Cross-sectional estimation of *Babesia bovis* antibody prevalence in cattle in two contrasting dairying areas in Tanzania

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ABSTRACT

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The crude prevalence of antibodies to *Babesia bovis* infection in cattle was estimated by serology using indirect ELISA during the period January to April, 1999. Sera were obtained from 1 395 dairy cattle (of all ages, sexes and breeds) on smallholder farms, the majority being kept under a zero grazing regime. The crude prevalence of antibodies to *Babesia bovis* was 6 % for Tanga and 12 % for Iringa. The forces of infection based on the age sero-prevalence profile, were estimated at six for Iringa and four for Tanga per 100 cattle years-risk, respectively. Using random effect logistic regression as the analytical method, the factors (variables) of age, source of animals and geographic location were hypothesised to be associated with sero-positivity of *Babesia bovis* in the two regions.

Keywords: *Babesia bovis*, dairy cattle, epidemiology, force of infection, sero-prevalence, smallholder, Tanzania

INTRODUCTION

Cattle ticks of the genus *Boophilus* are the only vectors of *Babesia bovis* infection (Mahoney 1979), and of these, *Boophilus microplus* larvae have been singled out as the most efficient transmitting agents of *Babesia bovis* in areas were this infection exists (Riek 1966). The classic epidemiological model of babesiosis in cattle is based on the long lasting immunity induced by primary infection in calves up to 7–9 months old (Riek 1968) when they are naturally resistant to the clinical effects of primary infection. In those herds subjected to *Babesia bovis* infections are from 0.0005 to 0.005 infective bites/head/day, are in endemic instability, while the risk is low, below or above those limit (Mahoney & Ross 1972).

In this article we present the results of a cross-sectional study estimation of the prevalence of *Babesia bovis* antibodies in dairy cattle on the two contrasting and diverse smallholder dairying regions in Tanzania where *Boophilus microplus* is often thought not to exist (Lynen, Bakuname & Sanka 1999). The objective were to quantify the occurrence of *Babesia bovis* through a serological survey, estimate the rate of infection of it and explore the possible relationship between the it and some animal/ farm level variables.

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MATERIALS AND METHODS

Area and study population

The study was carried out in five administrative districts of Tanga region and two in the Iringa region of Tanzania. Tanga region is situated on the North Eastern corner of Tanzania lying between longitude 36 and 38E and latitude 4 and 6S. The region has heterogeneous physical and climatic features varying from hot, humid coastal lowlands in the east to the cool Usambara Mountains in the north and semi-arid plain in the southwest. There are two rainy seasons, the long rains occurring between March and May and the short rains occurring between September and November. Rainfall varies widely from 500 mm in semi-arid areas to 1400 mm in coastal areas and up to 2000 mm in some inland mountain areas. Daytime temperatures vary from 23–28 °C during the cool season (May to September) to 30–33 °C during the hot season (December to March).

Iringa region is one of three in the Southern highland zone of Tanzania and lies between latitude 7 and 8S and longitude 35 and 36E. The region lies between 1 340 and 2 090 m above sea level. Rainfall is annually bimodal and ranges between 600 and 1 600 mm per annum, with most rain falling between March and June and occasional light rain between August and September. In both regions, type of cattle kept includes *Bos taurus* breed (Friesian, Ayrshire, Jersey, Simmental) and crosses of these breeds with *Bos indicus* breeds (Tanzania shorthorn zebu, Boran and Sahiwal).

Study design

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 (Gitau, McDermott, Walter-Toews, Lissemore, Osumo & Muruki 1994; French & Tyrer 1997). The farms in each study region were randomly (Epi-Info version 6.04b) selected in October 1998, 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. The Dutch and Swiss governments have been supporting dairy schemes in Tanga and Iringa regions, respectively. Over the last 15 years (1983–1998) of support, huge data bases have been generated.

As the antibody prevalence was not known *a priori*, a 50 % prevalence was assumed when calculating the total number of farms required for the study, with a 10% allowable error. The farms in both study sites were estimated (on previous experience) to have an average of three to four dairy stock of any age, breed or sex. A farm sample size of 200 in each study region was considered necessary to provide between 600 and 800 animals for the study and to allow for a “design effect” of 2.0. Farms having more than ten animals were excluded from the selection process because such herds are not considered as “smallholder” enterprises (Tanga Dairy Development Programme 1999).

Questionnaire design and administration

One person in each region collected most farm-level and some animal-level data using a structured questionnaire, which was completed by the smallholder on all the selected farms on a single visit. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation and improve precision of responses (Thrusfield 2000). Questionnaire administration and collection of data from animals were carried out by two separate teams, during the period of January to April 1999, in the two regions. Although comparisons are made between the two regions, the data were not combined for analyses to allow any unexplained variation due to variations in the precise way that data were collected to be estimated.

