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Seroprevalence of *Babesia bovis* and *Babesia bigemina* in cattle in the Soutpansberg region, Limpopo Province, South Africa, associated with changes in vector-tick populations

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ABSTRACT

A survey was conducted at 30 communal dip tanks and on 5 commercial farms in Limpopo Province, South Africa, during 1999 and 2000 to determine the seroprevalence of antibodies to *Babesia bovis* and *Babesia bigemina*. Cattle seropositive for *B. bovis* were found in 97 % of the herds on communal land; the overall seroprevalence changed little between 1999 (63.3 %) and 2000 (62.4 %). All herds surveyed were infected with *B. bigemina*, and overall seroprevalence decreased significantly from 56.1 % in 1999 to 49.3 % in 2000. In herds on communal land in Sour Lowveld Bushveld, overall seroprevalence of *B. bovis* increased from 70 % in 1999 to 80 % in 2000, while seroprevalence of *B. bigemina* decreased from 70 % in 1999 to 30 % in 2000. This was possibly due to an influx of *Rhipicephalus (Boophilus) microplus* that occurred at the time. In commercially farmed herds the seroprevalence to *B. bovis* increased significantly from 19 % in 1999 to 57.5 % in 2000. All commercial herds in the survey tested positive to *B. bigemina*, with a seroprevalence of 48.3 % in 1999 and 47.5 % in 2000. During 1999, cattle in 60 % of the dip tank/farm herds with only *R. (B.) microplus* present were approaching endemic stability to both *B. bovis* and *B. bigemina*. In 2000, 60 % of the herds with only *R. (B.) microplus* present were approaching endemic stability for *B. bovis*, while only 45 % were approaching endemic stability for *B. bigemina*. Those dip tanks/farms where only *R. (B.) microplus* was recorded had a significantly higher seroprevalence of *B. bovis* than those where both tick species were present.

Key words: *Babesia bigemina, Babesia bovis,* endemic stability, seroprevalence.

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INTRODUCTION

Bovine babesiosis or redwater occurs worldwide, with the exception of a few countries where it is too cold for the tick vectors to survive[4,10,17]. One-host ticks of the genus *Rhipicephalus (Boophilus)* transmit *Babesia bigemina* and *Babesia bovis*, the 2 *Babesia* species found in cattle in South Africa[19]. *Rhipicephalus (Boophilus) decoloratus* transmits only *Babesia bigemina*, while *Rhipicephalus (Boophilus) microplus* transmits both *B. bigemina* and *B. bovis*[19]. *Babesia bigemina* could also be transmitted by *Rhipicephalus evertsi evertsi* under experimental conditions[3], but the latter is probably not a significant vector in the field.

A serological survey found no evidence of *Babesia bovis* in Limpopo Province, South Africa, in the early 1980s[9]. De Vos and Potgieter[7] reported that *Babesia bigemina* was present on 26 farms in the Northern Transvaal (now Limpopo Province) in 1983, but they found no evidence of the presence of *Babesia bovis* during their survey. Making use of CLIMEX, a computer-based climatic model, to mimic the tick's ecological requirements in South Africa, Sutherst[21] predicted that the northeastern part of the Limpopo Province was a possible area where *R. (B.) microplus* might successfully establish itself.

The study area was endemic for *Babesia bigemina*, but over the past 15 years, outbreaks of redwater, attributed to *Babesia bovis*, had been reported from the eastern part of the Soutpansberg and Venda districts of Limpopo Province (P Loock, state veterinarian, Makhado, pers. comm., 1999). In Venda, redwater is mainly transmitted during the rainy season (October to May) when tick numbers are higher[5]. Only 10 % of the 144 redwater cases reported in Venda between 1997 and 1999 occurred in winter, and most of these cases (74 %) were due to *Babesia bovis* (P Loock, pers. comm., 1999). As many clinical cases were not reported by the farmers in the area, it was presumed that the actual morbidity and mortality due to redwater was much higher (P Loock, pers. comm., 2000).

