Laboratory and field investigations into the Theileria parva carrier-state in cattle in Zimbabwe

A.A. LATIF1, T. HOVE2, G.K. KANHAI3 and S. MASAKA3

ABSTRACT


The Theileria parva carrier-state in cattle on commercial farms on Zimbabwe was investigated using parasitological and serological methods. The proportion of cattle showing Theileria piroplasms on two farms, which had recent histories of disease outbreaks, were 64% (n = 106, total of heifers and weaned calves examined) and 71.5% (n = 60) while the proportion of T. parva antibodies for the same animals were 59% and 98.5%, respectively. On four farms where no cases of the disease occurred for over 10 years, the average proportion of animals showing piroplasms and antibodies were 55.4% (range 32-82, n = 223) and 73% (range 47-91, n = 223), respectively. However, on another three farms which had no history of theileriosis outbreaks these proportions were very low, being 11.4% (0-24, n = 157) for piroplasms and 12.2% (5-23, n = 157) for antibodies. The mean infection rate in unfed Rhipicephalus appendiculatus adults collected from farms with a high prevalence of cattle which were carriers of Theileria piroplasms during the tick activity season was 29% (range 12-60%) with 9.3 (range 2-18.7) mean infected acini per infected tick. The infectivity of different tick batches to susceptible cattle produced a wide spectrum of theileriosis reactions. Laboratory controlled experiments were carried out to study the persistence of T. parva (Boleni) piroplasms in cattle immunized with this strain as well as its infectivity for ticks and its subsequent transmissibility to cattle. Examination of the salivary glands of 15 batches of ticks collected from six immunized cattle on three different occasions over 18 months showed that none were infected with Theileria parasites. However, the infectivity of other ticks in the same batches to susceptible animals was demonstrated 6, 10 and 18 months after cattle had been immunized with Boleni stabilate.

Keywords: Carrier-state, cattle, epidemiology, Theileria parva

INTRODUCTION

In Zimbabwe, in spite of over 80 years of intensive tick control by chemicals, the annual number of theileriosis outbreaks during the years 1954-1988 has increased (Koch 1990; Anon. 1993) and it has proved impossible to eradicate ticks (Norval 1982; 1983). More recently, however, there has been a move away from strict tick control to one of selective control and the establishment of endemic stability in cattle belonging to the communal sector (Thomson 1985; Madzima & Mutugi 1996). Immunization against Theileria parva and other tick-borne diseases could play a major role in an integrated control programme (Lawrence 1985). An initial immunization trial using the locally isolated T. parva Boleni stock was conducted successfully in 1990 by Koch, Kambeva, Ocama, Munatswa, Franssen, Uilenberg, Dolan & Norval. The infection and treatment method of immunization has also been shown to be effective in Malawi (Musisi, Quiroga, Ngulube & Kanhai 1989) and Zambia (Berkvens, Geysen & Lynen 1989). However, there has been considerable concern about the application of the method particularly identifying the
role of immunized carrier-cattle in the overall epidemiology of the disease and the magnitude of the present *Theileria* carrier-state in the cattle population on different farms. Therefore, controlled laboratory experiments on the immunizing *T. parva* Boleni stock and field investigations on cattle theileriosis were carried out in this study to address these epidemiological concerns.

**MATERIALS AND METHODS**

**Prevalence of *Theileria* piroplasms and antibodies in cattle on commercial farms**

Seven farms that had no history of theileriosis outbreaks and two, which had a history of outbreaks (Anon. 1993), were selected to investigate the prevalence of *Theileria* piroplasms and antibodies in their cattle. Blood for serum and thin blood smears were taken from 30–56 animals from each of the weaner (7 months old) and the heifer (1–2 years old) groups on each farm. Sera were screened for *T. parva* schizont antibodies using the indirect immunofluorescent antibody test (IFA) (Burnidge & Kimber 1972). Blood smears were stained with Giemsa's stain and examined microscopically for *Theileria* piroplasms.

**Infection rates with *Theileria* parasites in ticks collected from five farms and their infectivity to cattle**

Unfed *Rhipicephalus appendiculatus* adults were collected from the grass on five commercial farms on the Highveld which had reported incidence of theileriosis outbreaks. A total of 12 collections were made from five farms between January and April in 1991 and 1992, the season of adult tick activity (Short & Norval 1981). The infection rates in ticks from 11 collections from four farms were assessed using Feulgen's staining of whole salivary glands (Blewett & Branagan 1973). The number of ticks examined varied from 36–66 and in one occasion only 15 ticks were examined since most of them were found engorged and unsuitable for dissection. The infectivity of five tick collections from four farms to susceptible cattle was assessed using either live ticks (50–200 were applied per animal) or by injection of stablate (1.0–2.0 mL) prepared from these ticks. One to two susceptible animals were infected with ticks or stablate from each collection. The method of preparation of the *Theileria* stablate has been previously described (Anon. 1993).

