



## A serological survey of bovine babesiosis in northern and eastern Zimbabwe

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### ABSTRACT

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The geographical distribution of *Babesia bovis* and *Babesia bigemina* antibodies in communal herds in northern and eastern Zimbabwe was determined using the ELISA technique. The animals in different herds in the study region had different levels of natural exposure to *B. bovis* (mean 32%, range 0–79%) and *B. bigemina* (mean 52%, range 5–92%) infections. The majority of herds (90%) were endemically unstable for *B. bigemina* and 62% were unstable for *B. bovis*. Natural region 5 and Manicaland province had the highest seroprevalence of *B. bovis* infection, while natural region 5 and Masvingo province had the highest seroprevalence of *B. bigemina* infection.

**Keywords:** *Babesia bigemina*, *Babesia bovis*, communal herds, distribution, Zimbabwe

### INTRODUCTION

Bovine babesiosis is a haemoparasitic tick-borne disease (TBD) caused by *Babesia bigemina* and *Babesia bovis* (Riek 1964; Riek 1966). The disease is among the major TBDs of economic importance that are a constraint to livestock production in many countries of the world (McCosker 1981; Pipano 1995), including Zimbabwe.

Integrated tick and TBD control (ITTC) methods based on the concepts of endemic stability (Mahoney & Ross 1972; Callow & Dalgliesh 1982) and immunization (Tatchell & McCosker 1984) have gained recognition worldwide. The Department of Veterinary Services (DVS) in Zimbabwe is interested in implementing the ITTC programme to control bovine babesiosis and other TBDs in communal herds. However, before such a programme can be implemented it is important to know the level of natural exposure to TBDs in cattle populations as well as the distribution of these diseases in the country. This information will be used to target suitable herds in the cattle population for immunization.

Although *B. bigemina* and its main tick vector, *Boophilus decoloratus* (Riek 1964) have been known to be widespread throughout Zimbabwe (Lawrence & Norval 1979; Norval, Fivaz, Lawrence & Daillecourt 1983), the current endemic status of cattle to this disease is unclear. Outbreaks of babesiosis continue to be reported in communal cattle throughout the country (DVS, unpublished field reports) even though these cattle are mainly indigenous Mashona animals of *Bos indicus* origin which are widely believed to be

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resistant to this disease (Daly & Hall 1955; Johnston 1967; Lohr 1973; Johnston & Sinclair 1980; Copeman 1983; Callow 1984; Parker, Shepherd, Trueman, Jones, Kent & Polkinghorne 1985; Bock, de Vos, Kingston & McLellan 1997).

The distribution of *B. bovis* in Zimbabwe is not clearly known. Recent studies in Zimbabwe revealed that *Boophilus microplus* ticks, which are the main vectors of this disease (Riek 1966), were limited mainly to eastern and northern parts of the country (Katsande, Mazhowu, Turton & Munodzana 1996). The same tick distribution was observed by Norval *et al.* (1983) who also reported isolated cases of *B. bovis* at some localities in the western parts of Zimbabwe. It was generally believed that after three successive years of drought between 1981 and 1984, *B. microplus* and *B. bovis* disappeared completely from the whole country (R.A.I. Norval unpublished data, cited by Norval, Perry & Hargreaves, 1992). This information may not have been correct or the situation may have changed given that cases of *B. bovis* have been diagnosed at the Central Veterinary Laboratory (CVL) in Harare (Katsande *et al.* 1996). In recent years, more cases of *B. bovis* have been diagnosed by field veterinarians in the northern and eastern districts of the country (DVS, unpublished field reports).

The results of a study on the distribution of antibodies against *B. bovis* and *B. bigemina* in communal herds in northern and eastern Zimbabwe between July and November 1997 are reported in this paper. On the basis of the findings, implications of the level of natural disease exposure and endemic stability in communal herds in the study region are discussed.

## MATERIALS AND METHODS

### Study area description

The study was carried out between July and November 1997 in northern and eastern Zimbabwe. This area has been divided into Natural Regions (NRs) 1, 2a, 2b, 3, 4 and 5 by the Zimbabwean government according to agricultural potential and recommended land use (Vincent & Thomas 1961; Anon 1996) (Table 1A). NRs 1, 2a, 2b and 3 are located in the highveld region with an altitude of greater than 900 m, while NRs 4 and 5 are in the lowveld region with an altitude of less than 900 m.