In a previous paper we reported on displacement of *R. (B.) decoloratus* by *R. (B.) microplus* in the Soutpansberg region[26]. Here we report on changes in seroprevalence of *B. bovis* and *B. bigemina* at the same collection sites.

MATERIALS AND METHODS

Survey areas

The serological survey was conducted between May 1999 and December 2000 in the Soutpansberg, Dzanani, Mutale, Thohoyandou and Vuvani districts of Limpopo Province, South Africa (Fig. 1). The region was chosen because of recent outbreaks of bovine babesiosis caused by *Babesia bovis* (P Loock, pers. comm., 1999). The area borders the Kruger National Park (KNP) to the east, Zimbabwe to the north, the Vivo–Dendron road (R 521) to the west and the Polokwane (Pietersburg) –Giyani road (R 81) to the south. Sibasa and Makhado (Louis Trichardt) are the administrative centres of the government veterinary services in this area.

There are 2 distinct types of land tenure in the study area. Commercial farms are fenced, divided into camps and are individually owned. (Mara Research Station met these requirements and was grouped with the commercial farms.) Communal land, on the other hand, is unfenced and grazing is shared by cattle of all owners legally residing in the area. The communal farming areas are divided into wards serviced by animal health technicians

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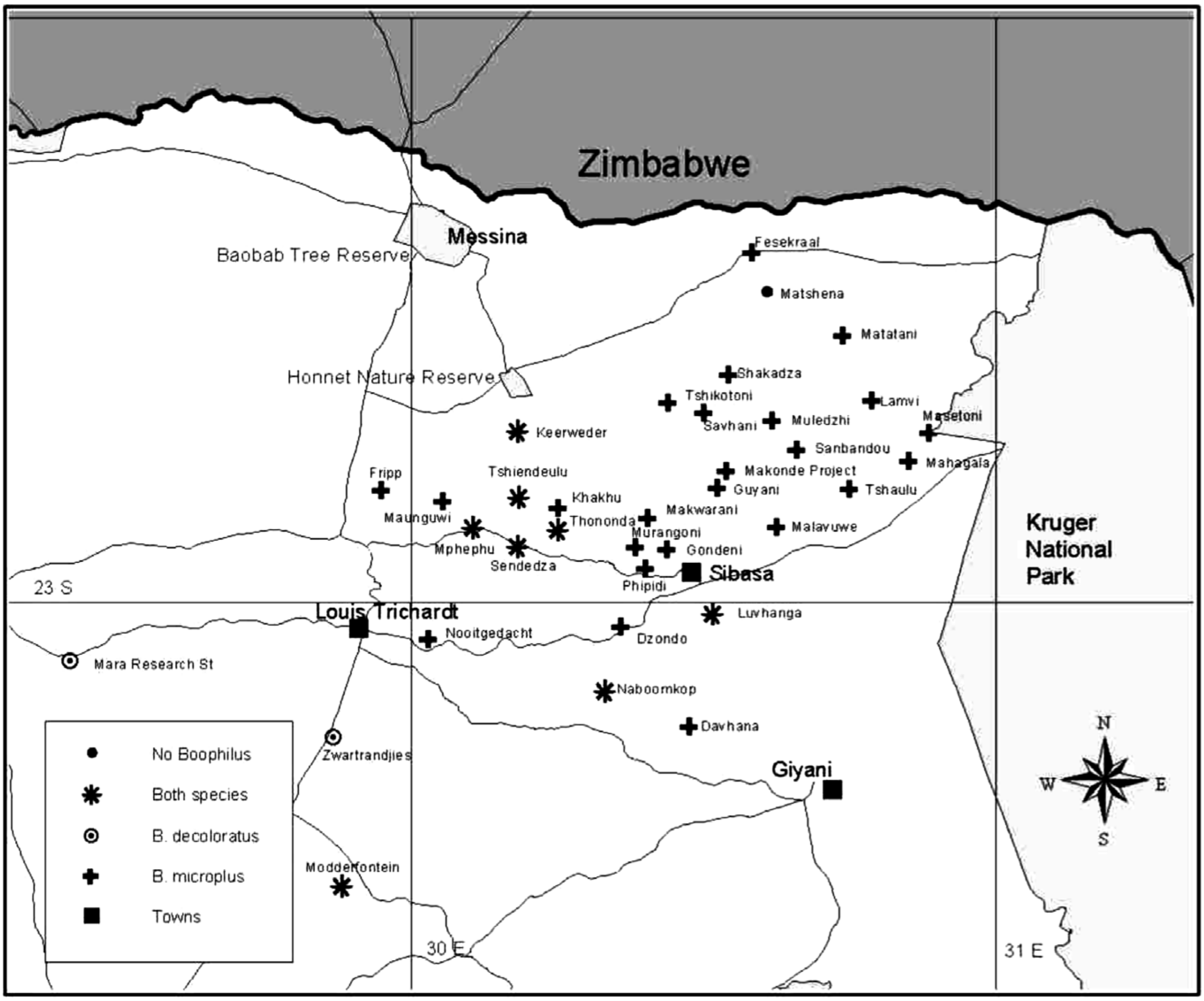