**The infectivity of *Theileria* parasites derived from primary infections of theileriosis on two farms to susceptible cattle**

Ten Hereford cattle (6–8 months old) were obtained from a farm on which no cases of theileriosis had occurred for over 16 years. Blood smears prepared and examined from these animals were free of piroplasms and their sera were negative for antibodies to *T. parva* schizont antigens in the IFA test. Five cattle were released on Ayrshire and five on Merton Park farms during the season of adult *R. appendiculatus* activity and were allowed to graze with the farm animals. Confirmed outbreaks of theileriosis had occurred on both farms during the preceding two years. If any of the experimental cattle on the two farms showed clinical signs of theileriosis which was confirmed by lymph node biopsy smears and blood smear examination they were isolated and uninfected *R. appendiculatus* nymphs from the laboratory colony maintained at the Veterinary Research Laboratory in Harare were applied to their ears. Tick pick-up of *Theileria* parasites was successful in two cases, bovines number A32, from Ayrshire and number MP-1 from Merton. The *Theileria* infection rates in adult ticks dropped as engorged nymphs from the two animals were assessed as described above. The infectivity of the adult ticks was also determined by applying ticks from each batch to the ears of one susceptible calf. The theilerial reactions in cattle was assessed according to those described by Hove, Musselsi, Kanhai, Latif, Masaka, Munatswa, Pegram, Kamwendo, Quiroga, Mwangondwe & Dolan (1995).

**Dynamics of transmission of *Theileria parva* Boleni parasites in carrier animals under laboratory conditions**

Experiments were carried out using cattle immunized with *T. parva* Boleni to determine for how long the *Theileria* piroplasms in these animals could be picked-up by ticks and subsequently transmitted to susceptible animals. Six Hereford calves, 7–8 months old, were immunized with the *T. parva* Boleni stock (Lawrence & Mackenzie 1980; Anon. 1993) by the infection and treatment method as has been previously described (Anon. 1993). They were kept free of ticks for 18 months. Uninfected *R. appendiculatus* nymphs from the laboratory colony maintained at the Veterinary Research Laboratory in Harare were used to pick-up the *Theileria* piroplasms from the immunized animals after 6, 10 and 18 months of immunization. The infection rates with *Theileria* parasites in the subsequent adult ticks were determined as described above. The infectivity of each batch of ticks obtained from an individual immunized animal was tested in one susceptible cattle. The six immunized cattle received homologous challenges with the *T. parva* Boleni stablate after 19 months of their initial immunization to ascertain their immunity. Similarly, the six animals receiving the ticks derived from the 18-month pick-up and recovered were also challenged after 2 months with the same Boleni stablate to assess their immunity. The animal reaction to theileriosis was assessed as described above.
RESULTS

Prevalence of *Theileria* piroplasms and antibodies in cattle in commercial farms

The proportion of cattle that were parasitologically and serologically positive to *Theileria* piroplasms and *T. parva* antibodies is shown in Table 1. On the two farms (Ayrshire, Dunninue) on which cases of theileriosis had previously occurred the prevalence of *Theileria* piroplasms in blood smears of heifers was 60% and 80% and antibodies was 68% and 100%, respectively. The prevalence rate in the weaner group on the two farms was also high, being 68% and 63% for piroplasms and 50% and 97% for antibodies. On the farms which had no history of previous outbreaks of theileriosis, the proportion of animals showing piroplasms and antibodies in four out of the seven farms was 55.4% (range 32–82, n = 223) and 73% (range 47–91, n = 223), respectively. These proportions were very low in the remaining three farms where 11.4% (range 0–24, n = 157) and 12.2% (range 5–23, n = 157) of animals were found to be positive, respectively.