The information gathered concerned farm and animal events that had occurred during 1998 and included cattle location, source (homebred or brought-in), mode of acquisition of dairy stock, sex, level of exotic blood (Filial generation), breed codes, age and housing practices, as well as whether or not a system of zero-grazing was practised of if the cattle had been allowed to graze on pastures in the 3 months prior to sampling. The detailed variables studied have been described by Swai (2002). The responses to many of these questions were investigated as explanatory variables in the analyses of sero-conversion to *Babesia bovis.*

Collection of sera and their analysis

During the visit to each farm, blood samples were collected by jugular venipuncture into 10 mℓ “Vacutainer” tubes (Becton Dickson, UK) from all animals on the farm. These were labelled and transported in a refrigerated cool box to local laboratories where aliquots of sera were obtained by centrifugation at 3 000 *g* for 20 min after which they were then stored at –20 °C at Sokoine University of Agriculture (SUA) prior to dispatch in refrigerated containers to the International Livestock Research Institute (ILRI) in Nairobi, Kenya where they were subjected to indirect enzyme-linked immunosorbent assay (ELISA) in order to evaluate the level of antibodies to *Babesia bovis* (Katende, Goddeeris, Nkonge, Morzaria & Musoke 1990; Katende,Toye, Skilton, Nene, Morzaria & Musoke 1998). The ELISA has shown a sensitivity of 99 % and a specificity of 98 % (Katende *et al.* 1998). The results were expressed as percent positivity (PP) values of optical densities (Wright, Nilsson, Van Rooij, Lelenta & Jeggo 1993), relative to that of a strongly positive control serum. Each test serum sample was analysed in duplicate and that of the control sera in triplicate. The threshold level of PP for positivity was 25 % for *Babesia bovis*.

Statistical methods

Descriptive statistics for the animal- and farm-level explanatory variables examined in the study were developed using Epi-Info, version 6.04d. The relationships between explanatory variables and outcome response (sero-conversion to *Babesia bovis*) were investigated in two steps by logistic regression (using Egret for Windows version 2.0, Seattle, USA) with "farm" as a random effect because animals on one farm may not have been statistically independent of one another (Kristula, Curtis, Galligan & Bartholomew 1992). In the first step, the relationships between each explanatory and outcome variable were individually investigated. In the second step, any variables that were significantly associated at the $P < 0.25$ level were included in multivariable models producing, by forwards and backwards substitution and elimination, the most parsimonious models in which all explanatory variables remained significant at the $P < 0.05$ level. The criteria for inclusion and exclusion were a change of in deviance significant at the 5 % level according to the maximum likelihood ratio test—Chi square distribution.

Forces of infection were estimated from age sero-prevalence profiles using Maximum Likelihood Methods (MLM) in Excel (Microsoft, USA) with solver add-in (Thrusfield 2000). Assuming a stable population size and age structure and a constant force of infection across all age groups, the log likelihood was derived using the following formula:

$$\textit{Loglikelihood } L = \sum_{i=1}^{a} R_i\, \ell n e^{-\lambda i} + (N_i - R_i)\, \ell n\, (1 - e^{-\lambda i})$$

where R_i = number of sero-positive in group *i*, N_i = number tested in age group *i* and λ = the force of infection

RESULTS

Farm participation

All selected 200 farms from each of Tanga and Iringa regions were visited and farmers interviewed during the period of January 1999 to April 1999 (a 100 % response rate). In Tanga, a total of 697 animals kept on 185 (92.5 %) farms were examined. Fifteen farms (7.5 %) had no animals during the actual survey period. In Iringa, a total of 698 animals from 195 (97.5 %) farms were examined. Three farms (1.5 %) had no animals and two farms (1 %) could not be sampled because the owner could not be traced. The number of animals examined per herd was 3 ranged from 1 to 9 animals. The distribution of cattle amongst categories of each variable investigated is summarised in Table 1.

Serological responses to *Babesia bovis*

The crude sero-prevalence of antibodies to *Babesia bovis* was 6 % (4.7, 8.6) and 12 % (9.6, 14.7) for Tanga and Iringa, respectively (Table 2).

The estimated force of infection was 0.04 and 0.06 per animal years-risk for Tanga and Iringa, respectively. The graphical forces of infection are shown in Fig.1 and 2. The estimated force of infection was slightly higher in Iringa than in Tanga.