Fig. 1: **Map of the Soutpansberg region, Limpopo Province, South Africa, showing the dip tanks and commercial farms where bovine serum was collected. The occurrence of *Rhipicephalus (Boophilus) decoloratus* and *Rhipicephalus (Boophilus) microplus* at the various collection sites is also shown.** (Note name changes: Louis Trichardt = Makhado; Messina = Musina.) (Reprinted with permission from Tønnesen M H *et al.* 2004, *Experimental and Applied Acarology* 32: 199–208.)

who oversee the dipping of cattle at the communal dip tanks.

Experimental design

A 2-stage non-probability cluster sampling method was used to sample the cattle[22]. The farms/dip tanks in the Northern Province were the primary units and the individual animals at each dip tank/farm were the secondary sampling units. The sampling method was non-probability (convenience) sampling[22], and the farms/dip tanks were selected according to the following criteria:

- History of occurrence of *Babesia bigemina/Babesia bovis* in the area.
- Number of cattle at the dip tank / on the farm.
- Geographical location.
- Usability of the crush.
- Farmers' willingness to participate in the study.

Individual animals from each dip tank/farm were selected randomly, but an attempt was made to sample at least 1 animal from each kraal.

Study population

Dip tanks

The cattle population in Dzanani, Mutale, Thohoyandou and Vuvani districts totaled 103 252 head, distributed among 142 dip tanks (1999 South African Census). The cattle were not vaccinated against tick-borne diseases. Compulsory, free dipping of all cattle at prescribed intervals was discontinued in the early 1990s. Owners generally only present their cattle for dipping if, in their opinion, tick loads are excessive. This is obviously a subjective assessment. The number of cattle dipped at each dip tank varied from 200 to 1500. Thirty dip tanks were selected for inclusion in the study, 11 in 1999 and 19 in 2000 (Fig. 1). As a rule 30 cattle between 4 and 14 months and 30 cattle older than 18 months were sampled at each dip tank/farm, but at one dip tank only 11 animals were sampled due to poor owner compliance.

The majority (n = 20) of the dip tanks were located in Sour Lowveld Bushveld (Fig. 1), 8 were in Soutpansberg Arid Mountain Bushveld (Matshena, Shakadza, Matatani, Savhani, Mphephu, Keerweeder, Fripp and Maunguwi), while Fesekraal was located in Mopani Bushveld and Davhana in Mixed Lowveld Bushveld.

The occurrence of both vectors on cattle at dip tanks is shown in Fig. 1 and is reported in more detail elsewhere[25,26].

Commercial farms

There were 595 commercial farm units in the Soutpansberg district with a total cattle population of 128 200. The number of cattle on the commercial farms in the survey varied from 160 to 800 per farm. Four commercial farms (Zwartrandjes, Modderfontein, Nooitgedacht and

Naboomkop) and Mara Research Station (Fig. 1) were selected in 1999 for inclusion in the study and cattle on 2 of these farms were also sampled in 2000. The cattle were not vaccinated against tick-borne diseases. Strategic dipping was practised on all properties, *i.e.* no set regimen was followed, but acaricides were applied when through subjective assessment, tick burdens were deemed to be excessive. The cattle to be sampled were selected in the same way as with the communally grazed cattle. On 1 commercial farm some young cattle had been sold off, so more adult cattle were sampled to make up the sample to 60 cattle.