Infection rates in ticks collected from different farms and their infectivity to susceptible cattle

Unfed *R. appendiculatus* adults were found questing on grass tips on the farms from January to April. The mean infection rate with *Theileria* parasite masses in the ten tick batches collected from Ayrshire, Chikeya and Botha farms was 29% (n = 410) and ranged between 11.7–60%. The mean infected acini per infected tick was 9.3 (range 2–18.7) (Table 2). The infection rate was low in ticks collected from Mazuri.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Type</th>
<th>History of theileriosis</th>
<th>Piroplasm %</th>
<th>Ab * %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weaner (n)</td>
<td>Heifer (n)</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>Beef</td>
<td>Case</td>
<td>68 (56)</td>
<td>60 (50)</td>
</tr>
<tr>
<td>Dunninue</td>
<td>Dairy</td>
<td>Case</td>
<td>63 (30)</td>
<td>80 (30)</td>
</tr>
<tr>
<td>Windsor</td>
<td>Dairy</td>
<td>No-case</td>
<td>–</td>
<td>67 (30)</td>
</tr>
<tr>
<td>Rydale</td>
<td>Dairy</td>
<td>Mo-case</td>
<td>60 (25)</td>
<td>82 (33)</td>
</tr>
<tr>
<td>Highburry</td>
<td>Beef</td>
<td>No-case</td>
<td>32 (28)</td>
<td>57 (47)</td>
</tr>
<tr>
<td>Woodleigh</td>
<td>Beef</td>
<td>No-case</td>
<td>50 (30)</td>
<td>40 (30)</td>
</tr>
<tr>
<td>Magog</td>
<td>Beef</td>
<td>No-case</td>
<td>20 (30)</td>
<td>24 (30)</td>
</tr>
<tr>
<td>Gomo Estate</td>
<td>Beef</td>
<td>No-case</td>
<td>0 (30)</td>
<td>13 (30)</td>
</tr>
<tr>
<td>Mazoe Station</td>
<td>Mixed</td>
<td>No-case</td>
<td>0 (37)</td>
<td>–</td>
</tr>
</tbody>
</table>

(n) Number examined
Ab * Antibody titre 1/640 or higher was considered significant in the IFA test
Case Farms on which theileriosis had previously occurred
No-case Farms on which theileriosis had not previously occurred

<table>
<thead>
<tr>
<th>Farm</th>
<th>Date tick collection</th>
<th>Number examined</th>
<th>Infection rate (%)</th>
<th>Mean infected acini/tick</th>
<th>Maximum parasite masses in single tick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayrshire</td>
<td>Feb. 1991</td>
<td>52</td>
<td>34.9</td>
<td>16.4</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Apr. 1991</td>
<td>56</td>
<td>51.8</td>
<td>18.7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Jan. 1992</td>
<td>41</td>
<td>24.4</td>
<td>09.3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Feb. 1992</td>
<td>42</td>
<td>21.4</td>
<td>06.5</td>
<td>25</td>
</tr>
<tr>
<td>Mazuri</td>
<td>Feb. 1991</td>
<td>66</td>
<td>32.0</td>
<td>09.4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Apr. 1991</td>
<td>15</td>
<td>60.0</td>
<td>10.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Jan. 1992</td>
<td>39</td>
<td>25.6</td>
<td>05.3</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Feb. 1992</td>
<td>36</td>
<td>11.7</td>
<td>02.0</td>
<td>3</td>
</tr>
<tr>
<td>Botha</td>
<td>Feb. 1992</td>
<td>38</td>
<td>34.2</td>
<td>13.3</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Mar. 1992</td>
<td>40</td>
<td>13.0</td>
<td>03.0</td>
<td>5</td>
</tr>
<tr>
<td>Chikeya</td>
<td>Mar. 1992</td>
<td>50</td>
<td>03.2</td>
<td>03.7</td>
<td>7</td>
</tr>
</tbody>
</table>

Infection rate in ticks from the 5th farm was not determined
Theileria parva carrier-state in cattle in Zimbabwe

Table 3

Infectivity in susceptible cattle of *R. appendiculatus* adults collected from the pasture on four of the commercial farms

<table>
<thead>
<tr>
<th>Source of ticks</th>
<th>Susceptible animal no.</th>
<th>Tick infection rate %</th>
<th>Stablate/tick challenge</th>
<th>Animal reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayrshire Farm</td>
<td>310 5-89</td>
<td>34.9 (16.4)</td>
<td>1.0 ml</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chikeya Farm</td>
<td>B14</td>
<td>11.2 (2)</td>
<td>120 ticks</td>
<td>Severe/treated day 24</td>
</tr>
<tr>
<td>Mazuri Farm</td>
<td>B19</td>
<td>3.2 (3.7)</td>
<td>50 ticks</td>
<td>No</td>
</tr>
<tr>
<td>Felton Farm</td>
<td>084</td>
<td>Not done</td>
<td>200 ticks</td>
<td>Mild</td>
</tr>
</tbody>
</table>

Number of infected acini per infected tick shown between brackets

Table 4

Infectivity in susceptible cattle of *R. appendiculatus* adults derived from theileriosis primary infections in commercial farms