### Reference population and unit of interest

In this investigation, the reference population is all communal cattle herds in northern and eastern Zimbabwe. The unit of interest is the dip tank or communal herd cluster. A dip tank is defined as a communal dipping facility to which communal farmers bring their cattle on a regular basis for acaricide treatment. Dip tanks are fairly evenly distributed through-

out the study region and the cattle presented at these locations are primarily indigenous Mashona cattle (Sanga type) of *B. indicus* origin.

### Dipping frequency

Dipping of cattle at the study dip tanks was generally done according to recommendations by DVS. Depending on eco-climatic conditions and tick challenge some dip tanks followed either a weekly or fortnightly dipping interval during the wet season and fortnightly or monthly interval during the dry season. This dipping interval was, however, occasionally disrupted throughout the year mainly due shortage of water and/or acaricides.

### Sampling technique

A three stage sampling regime was used in the study. NRs, dip tanks and individual animals were the primary, secondary and tertiary sampling units, respectively. The sampling frame for the selection of dip tanks was obtained from the DVS in Harare. Dip tanks were stratified by NR and selected using a simple random method by use of random numbers.

There were 885 dip tanks in the study area. Based on an estimated herd prevalence of 20%, we calculated that a random sample of 60 dip tanks would enable us to be 95% confident of being within 10% of the true prevalence (Dean 1994, Statcalc, Epi Info Version 6.04a). The number of dip tanks to be sampled in each NR strata was then determined by proportional allocation.

### Sampling of animals at the dip tank

The required number of animals to be sampled at each dip tank was calculated using the Freecal software (Cameron 1996), based on the assumption that the sensitivity and specificity of the *B. bovis* and *B. bigemina* ELISAs were each 95% (Molloy, Bowles, Bock, Turton, Katsande, Katende, Mabikacheche, Waldron, Blight & Dalgliesh 1998a; Molloy, Bowles, Jeston, Bruyars, Bowden, Bock, Jorgensen, Blight & Dalgliesh 1998b). Using this software and based on expected minimum prevalence in infected herds of 25%, we calculated that a random sample of 25 from a population of approximately 200 eligible animals would be required. In this situation, if three or fewer reactors were found, we could be at least 95% confident that the herd was in fact disease free.

Samples were collected from animals aged between 6–18 months old using a sampling method based on convenience. Although an attempt was made to obtain a representative sample by sampling at least one animal from every village or kraal, the sampling method generally depended on the cooperation of farmers. The age of each sampled animal was

determined from the farmer and/or from dentition. The first molar (M1), which erupts at 5–6 months, and milk teeth which are found in animals less than 18 months old were used as landmarks to identify animals aged between 6–18 months old (Bryant & Norval 1984)

### Sample collection and processing

Animals were physically restrained with the help of the owners and DVS field staff and about 10 ml of blood was collected by jugular veni-puncture using an 18 gauge needle and a 10 or 20 ml syringe. The blood was poured into Terumo vacutainers (Terumo Vacuum blood collect, Venoject II, P200s, Banksia Scientific, Australia). Each sample was labelled with a date, dip tank name and sex of the animal. Samples were left in the shade for at least 2 h before being transported to the nearest Veterinary Office where they were kept at 4 °C for 1–6 d before being transported to CVL in a cooler box with ice.

In the laboratory sera were separated from clotted blood samples by centrifugation at 3000 rpm for 30 min. Each sample was divided into four by 2 ml aliquots and stored at –20 °C or –80 °C for short or long term storage, respectively.

### Serological tests

Serum samples were tested for antibodies directed against *B. bovis* and *B. bigemina* using the *B. bovis* and *B. bigemina* ELISAs as described by Molloy *et al.* (1998a) and Molloy *et al.* (1998b), respectively, with the following modifications. Five percent skim milk powder was used instead of 5% normal horse serum in the *B. bovis* ELISA. In the *B. bigemina* ELISA, monoclonal antibody B9 was diluted 1:750 in carbonate buffer instead of 1:1 000. Horse Radish Peroxidase Streptavidin was diluted 1:3 000 in buffer instead of 1:5 000.

### Data management and analysis

Epidemiological data were entered into Epi Info v6.04a (Dean 1994). Analyses were conducted using Epi Info, Statistix v4.0 (Analytical Software, Tallahassee, FL, USA), Microsoft Excel for Windows v7.0 and Exactbin Version 1.0 (Staebling & Sullivan 1988).