The occurrence of both vectors on cattle on the commercial farms is shown on Fig. 1 and is reported in more detail elsewhere[25,26].

Blood collection

As the prevalence of tick-borne diseases in the survey areas was unknown, 50 % prevalence was estimated with a desired confidence level of 95 %[22]. Sixty cattle were bled at each dip tank and commercial farm, which had been randomly selected according to the number of cattle at the dip tank/farm. Each animal was held in a crush prior to dipping and blood samples were collected from the tail vein (*V. caudalis mediana*) into a 10 mℓ Monoject® Vacutainer tube without anti-coagulant. The blood samples were carefully labelled, making sure that the age group was clearly indicated.

The blood samples were stored at room temperature for 4 hours to allow clotting and were then centrifuged at 3000 rpm for 20 min. The sera were decanted into 4 mℓ cryotubes (Cryovial®), which were clearly marked with the year, date, dip tank/farm and age of the animal and then stored at –10 °C at the Veterinary Laboratories at Sibasa or Makhado. The cryotubes were later transferred on ice to the Onderstepoort Veterinary Institute (OVI), where the serum samples were analysed for antibodies against *B. bigemina* and *B. bovis* using the Indirect Fluorescent Antibody test (IFAT)[1]. This is the standard test in use at the OVI.

Endemic stability

Mahoney and Ross[16] and Norval *et al.*[18] developed different models for endemic stability to bovine babesiosis, which were designed from serological data from cattle up to 9 months of age. Herds >80 % seropositive are generally regarded as endemically stable, those 60–80 % seropositive are regarded as approaching endemic stability, while those <60 % seropositive are regarded as endemically unstable. In the present study the serological data from the entire herd was used for the model.

Statistical analysis

The chi-square test was used to compare observed values with expected ones.

RESULTS

A total of 2201 blood samples were collected (Tables 1–4). When all the animals sampled are considered, there was an overall significant increase ($P = 0.0001$) in seroprevalence of *B. bovis* from 1999 to 2000. The overall seroprevalence for *B. bigemina* decreased significantly ($P = 0.0366$) from 1999 to 2000, a non-significant ($P = 0.6804$) decline in the older cattle being offset by a significant decline ($P = 0.0130$) in the younger animals.

Communally grazed cattle

Babesia bovis

The seroprevalence of young and older cattle is shown in Table 1. During 1999 *B. bovis* seropositive cattle were found in 100 % of the herds tested, 64 % of which were endemically stable or approaching stability, while 36 % were in an unstable situation. During 2000, positive animals were found in 97 % of the herds, with 58 % of the herds being endemically stable or approaching stability, 42 % being unstable. The mean seropositivity of the various age classes did not change significantly from 1999 to 2000.

In 1999 most dip tanks (10/11) were located in the Sour Lowveld Bushveld veld type[14], and half (10/19) of the dip tanks were located in the same veld type in 2000. When results from these dip tanks were compared (Table 5), the number of herds which were endemically stable or were approaching stability for *B. bovis* increased slightly, from 70 % in 1999 to 80 % in 2000. There were also significant increases in seroprevalence in both young ($P = 0.0004$) and older cattle ($P = 0.0202$).

Babesia bigemina

The seroprevalence of young and older cattle is shown in Table 2. All herds were positive for *B. bigemina* in 1999 and 2000. The mean seroprevalence in 1999 (56.1 %) decreased significantly ($P < 0.006$) to 49.3 % in 2000 (Table 2). In 1999, 64 % of these herds were endemically stable or approaching stability, while 36 % were in a minimal disease situation. During 2000 only 37 % of the herds were endemically stable or approaching stability, 47 % were in an unstable situation and 16 % in a minimal disease situation.