<table>
<thead>
<tr>
<th>Source of ticks</th>
<th>Parasite stock</th>
<th>Calf no.</th>
<th>Tick infection rate</th>
<th>Stablate/tick challenge</th>
<th>Animal reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayrshire Farm</td>
<td>A32</td>
<td>89-4</td>
<td>90 (19.2)</td>
<td>2.0 ml</td>
<td>S/F, D14</td>
</tr>
<tr>
<td>Merton Farm</td>
<td>MP-1</td>
<td>317</td>
<td>38 (2.2)</td>
<td>60 ticks</td>
<td>S/prolonged</td>
</tr>
</tbody>
</table>

Number of infected acini per infected tick shown between brackets

S: Severe reaction
F: Fatal
D: Days to death

Farm (3.2%). The infectivity in susceptible cattle of the four batches of ticks collected in the field is shown in Table 3.Ticks collected from Ayrshire and Chikeya farms produced severe reactions in susceptible animals, but the ticks collected from Mazuri and Felton farms induced only mild reactions.

**Infectivity of ticks derived from primary infections of theileriosis on the two farms**

The infectivity in susceptible cattle of *R. appendiculatus* adults derived from theileriosis primary infections in two commercial farms is shown in Table 4. Ticks dropped from animal number A32 in Ayrshire and MP-1 in Merton Park farms when they were showing severe theileriosis reactions had high infection rates, 90% and 38%, respectively and produced severe infections in susceptible animals (Table 4).

**Dynamics of transmission of the Theileria Boleni parasites in carrier cattle under laboratory conditions**

The results of the tick pick-up and transmission attempts from the six immunized cattle over 18 months period are shown in Table 5. None of the ticks in the 15 batches harvested from the six immunized cattle on three occasions showed any infections with *Theileria* parasites in their salivary glands. However, the infectivity of the these ticks to susceptible cattle was demonstrated in five out of six batches six months after immunization, one out of three batches ten months after immunization and four out of six batches 18 months after immunization. The theileriosis reactions in animals were moderate in five batches (330 ticks dropped per animal), mild in five batches (302 ticks dropped per animal) and five tick batches were not infective (326 ticks dropped per animal). The six cattle that were infected with the tick batch derived from the immunized animals 18 months after immunization were challenged with *T. parva* Boleni, the immunizing stablate. Four out of the six animals, which had previously reacted to the carrier-parasites were found to be immune. The remaining two animals, which had not reacted, together with the two susceptible controls were proved to be fully susceptible. At the same time, all the six immunized cattle were found to be immune when challenged 19 months after immunization.

**DISCUSSION**

This parasitological and serological survey on theileriosis in Zimbabwe has yielded valuable epidemiological information on the disease. The data obtained on antibody titre and piroplasm prevalence rates were comparable. The piroplasms were associated to *T. parva* and *T. taurotragi* which are transmitted by *R. appendiculatus* while the vectors for other *Theileria* species of cattle are not present on the farms used in this investigation. In six of the nine farms surveyed, the prevalence rates were high suggesting that the majority of the cattle population were exposed to
transmission of the disease. Previous serological sur-
theilerial infections. Higher prevalence rates of theile-
the IFA test as the two species cross-react (Uilen-
prevalence rates of theileriosis were due to pathogenic
outbreaks as well as on farms on which no cases had
spread including areas where theileriosis outbreaks
had not been officially reported. Whether these in-
fected due to pathogenic *T. parva* or to the non-
pathogenic *T. taurotragi* could not be ascertained in
the IFA test as the two species cross-react (Uilen-
berg, Perie, Lawrence, De Vos, Paling & Spanjer
1982). However, it has been possible to character-
ize these infections on some of the farms using mole-
cular techniques (Bishop, Spooner, Kanhai, Kiarie,
Latif, Hove, Masaka & Dolan 1994) confirming the
presence of *T. taurotragi*.

Another important component in the epidemiology of
theileriosis related to carrier animals is to determine
the infection rates in field ticks (Young 1981; Young,
Leitch & Newson 1981). These infection rates need
to be determined before a quantitative study of the
epidemiology can be undertaken (Walker, Young &
Leitch 1981). *Rhipicephalus appendiculatus* ticks
collected from farms with previous histories of theile-
riosis had very high average infection rates (29 %)
and a high percentage of infected acini. However, in
Zimbabwe *T. taurotragi* is widespread (Bishop et al.
1994) and one tick batch or a single tick may harbour
and transmit more than one *Theileria* species. There-
fore, in the present study, field ticks were applied to
infect susceptible cattle to partly confirm the identity
of the *Theileria* species. The infectivity of the field
ticks in susceptible cattle produced a wide spectrum
of clinical signs of theileriosis infections confirming
the earlier results obtained by Matson (1967). In the
present investigation only conventional methods
have been used to determine some of the important
factors related to the epidemiology of theileriosis.
However, improved methods using molecular tech-
niques are now available which can detect infection
rates in ticks collected from cattle carrying very low
levels of piroplasm parasitaemia (d'Oliveira, Van der
Weide, Jacquiet & Jongejan 1997), as well as mixed
infections and carrier animals (Gubbels, De Vos, Van
der Weide, Viseras, Schouls, De Vries & Jongejan
1999).