The results of the final logistic regression model are detailed in Tables 3 and 4.

In both Tanga and Iringa regions sero-prevalence was significantly greater in grazed animals than in zero-grazed animals. In Iringa region animals located in Iringa urban district were significantly more likely to be sero-positive for *Babesia bovis* than animals on farms in Iringa rural district (OR = 5.83, $P = 0.010$). In both regions cattle brought onto the farms were significantly more likely to be sero-positive than animals born on-farm (OR = 2.43, $P = 0.035$ and OR = 3.1, $P = 0.016$, respectively for Iringa and Tanga regions).

DISCUSSION

The prevalence of *Babesia bovis* infection as reflected by ELISA appears to be comparatively higher in Iringa (12 %) than in Tanga (6 %). However, the

TABLE 1 The proportions of cattle in each category of each variable investigated during the study

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	1 (0.1)	12 (2)
	Simmental cross	0	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 1998	Zero grazing	489 (70)	631 (90)
	Semi/free grazing	209 (30)	66 (10)
Farm-level variables			
Farm classification	Peri-urban	109 (16)	117 (17)
	Urban	391 (56)	318 (46)
	Rural	198 (28)	262 (37)
District (Iringa)	Iringa urban	461 (66)	NA
	Iringa rural	237 (34)	
District (Tanga)	Tanga	NA	341 (48.8)
	Muheza		185 (26.5)
	Pangani		26 (4)
	Korogwe		64 (9)
	Lushoto		81 (12)
Mode of acquisition	Bought cash	471 (67)	268 (38)
	Credit (HIT)	117 (17)	356 (51)
	Others (gift)	110 (16)	73 (11)

TABLE 2 The prevalence (with +/– 95 % confidence intervals) of cattle sero-positive for *Babesia bovis* in the study regions (January to April 1999)

Region	No tested	No positive	Prevalence (%)	95 % CI	
				Lower	Upper
Tanga	697	42	6	4.7	8.6
Iringa	698	84	12	9.6	14.7

TABLE 3 Final logistic binomial multiple regression for sero-prevalence of *Babesia bovis* in dairy cattle, Tanga, Tanzania (January to April 1999)

Factor	β(SE)	OR	Lower to upper 95 % CI	Wald *P*	Likelihood ratio *P*
Constant	–4.33 (0.56)				
Grazing vs zero grazing	1.84 (0.57)	6.34	2.05–19.56	0.001	< 0.001
Brought vs Homebred	1.14 (0.47)	3.15	1.23–8.03	0.016	< 0.001
Random term	1.44 (0.38)				

TABLE 4 Final logistic binomial multiple regression for sero-prevalence of *Babesia bovis* in dairy cattle, Iringa, Tanzania (January to April 1999)

Factor	β(SE)	OR	Lower to upper 95 % CI	Wald *P*	Likelihood ratio *P*
Constant	–5.57 (0.82)				
Grazing vs zero grazing	1.29 (0.48)	3.65	1.40–9.49	0.007	< 0.001
Brought vs Homebred	0.88 (0.42)	2.43	1.06–5.56	0.035	< 0.001
Iringa Urban vs Iringa Rural	1.76 (0.68)	3.83	1.51–22.53	0.010	0.032
Age centred	0.008 (0.006)			0.062	
Random term	2.06 (0.40)				

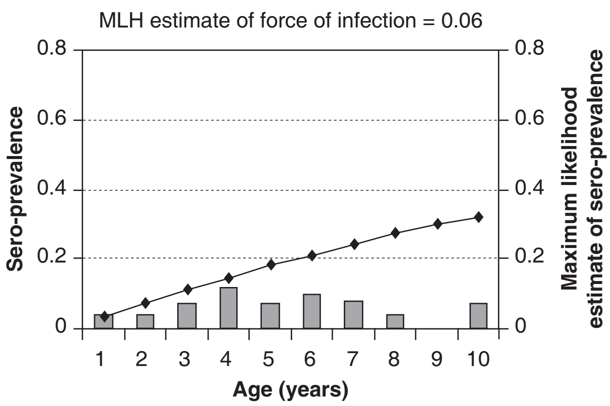

FIG. 1 Force of infection estimates for *Babesia bovis* infection in dairy cattle—Iringa, Tanzania (January to April 1999)