Differences in prevalence rates among provinces and NRs were determined by comparing 95% confidence intervals. Prevalence rates with overlapping confidence intervals were considered to be not significantly different.

### Classification of seroprevalence

In this study herd seroprevalence was classified into five categories according to definitions of Norval *et al.* (1983) as follows:

Endemically stable (S)	= 81–100%	animals positive
Approaching endemic stability (A)	= 61–80%	animals positive
Endemically unstable (U)	= 21–60%	animals positive
Minimum disease (M)	= 11–20%	animals positive
Disease free (F)	= 0–10%	animals positive

## RESULTS

A total of 61 dip tanks was visited during the study. At each of these dip tanks, blood samples were collected from an average of 37 animals. With the exception of two dip tanks where, due to poor turnout, only 20 and 21 animals were sampled, a range of 26–40 animals were sampled. A total of 60 questionnaires were answered from the original sample of 61 dip tanks. Fig. 1 shows the locations of dip tanks that were sampled during the study. The results of the seroprevalence of bovine babesiosis and some factors affecting seroprevalence of this disease are presented in this paper.

### Prevalence of *B. bovis* and *B. bigemina* infection

#### *B. bovis*

A total of 2 237 blood samples collected from 61 herds in the study region was processed and tested for antibodies against *B. bovis* using the ELISA. The mean prevalence of positive animals was 32.3% (median 17, 25<sup>th</sup> percentile 5, 75<sup>th</sup> percentile 63, range 0–79). A substantial proportion of the herds (37.7%) were free from *B. bovis* infection. None of the dip tanks had an endemically stable situation, 27.9% were approaching endemic stability, 21.3% were unstable and 13.1% had minimum disease. The geographical distribution of antibodies against *B. bovis* by NR is shown in Fig. 2. Table 1A shows the animal and herd-level prevalence results of *B. bovis* by natural region.

#### *B. bigemina*

A total of 2 266 blood samples from the same herds as above were tested for antibodies against *B. bigemina*. The mean prevalence of positive animals was 52.4% (median 63, 25<sup>th</sup> percentile 30.5, 75<sup>th</sup> percentile 70, range 5–92). About 7% of the dip tanks were stable, 44.3% were approaching endemic stability, 29.5% were unstable, 16.4% had minimum disease and 3.3% were free from *B. bigemina* infection. The geographical distribution of antibodies against *B. bigemina* by NR is shown in Fig. 3. Animal and

TABLE 1A Prevalence of *B. bovis* in 6 to 18-months-old cattle by natural regions and agricultural production systems in northern and eastern Zimbabwe during July to November 1997

Natural region	Average rainfall (mm)	Agricultural production system		Animals tested	Animal seroprevalence			Herds tested	% of herds in each category <sup>cd</sup>				
		Livestock	Crops		% <sup>a</sup>	95 % CI <sup>b</sup>	S		A	U	M	F	
1	> 900	Intensive	Forestry and fruit	87	13,8 <sup>x</sup>	7,3,	22,8	3	0	0,0	33,3	0,0	66,7
2a	750–1000	Intensive	All crops	298	19,8 <sup>x</sup>	15,4,	24,8	8	0	12,5	12,5	25,0	50,0
2b	750–1000	Intensive	All crops	508	38,8 <sup>y</sup>	34,5,	43,2	14	0	42,9	28,6	0,0	28,6
3	600–800	Semi-extensive	Maize, tobacco and cotton	668	38,0 <sup>y</sup>	34,3,	41,8	18	0	27,8	27,8	5,6	38,9
4	450–650	Semi-extensive	Drought resistant crops	532	21,4 <sup>x</sup>	18,0,	25,2	14	0	21,4	0,0	35,7	42,9
5	<450	Extensive	Unsuitable	144	60,4 <sup>z</sup>	51,9,	68,4	4	0	50,0	50,0	0,0	0,0
Total/overall				2237	32,3	30,4,	34,3	61	0	27,9	21,3	13,1	37,7

<sup>a</sup> The percentage of animals that tested positive

<sup>b</sup> Exact binomial 95 % Confidence Intervals

<sup>c</sup> Herd seroprevalence was classified into five endemic stability categories as follows:

Endemically stable (S) = 81–100 % animals positive

Approaching endemic stability (A) = 61–80 % animals positive

Endemically unstable (U) = 21–60 % animals positive

Minimum disease situation (M) = 11–20 % animals positive

Disease free (F) = 0–10 % animals positive

<sup>d</sup> A herd was considered positive if more than four animals in the herd tested positive in the *B. bovis* or *B. bigemina* ELISA as determined by FreeCalc (Cameron 1996), assuming a minimum expected prevalence of 25 % in infected herds, average sample size of 35 animals per herd, average population size of weaners of 200 and sensitivity and specificity of ELISAs of 95 %

<sup>xyz</sup> Percentages with different superscripts are significantly different at  $P < 0,05$  as determined by non overlapping 95 % confidence intervals

TABLE 1B Prevalence of *B. bigemina* in 6 to 18-months-old cattle by natural region in northern and eastern Zimbabwe during July to November 1997

Natural region	Animals tested	Animal seroprevalence		Herds tested	% of herds in each category <sup>cd</sup>				
		% <sup>a</sup>	95 % CI <sup>b</sup>		S	A	U	M	F
1	92	38,0 <sup>x</sup>	28,1, 48,8	3	0,0	0,0	100,0	0,0	0,0
2a	302	57,0 <sup>y</sup>	51,2, 62,6	8	0,0	75,0	0,0	5,0	0,0
2b	514	49,4 <sup>xy</sup>	45,0, 53,8	14	0,0	35,7	50,0	14,3	0,0
3	668	53,1 <sup>y</sup>	49,3, 57,0	18	11,1	38,9	33,3	16,7	5,6
4	539	49,7 <sup>xy</sup>	45,4, 54,0	14	14,3	35,7	14,3	35,7	7,1
5	151	68,2 <sup>z</sup>	60,1, 75,5	4	0,0	100,0	0,0	0,0	0,0
Total/overall		52,4	50,3, 54,5	61	6,6	44,3	29,5	16,4	3,3