In the experiments that were carried out to examine
the persistence of *T. parva* Boleni in immunized cat-
tle, its infectivity for ticks and the subsequent trans-
missibility to cattle, the immunized cattle maintained
sporadic infection rates in ticks for 18 months and the
infectivity of these parasites in susceptible cattle was
demonstrated. The regulating factor, which was re-
sponsible for low infection rates in ticks, might have
been the very low piroplasm parasitaemia in the
immunized carrier cattle. In contrast, higher infection
rates were demonstrated in ticks collected from the
field and some batches produced severe theileriosis
infections in susceptible cattle. In the field, however,
superinfection of ticks can occur (Young 1987). Koch,
Norval, Ocama & Munatswa (1992) compared the
pathogenicity of *T. parva* Boleni carrier-parasites
derived from carrier cattle either kept tick-free or main-
tained in the field. The study showed that ticks derived
from carriers in the field produced severe reactions
in susceptible cattle.

In conclusion, the present epidemiological studies on
the carrier-state of theileriosis in cattle have pro-
duced valuable information, which is central in the
future design of a theileriosis control policy in Zim-
babwe. They have shown that the carrier-state is
widespread and that long-term tick control has not
reduced the rate and frequency of transmission.
Moreover, field ticks can be highly infective for cattle

---

### TABLE 5 Dynamics of transmission using *R. appendiculatus* from cattle immunized with *Theileria parva* Boleni after 6, 10 and 18 months of initial immunization, to susceptible animals

<table>
<thead>
<tr>
<th>Immune animal</th>
<th>Suscept. animal no.</th>
<th>No. ticks fed</th>
<th>Animal reaction</th>
<th>Suscept. animal no.</th>
<th>No. ticks fed</th>
<th>Animal reaction</th>
<th>Suscept. animal no.</th>
<th>No. ticks fed</th>
<th>Animal reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>079</td>
<td>4408</td>
<td>230</td>
<td>Mo (13)</td>
<td>NC</td>
<td>ND</td>
<td>ND</td>
<td>4000</td>
<td>22</td>
<td>M (5)</td>
</tr>
<tr>
<td>197</td>
<td>057</td>
<td>15</td>
<td>Mo (12)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4420</td>
<td>144</td>
<td>Mo (6)</td>
</tr>
<tr>
<td>200</td>
<td>110</td>
<td>156</td>
<td>Mo (13)</td>
<td>325</td>
<td>130</td>
<td>NR</td>
<td>4414</td>
<td>170</td>
<td>NR</td>
</tr>
<tr>
<td>253</td>
<td>032</td>
<td>228</td>
<td>Mo (13)</td>
<td>323</td>
<td>74</td>
<td>NR</td>
<td>4430</td>
<td>77</td>
<td>M (5)</td>
</tr>
<tr>
<td>254</td>
<td>084</td>
<td>305</td>
<td>M (3)</td>
<td>324</td>
<td>212</td>
<td>Mo (9)</td>
<td>4919</td>
<td>123</td>
<td>Mo (7)</td>
</tr>
<tr>
<td>294</td>
<td>065</td>
<td>270</td>
<td>Mo (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Animal reactions:
- NR: no reaction
- M: Mild
- Mo: Moderate
- ND: Not done

Number between brackets indicates duration of schizont parasitosis.
Theileria parva carrier-state in cattle in Zimbabwe

and constitute continuous risks for the cattle industry. The immunization method of infection and treatment using the T. parva Boleni stock produced a carrier-state in the immunized cattle for over 18 months. The carrier parasites transmitted to susceptible cattle produced mild theileriosis reactions under controlled laboratory experiments but afforded solid immunity in recipient animals when challenged with the original stock.

ACKNOWLEDGEMENTS

We are grateful to the commercial farms owners for their cooperation. We thank Prof. F. Jongejan who made valuable comments on the manuscript. The work was supported by the Food and Agriculture Organization (FAO), DANIDA and the Government of Zimbabwe.

REFERENCES