Table 1: **Seroprevalence of antibodies to *Babesia bovis* in cattle bled at communal dip tanks during 1999 and 2000.**

	Cattle 4–14 months	Cattle >18 months	All cattle
1999	61.2 % (202/330)	65.5 % (216/330)	63.3 % (418/660)
2000	57.0 % (314/551)	67.7 % (386/570)	62.4 % (700/1121)

Table 2: **Seroprevalence of antibodies to *Babesia bigemina* in cattle bled at communal dip tanks during 1999 and 2000.**

	Cattle 4–14 months	Cattle >18 months	All cattle
1999	60.0 % (198/330)	52.1 % (172/330)	56.1 % (371/660)
2000	47.0 % (259/551)	51.6 % (294/570)	49.3 % (553/1121)

Table 3: **Seroprevalence of antibodies to *Babesia bovis* in cattle bled on commercial farms during 1999 and 2000.**

	Cattle 4–14 months	Cattle >18 months	All cattle
1999	17.4 % (24/138)	20.4 % (33/162)	19.0 % (57/300)
2000	65.0 % (13/60)	50.0 % (30/60)	57.5 % (69/120)

Table 4: **Seroprevalence of antibodies to *Babesia bigemina* in cattle bled on commercial farms during 1999 and 2000.**

	Cattle 4–14 months	Cattle >18 months	All cattle
1999	44.2 % (61/138)	51.9 % (84/162)	48.3 % (145/300)
2000	51.7 % (31/60)	43.3 % (26/60)	47.5 % (57/120)

Table 5: **Seroprevalence of antibodies to *Babesia bovis* in cattle bled during 1999 and 2000 at communal dip tanks situated in the Sour Lowveld Bushveld.**

	Cattle 4–14 months	Cattle >18 months	All cattle
1999	62.7 % (188/300)	69.7 % (209/300)	66.2 % (397/600)
2000	76.2 % (214/281)	78.0 % (234/300)	77.1 % (448/581)

Table 6: **Seroprevalence of antibodies to *Babesia bigemina* in cattle bled during 1999 and 2000 at communal dip tanks situated in the Sour Lowveld Bushveld.**

	Cattle 4–14 months	Cattle >18 months	All cattle
1999	62.0 % (186/300)	57/0 % (171/300)	59.5 % (357/600)
2000	53.0 % (149/281)	58.3 % (175/300)	55.7 % (324/581)

When dip tanks in the Sour Lowveld Bushveld compared (Table 6), the number of herds that were endemically stable or were approaching stability decreased markedly, down from 70 % in 1999 to 30 % in 2000. There was a significant decrease (P = 0.0287) in the seroprevalence of young cattle from 1999 to 2000.

Commercial herds

Babesia bovis

The seroprevalence of young and older cattle is shown in Table 3. All the commercial herds were positive for *B. bovis* in 1999 and 2000. The mean seroprevalence during 1999 was 19 %, and none of these herds had reached or were approaching endemic stability: 20 % of the herds were unstable, while 80 % were in a minimal disease situation. During 2000 the mean seroprevalence increased significantly (P < 0.0001) to 57.5 %, with 50 % of the herds being endemically stable or approaching stability and 50 % in an unstable situation.

Babesia bigemina

The seroprevalence of young and older cattle is shown in Table 4. All commercial herds were positive for *B. bigemina* in 1999 and 2000. The mean seroprevalence in 1999 of 48.3 % changed little in 2000.

Comparison between communally grazed cattle and commercial herds

In 1999 the seroprevalence of both *B. bigemina* and *B. bovis* was significantly higher (P = 0.0261 and P = 0.0001, respectively) in the communally grazed cattle (Tables 1, 2) when compared with the commercial herds (Tables 3, 4). In 2000, however, there was no significant difference between the 2 groups for either *B. bovis* (P = 0.2890) or *B. bigemina* (P = 0.7030).

Seroprevalence related to known occurrence of vectors

The mean seroprevalence at collecting sites where the 2 vector-tick species occur separately or together is shown in Table 7. Where only *R. (B.) microplus* was present, the mean seroprevalence to *B. bovis* increased between 1999 and 2000, while mean seroprevalence to *B. bigemina* remained virtually constant. In 1999, 60 % of the herds had reached endemic stability or were approaching stability for both *Babesia* species. In 2000 the figure remained the same for *B. bovis*, while for *B. bigemina* it decreased to 45 %. At sites where both tick-vector species occurred, mean seroprevalence for *B. bigemina* declined substantially between 1999 and 2000.