Notes for the superscripts are as shown in Table 1A

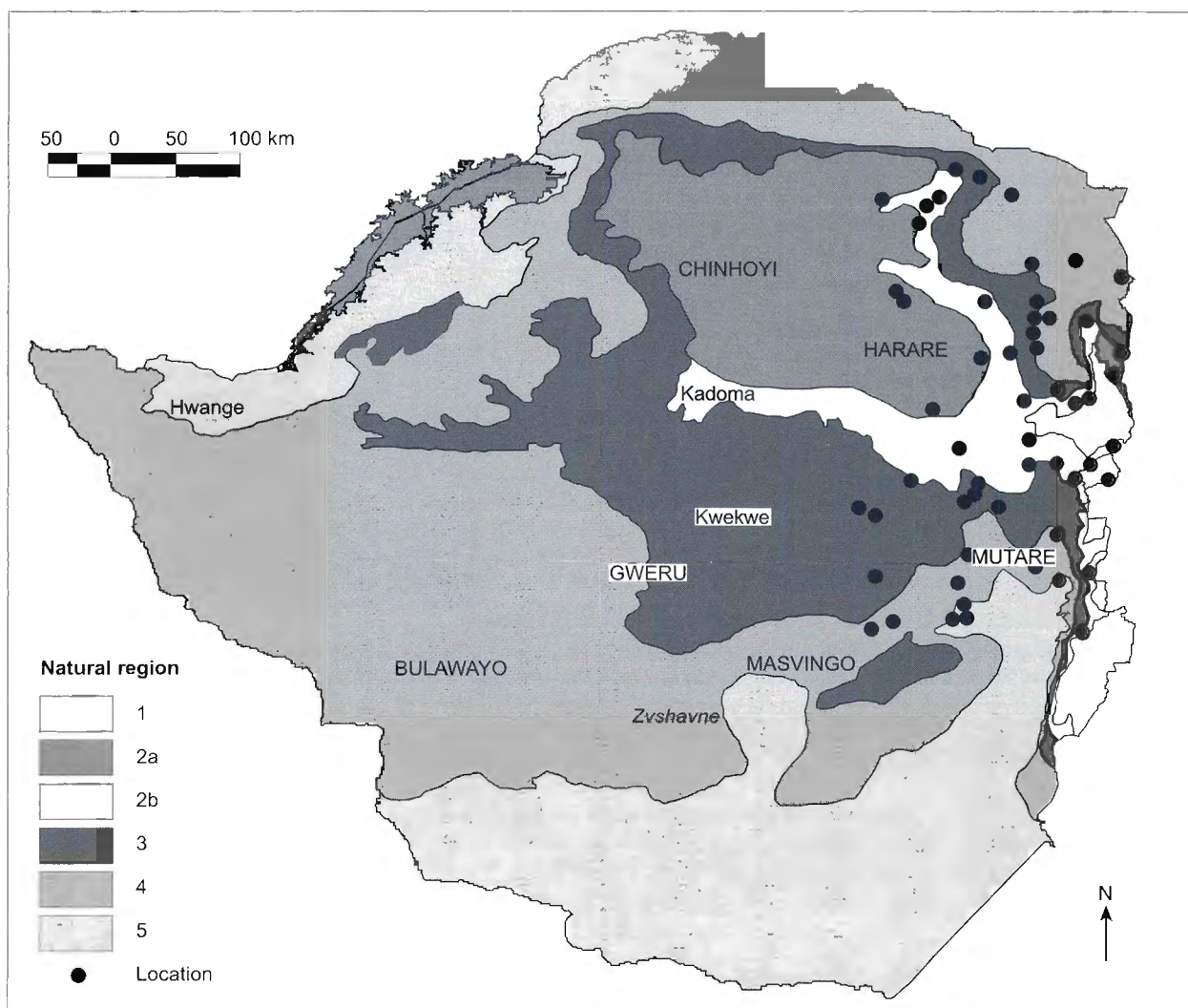


FIG. 1 Map of Zimbabwe showing the location of dip tanks at which cattle were sampled in the northern and eastern regions during July and November 1997 by natural region

herd-level *B. bigemina* seroprevalence results by natural region are shown in Table 1B.

## DISCUSSION

### Distribution of bovine babesiosis

The distribution of *B. bovis* has been reported to be limited to eastern Zimbabwe in association with the spread of its tick vector *B. microplus* from Mozambique during the 1970s (Norval *et al.* 1983). In support of this report, the current study demonstrated that herd-level seroprevalence to *B. bovis* was highest at those dip tanks situated close to Zimbabwe's eastern border with Mozambique (NR 2b and Manicaland province), and at those dip tanks located in areas where climatic conditions are generally dry (NR 5). The *B. bovis* seroprevalence was, however,

lowest in NR 1, despite the fact that this region is closest to the eastern border with Mozambique. This region receives the highest amount of rainfall in the country and water for dipping is readily available. It is therefore speculated that the dipping system in that region is relatively efficient, hence, the low prevalence of *B. bovis*.

The prevalence of *B. bigemina* infection in the study region was relatively higher and more uniform than that of *B. bovis*. This distribution was not unexpected because *B. bigemina* is transmitted by both *B. microplus* and *B. decoloratus* tick vectors (Riek 1966; Mahoney & Mirre 1979). In contrast, *B. bovis* is only transmitted by *B. microplus* (Callow & Hoyte 1961; Riek 1964; Dalgliesh, Stewart & Callow 1978). *B. decoloratus* is more widespread in Zimbabwe and probably occurs in higher numbers than *B. microplus* (Lawrence & Norval, 1979; Norval *et al.* 1983; W.