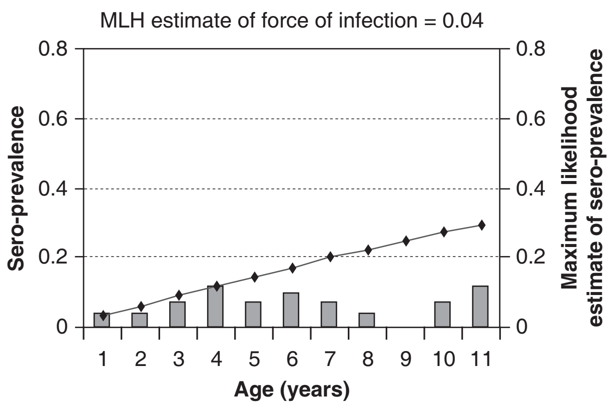

FIG. 2 Force of infection estimates for *Babesia bovis* infection in dairy cattle—Tanga, Tanzania (January to April 1999)

estimated prevalences were low when compared to the findings of 88 % by Woodford, Jones, Rae, Boid & Bell-Saikyi (1990) in Pemba, Tanzania; over 60% by Perez, Herrero, Jimenez, Carpenter & Buening (1994) in Costa Rica; and 73 % of dairy cattle by Maloo, Thorpe, Kioo, Ngumi, Rowland & Perry (2001) in coastal Kenya.

There was also evidence of wide spread of this pathogen within the studied districts, particularly in Iringa. The distribution of *Babesia bovis* infection was limited to some districts in Tanga. This distribution pattern may suggest that a situation of "endemic instability" prevails and that a similar distribution of the vector ticks (*Boophilus* spp.) occurs in the district where the *Babesia* infection is known to exist. The low serological prevalence for *Babesia bovis* infection may be due to its poor infectivity for *Babesia* (Mahoney & Mirre 1971) and subsequently the occurrence of lower inoculation rates in susceptible cattle (Mahoney 1979). The lower observed inoculation rates (force of infection), in the light of these findings, may predict an enhanced tick resistance (Mahoney 1979) or a genetically based immunity to *Babesia bovis* characteristic of zebu crosses, which results in low inoculation rates of this *Babesia* parasite.

As in other studies, in both Tanga and Iringa regions, a history of recent grazing prior to sampling was associated with a significantly higher likelihood of an animal being sero-positive to *Babesia bovis* infection, compared to zero-grazed animals (Maloo *et al*. 2001).

Sero-prevalence varied with the mode of acquisition of the animals. Accounting for age, brought-in animals were more likely to be sero-positive for *Babesia bovis* than were homebred animals. This would be consistent with the fact that most dairy stock available for sale in local markets are pregnant heifers from specialized large-scale crossbreeding ranches where they are mostly grazed rather than housed (Swai 2002).

Allowing for variations in sero-prevalence related to age and management factors, such as grazing, geographic variation in sero-prevalence of *Babesia bovis* infection was also determined. This variation was more conspicuous in the Iringa study region with the odds of infection being three fold for cattle situated in Iringa urban areas when compared to that in the cattle in the Iringa rural district. This implies that there must be geographical variations in either the density of tick vectors or the prevalence of *Babesia bovis* infection in host-seeking ticks, or both. Variations in sero-prevalence may partly be owing to cattle immune responses (Mahoney 1979). Geographic variation in sero-prevalence was more uniform and generally higher in the Iringa region. Babesiosis has not been reported to be a major clinical problem in both study regions (TDDP 1999; Southern Highland Dairy Development Programme 1995) but there might be underestimations of clinical babesiosis in these regions.

A diagnosis of bracken poisoning is frequently made by farmers and extension workers when cattle show clinical signs of “red water” (haemoglobinuria or haematuria) and in the light of the findings in this survey, some or many of these cases may, in fact, be misdiagnosed cases of babesiosis.

An increased trend of sero-positivity associated with age was evident in this study. These findings are consistent with previous reports (Hugh-Jones, Busch & Jones 1988) which showed young animals to be more resistant to primary infections.

We decided a *priori* to allow for intraherd correlation in the analysis by incorporating “farm” as a random-effect term in all models. However in common with similar studies (Gitau *et al.* 1994), there was little evidence of clustering at the level of farm, probably owing to the small number of animals per farm (McDermott & Schukken 1994)

CONCLUSION

The results of this study can be summarized as follows:

- Sero-prevalence of antibodies to *Babesia bovis* was low in smallholder dairy cattle in the study regions, most likely due to zero-grazing management, but there was evidence of a wide distribution in both the study regions.
- Consistent with this the force of infection varied geographically. It was, however, comparatively low and the likelihood of a bovine encountering the infection increased significantly with age.
- Farmer reporting of grazing significantly increased the likelihood of contact of cattle with infective ticks.
- The source of animals, particularly recently purchased animals, may be more likely to have encountered and recovered from *Babesia bovis* infection.

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