DISCUSSION

Distribution of *Babesia bovis* and *Babesia bigemina*

This survey demonstrated that both *B. bovis* and *B. bigemina* were widespread in the northeastern part of Limpopo Province. Earlier surveys from southern Africa had also shown *B. bigemina* to be widespread, but reported that *B. bovis* had a patchy distribution, following that of the tick vector *R. (B.) microplus*[2,5–9,11–13,18,20,23,25].

Communally grazed cattle

The seroprevalence of *B. bovis* found in the communally grazed cattle was higher than reported in most of the earlier surveys[2,8,11–13,18,20]. The situation resembled that found in Zimbabwe, where *B. bovis* was common at the dip tanks where *R. (B.) microplus* was present and where there was minimal tick control[18].

The seroprevalence of *B. bigemina* in the communally grazed cattle was lower than reported in other surveys. The significant decrease from 1999 to 2000 was probably due to a decrease in the infection rate in the young animals (Table 2).

The prevalence of *B. bovis* in the communally grazed areas in 1999 was not significantly higher than that of *B. bigemina*, but in 2000 the difference was significant. One would expect to find a higher transmission rate and seroprevalence for *B. bigemina* when compared with those of *B. bovis*, due to the higher infection rate of *B. bigemina* in the *Rhipicephalus (Boophilus)* ticks[5,7,15]. In contrast, several studies from southern Africa[2,18,20] (A M Spickett, Onderstepoort Veterinary Institute, pers. comm., 2001) have reported a higher seroprevalence of *B. bovis* compared with *B. bigemina* where both parasites co-exist. Tjornehoj *et al.*[24] monitored an outbreak of Asiatic redwater in Malawi where, within a period of 3 months, 75 % of previously negative animals had seroconverted to *B. bovis*, compared to only 36 % to *B. bigemina.* The increase in seroprevalence of *B. bovis* in our study may therefore probably be attributed to the general influx of *R. (B.) microplus* during the survey period[26]. The decline in seroprevalence of *B. bigemina* at dip tanks where only *R. (B.) microplus* was recorded may indicate that this piroplasm is transmitted less efficiently by *R. (B.) microplus* than by *R. (B.) decoloratus*[18].

Commercial herds

The seroprevalence to *B. bovis* was higher than in earlier studies in southern Africa[5,9,18]. The significant increase between 1999 and 2000 was probably mainly due to a higher transmission rate in the young animals (Table 3). The seroprevalence to *B. bigemina* remained around 50 % during the study.

Seroprevalence (10 %) of *Babesia bovis* in cattle where only *R. (B.) decoloratus* was found (Table 7) indicates that *R. (B.) microplus* must have been present, but had not been recovered from the cattle.

The commercial farms experienced a substantial increase in *R. (B.) microplus*

Table 7: **Seroprevalence (%) of antibodies to *Babesia bovis* and *Babesia bigemina* related to vector occurrence at collecting sites.**

	Only *R. (B.) microplus* present		Both *R. (B.) microplus* and *R. (B.) decoloratus* present		Only *R. (B.) decoloratus* present	
	1999	2000	1999	2000	1999	2000
B. bovis	60.8	68.3	50.0	49.2	10.0	–
B. bigemina	55.0	53.2	55.7	44.6	59.2	–

numbers during the survey period[25,26], and there were losses due to redwater among cattle in the 18–24-month-old group, as well as among cattle brought in from outside. High rainfall and subsequent flooding were experienced in February/March 2000. This prevented the commercial farmers from carrying out their normal tick control regimen and could have enhanced tick survival. During the flooding the commercial farmers had a wider choice of grazing areas available for their cattle than the communal farmers and were also able to graze their cattle on higher ground, which was less affected by the rising water. In these areas the *R. (B.) microplus* larvae would have been able to survive and multiply.

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