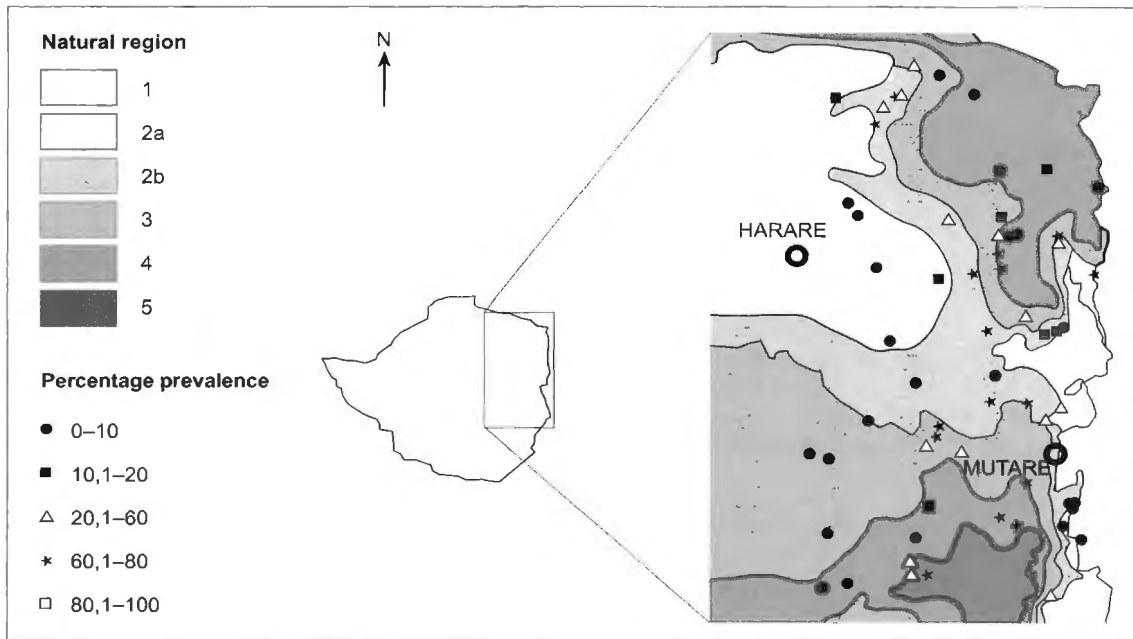


FIG. 2 Map of Zimbabwe showing the geographical distribution of herd prevalence of antibodies against *B. bovis* infection in the northern and eastern region during July to November 1997 by natural region

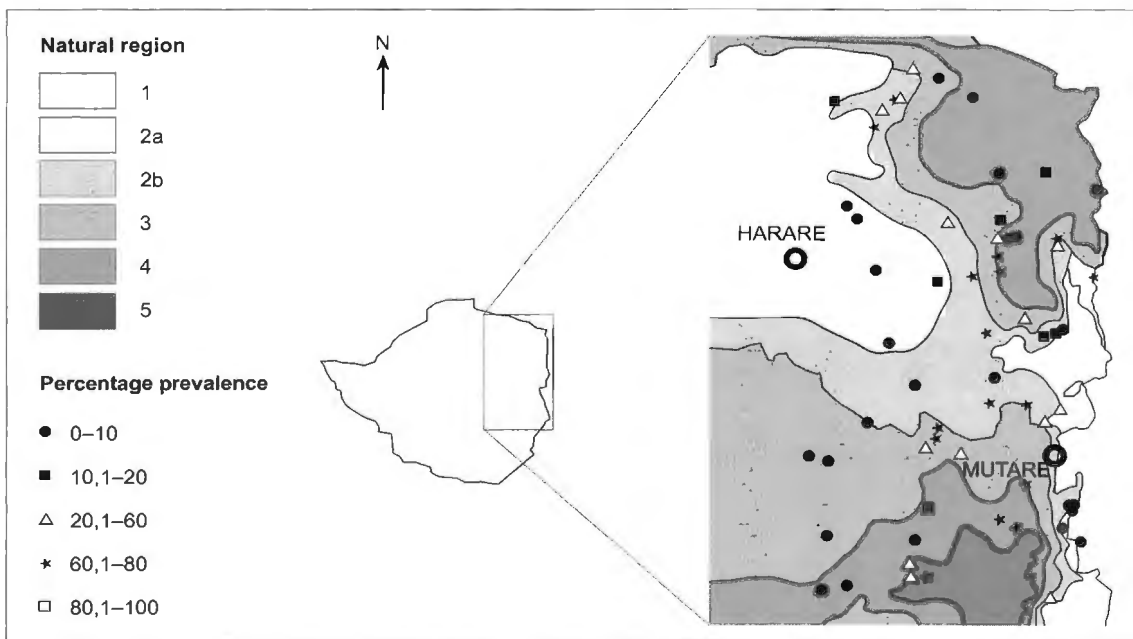


FIG. 3 Map of Zimbabwe showing the geographical distribution of herd prevalence of antibodies against *B. bigemina* infection in the northern and eastern region during July to November 1997 by natural region

Mazhovu, National Tick Survey 1989–1995, unpublished data), hence the higher and more uniform distribution of *B. bigemina*.

Although the distribution of *B. bigemina* was more uniform than that of *B. bovis*, there were some differences in the distribution of the disease among the different regions of the country. These differences

were probably attributed to distributions of the tick vectors and the differences in climatic conditions within the various regions. NR 5 and Masvingo province, which are the driest regions in the country, had the highest seroprevalence of *B. bigemina* infection. This distribution was probably associated with low dipping efficiency due to problems with water availability. It was also interesting to note that the four

herds that were endemically stable to *B. bigemina* were in NRs 3 and 4 (two in each NR). The high *B. bigemina* seroprevalence in these regions can be explained by the relatively dry climatic conditions in these regions and the possible overlapping distribution of the two tick vectors, *B. microplus* and *B. decoloratus*, responsible for transmitting this disease. Both tick vectors are probably found in NR 4, which shares the country's border with Mozambique in the north-east, and in NR 3 which lies on the boundary of the eastern highlands. It is therefore possible that the two vectors are contributing to the transmission of the disease in these regions, resulting in a high prevalence rate for *B. bigemina*.

