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

M H Tønnesena,b, B L Penzhornb, N R Brysona, W H Stoltszb and T Masibigiric

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.


INTRODUCTION
Bovine babesiosis or redwater occurs worldwide, with the exception of a few countries where it is too cold for the tick vectors to survive13,17. One-host ticks of the genus Rhipicephalus (Boophilus) transmit Babesia bigemina and Babesia bovis, the 2 Babesia species found in cattle in South Africa19. Rhipicephalus (Boophilus) decoloratus transmits only Babesia bigemina, while Rhipicephalus (Boophilus) microplus transmits both B. bigemina and B. bovis16. Babesia bigemina could also be transmitted by Rhipicephalus evertsi evertsi under experimental conditions2, 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 1980s5. De Vos and Potgieter7 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, Sutherst21 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 higher1. 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 region22. 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, Dzamani, Mutale, Thohoyandou and Vuvu 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–Dendoron 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.
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. 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, 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/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.

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, Nootgedacht and

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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.)
Antibodies against the serum samples were analysed for later transferred on ice to the Onderstepoort Veterinary Laboratory at –10 °C at the Veterinary Laboratories and age of the animal and then stored at room temperature for 4 hours to allow clotting. The sera were decanted into 4 mL Vacutainer tube without anti-coagulant. The blood samples were carefully labelled, making sure that the age group was clearly indicated.

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 %.27 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 Ross16 and Norval et al.14 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 enduringly stable, those 60–80 % seropositive are regarded as approaching endemic stability, while those <60 % seropositive are regarded as enduringly unstable. In the present study the serological

<table>
<thead>
<tr>
<th>Year</th>
<th>Cattle 4–14 months</th>
<th>Cattle &gt;18 months</th>
<th>All cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>61.2 % (202/330)</td>
<td>65.5 % (216/330)</td>
<td>63.3 % (418/660)</td>
</tr>
<tr>
<td>2000</td>
<td>57.0 % (314/551)</td>
<td>67.7 % (386/570)</td>
<td>62.4 % (700/1121)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Cattle 4–14 months</th>
<th>Cattle &gt;18 months</th>
<th>All cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>60.0 % (198/330)</td>
<td>52.1 % (172/330)</td>
<td>56.1 % (371/660)</td>
</tr>
<tr>
<td>2000</td>
<td>47.0 % (259/551)</td>
<td>51.6 % (294/570)</td>
<td>49.3 % (553/1121)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Cattle 4–14 months</th>
<th>Cattle &gt;18 months</th>
<th>All cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>17.4 % (24/138)</td>
<td>20.4 % (33/162)</td>
<td>19.0 % (57/300)</td>
</tr>
<tr>
<td>2000</td>
<td>65.0 % (13/60)</td>
<td>50.0 % (30/60)</td>
<td>57.5 % (69/120)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Cattle 4–14 months</th>
<th>Cattle &gt;18 months</th>
<th>All cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>44.2 % (61/138)</td>
<td>51.9 % (84/162)</td>
<td>48.3 % (145/300)</td>
</tr>
<tr>
<td>2000</td>
<td>51.7 % (31/60)</td>
<td>43.3 % (26/60)</td>
<td>47.5 % (57/120)</td>
</tr>
</tbody>
</table>

RESULTS
A total of 2201 blood samples were collected (Tables 1–4). When all the animals sampled were 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 enduringly 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 enduringly 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 type16, 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 enduringly 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 enduringly stable or approaching stability, while 36 % were in a minimal disease situation. During 2000 only 37 % of the herds were enduringly stable or approaching stability, 47 % were in an unstable situation and 16 % in a minimal disease situation.
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 79%, 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 was 35%, and none of these herds had reached or were approaching endemic stability: 30% of the herds were unstable, while 70% were in a minimal disease situation. During 2000 the mean seroprevalence increased significantly (P < 0.0001) to 55.7%, with 50% of the herds being endemically stable or approaching stability and 50% in an unstable situation.

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.

Communally grazed cattle

The seroprevalence of B. bovis found in the communally grazed cattle was higher than reported in most of the earlier surveys. 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.

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. In contrast, several studies from southern Africa found that B. bovis was in the communal dip tanks situated in the Sour Lowveld Bushveld. However, there was no significant difference between the 2 groups for either B. bovis or B. bigemina, although there was a higher seroprevalence to B. bigemina in the Rhipicephalus (Boophilus) ticks.

The decline in seroprevalence of B. bovis in our study may therefore be attributed to the general influx of R. (B.) microplus during the survey period. 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.

Commercial herds

The seroprevalence to B. bovis was higher than in earlier studies in southern Africa. 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 30% during the study. Seroprevalence (10%) of B. 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.

<table>
<thead>
<tr>
<th>Only R. (B.) microplus present</th>
<th>Both R. (B.) microplus and R. (B.) decoloratus present</th>
<th>Only R. (B.) decoloratus present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>2000</td>
<td>1999</td>
</tr>
<tr>
<td>1999</td>
<td>2000</td>
<td>1999</td>
</tr>
<tr>
<td>B. bovis</td>
<td>60.8</td>
<td>68.3</td>
</tr>
<tr>
<td>B. bigemina</td>
<td>55.0</td>
<td>53.2</td>
</tr>
</tbody>
</table>

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.

<table>
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<th>Cattle &gt;18 months</th>
<th>All cattle</th>
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<tbody>
<tr>
<td>1999</td>
<td>62.7 % (188/300)</td>
<td>69.7 % (209/300)</td>
</tr>
<tr>
<td>2000</td>
<td>76.2 % (214/281)</td>
<td>78.0 % (234/300)</td>
</tr>
</tbody>
</table>

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.

<table>
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<th>All cattle</th>
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</thead>
<tbody>
<tr>
<td>1999</td>
<td>62.0 % (186/300)</td>
<td>57.0 % (171/300)</td>
</tr>
<tr>
<td>2000</td>
<td>53.0 % (149/281)</td>
<td>58.3 % (175/300)</td>
</tr>
</tbody>
</table>
numbers during the survey period\textsuperscript{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 \textit{R. (B.) microplus} larvae would have been able to survive and multiply.

**ACKNOWLEDGEMENTS**

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