in some districts of Tanga Region, *i.e* Lushoto, where prevalence was high (55 %) and Amani, diagnosis of bracken fern poisoning is frequently made by farmers and extension workers when cattle show symptoms of 'red water' (haemoglobinuria or haematuria). In the light of these findings, some or many of these cases may in fact be misdiagnosed cases of babesiosis.

The mean prevalence of cattle that had seroconverted to *B. bigemina* was higher in Iringa than in Tanga Region. This may suggest that smallholder dairy cattle in Iringa Region are more at risk of *B. bigemina* infection. The mean *B. bigemina* seroprevalence of (27 %) in Tanga and (43 %) in Iringa was lower than those reported in recent studies of smallholder dairy cattle in similar coastal and highland regions of Kenya where tick-borne diseases (TBDs) including babesiosis are also considered a major constraint to livestock production[8,18]. The detected prevalence of infection in the cattle was intermediate compared with that observed in other studies in Africa *i.e.* from 29.4 % in Nigeria[1] and up to 65 % in N'dama cattle in Gambia[15]. Similarly, the mean seroprevalence obtained was low compared with the reported prevalence of 73 % in grazed cattle in coastal Kenya[19]; over 88 % in the Caribbean[11]; 55.5 % in Costa Rica[27]; 70 % in El Salvador[28]; 70 % in St Lucia[10]; up to 88 % on Pemba Island, Tanzania, that has a similar climate to the coastal area of Tanga[42].

The estimated seroprevalence, was considerably less than 70 % suggesting, that this pathogen exists in a state of 'endemic instability' as defined by Norval *et al.*[25] and Deem *et al.*[6]. A single cross-sectional study of seroprevalence can only serve as an indicator of the probability of

Table 3: **Variables associated with *Babesia bigemina* percentage seropositivity values from the logistic regression model for dairy cattle in Tanga, Tanzania** (variable considered significant at $P < 0.05$)**.**

Variable	β (SE)	OR	Lower–upper 95 % CI	Wald *P*	Likelihood ratio *P*
Constant	–1.57(0.206)				
Grazing *vs* zero-grazing	0.99(0.38)	2.71	1.28–5.71	0.0087	<0.001
Lushoto *vs* Tanga rural	1.44(0.38)	4.24	1.97–9.10	<0.001	<0.001
Age (centred) in years	0.01(0.004)			0.0017	
Random term	1.0(0.19)				

Table 4: **Variables associated with *Babesia bigemina* percentage seropositivity values from the logistic regression model for dairy cattle in Iringa, Tanzania** (variable considered significant at $P < 0.05$)**.**

Variable	β (SE)	OR	Lower–upper 95 % CI	Wald *P*	Likelihood ratio *P*
Constant	–1.67(0.28)				
Grazing *vs* zero-grazing	1.51(0.25)	4.53	2.70–7.52	<0.001	<0.001
Iringa Urban *vs* Iringa Rural	0.59(0.29)	1.81	1.02–3.24	0.040	0.038
Age (centred) in years	0.01(0.005)			0.008	
Random term	1.04 (0.19)				

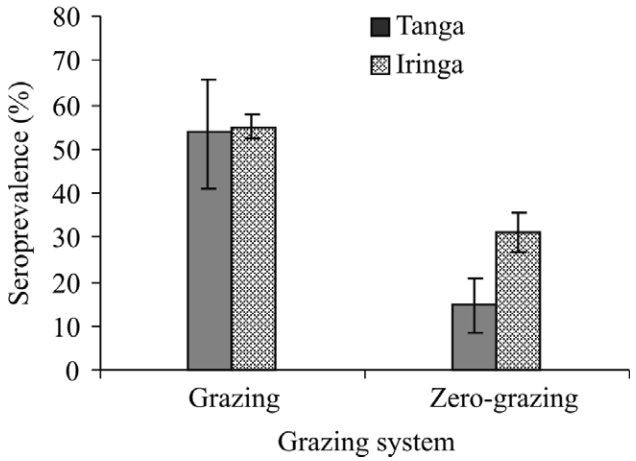

Fig. 3: **Mean serum antibody prevalence** (±95 % CI) **to *Babesia bigemina* by grazing management in Tanga** (grey) **and Iringa** (stippled) **– adjusted for farm effects.**

endemic stability because disease prevalence can vary quite substantially with climatic conditions[5,25,41]. Other factors such as the inherent resistance of cattle to ticks and TBDs, as well as virulence of the pathogen and the infection rate of ticks, may equally influence seropositivity threshold levels[3,18,30,44]. None the less, the levels of exposure to *B.bigemina* (in both study sites) seen in our study are well below any that could be consistent with endemic stability, yet they are sufficiently high to ensure that clinical disease would be a risk.

Consistent with other studies[9,19] in both study regions, a history of recent grazing prior to sampling was associated with a significantly higher likelihood of an animal being seropositive compared with zero-/or semi-grazed animals. Most farmers that practiced zero-grazing fed their cattle forage cut-and-carried from communal grazing pasturelands. It is likely that animals (zero-grazed) had acquired infective ticks *via* fodder brought in from these communal (traditional managed stock) grazing lands.

Results of this study showed a trend of increased seropositivity for *B. bigemina* infection with age. This may partly suggest for the lack of adequately infected numbers of ticks to successfully transmit the infection to calves before they reach 1 year of age. Consistent with previous reports[10], young animals are known to be more resistant to clinical disease than adult animals[2,39].

Variation due to farm effect in this study was less and evidence of clustering of *B. bigemina* within farms and repeated measures on the same animal was minimal. Very few risk factors (out of several) studied were found to be statistically significantly associated with variation in seroprevalence values in the final multivariable models. This was partly attributed to the small number of animals per farm (3–4), which would make clustering difficult to detect even if farm factors were important risk factors for *B. bigemina* infection. Furthermore, study data used was non-experimental in nature, with the attendant difficulties of establishing causal relationship[9,21]. Animal-to-animal transmission of the infection was therefore considered to be minimal.

Most farmers (>90 %) in both regions claimed to practice some form of tick control. However, farmers reported variation in the frequency of use and the methods of application of acaricide were not associated with variations in risk of exposure to *B. bigemina.* This may suggest widespread misuse of acaricides, either due to incorrectly diluting or incorrect application or some resistance of ticks to acaricides[26,34].

Accounting for age, grazing and animal location, none of the other factors investigated were associated with variation of prevalence of serum antibody to *B. bigemina.*

CONCLUSIONS

Seroprevalence was low in smallholder dairy cattle in both study regions, most likely due to zero-grazing management and frequent, intensive use of acaricide, but *B. bigemina* was common and has a wide distribution in the study regions. Under this system, attempts to increase tick burdens and attain 'endemic stability' of babesiosis are likely to be impractical. Grazing significantly increased the likelihood of contact of cattle with infective ticks.

Zero-grazing, even accompanied by high frequency acaricide treatments, did not fully protect the cattle from being exposed to ticks. The use, intensity of use and the type of acaricide application had no effect on the prevalence of serum antibody to *B. bigemina.*

Consistent with low seroprevalence, the likelihood of encountering *B. bigemina* infection increased significantly and logically with age.

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