### Assessment of endemic stability

The method that was used in this study to assess endemic stability was first established by Mahoney & Ross (1972) and Mahoney (1973 and 1974), and later redefined by Norval *et al.* (1983). These authors had each based their assessment on serological results from animals aged up to 9 months. In the current study, however, endemic stability was assessed using serological results from animals aged between 6–18 months. The inclusion of 10–18 month-old animals in the study probably resulted in an over-estimation of endemically stable herds because estimates of endemic stability should be done on 6–9 month-old animals (Mahoney & Ross 1972). Ideally, the level of endemic stability in a herd should be determined from a model based on determination of inoculation rates (Mahoney & Ross 1972; Mahoney 1973 and 1974). This model was developed and validated in *B. taurus* cattle. The application of this model in *B. indicus* cattle is, however, questionable. In Venezuela (James, Coronado, Lopez, Melendes & Ristic 1985), Zambia (Jongejan, Perry, Moorhouse, Musisi, Pegram & Snacken 1988), Lesser Antilles (Camus & Montenegro-James 1994) and South Africa (Tice, Bryson, Stewart, du Plessis & de Waal 1998), the majority of the inoculation rate values (*h* values) in *B. indicus* cattle herds fell within the area of instability with maximum risk. Huge disease outbreaks were, however, not reported. For this reason, this model was not used in this study as the majority of the cattle population in the study region are of *B. indicus* breed.

### Herd prevalence and endemic stability

Our results on herd prevalence differ from those obtained by Norval *et al.* (1983). They reported that antibodies to *B. bovis* were detected in more than 80% of the sera at 7% of the communal dip tanks, compared to 0% in this study. Norval *et al.* (1983) also reported antibodies to *B. bigemina* to be much more common than was observed in the this study, with 58% of the localities having more than 80% positive sera. In this study only 7% of the localities sampled had more than 80% positive sera.

The timing of the two studies relative to the Zimbabwean war of independence is a possible explanation for the difference in the level of natural exposure to bovine babesiosis in communal herds between this study and that of Norval *et al.* (1983). During the 1970s, dipping was greatly disrupted as a result of this war. Following the country's independence in 1980, intensive dipping recommenced and has continued until the present day. As a result of the disruption in dipping, and as further confirmed in the study of Norval *et al.* (1983), it was demonstrated that a relatively high proportion of communal cattle had gained endemic stability to *B. bigemina* infection. Furthermore, a small proportion gained endemic stability to *B. bovis* infection, probably due to the limited abundance of the tick vector *B. microplus* in the country. It is believed that the continuation of intensive dipping in these herds disrupted this endemic stability resulting in a highly unstable situation as demonstrated by this study.

The implications of the endemically unstable babesiosis situation in communal cattle are not clearly known. In South Africa, Tice *et al.* (1998) reported that clinical babesiosis was rare in communal cattle herds that moved from an endemically unstable to a stable situation. They cited low pathogenicity of the babesia organisms, resistance of the local cattle breed and under-reporting as some the possible reasons for the apparent absence of clinical disease. This report is, however, contrary to the situation in Zimbabwe during the independence war between 1974–1979 when dipping collapsed in endemically unstable communal herds. Huge losses from babesiosis and other TBDs were reported during this period (Norval, 1978 and 1979). Over the last decade the dipping service in Zimbabwe has been erratic due to shortages of water from the recurrent droughts and the inconsistent supply of acaricides by government. Although losses from babesiosis and other TBDs have been reported in some areas especially in the highveld, no major TBD outbreaks have come to the attention of the DVS. It is possible that the same reasons for the absence of major outbreaks as those cited by Tice *et al.* (1998) also apply to the current situation in Zimbabwe. It is therefore difficult to speculate on what might happen if the current government subsidized dipping service completely collapses. Further epidemiological research which focuses on active reporting of TBD-related morbidity and mortality in communal cattle in Zimbabwe, resistance or susceptibility of communal cattle to TBDs and economic impact assessment of alternative TBD control strategies is required.

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