INTRODUCTION

The South African bivalent unfrozen redwater vaccine containing *Babesia bigemina* and *Babesia bovis* infected blood was sold until April 1998, when it was finally discontinued and replaced by the two frozen monovalent African (*Babesia bigemina*) and Asiatic (*Babesia bovis*) redwater vaccines.

Diminazene aceturate has been shown to be highly effective against *B. bigemina* at levels from 0.5 to 3.0 mg/kg (Kutler 1981). Therefore, a third (1.16 mg/kg) of the prescribed dose of the antibabesial drug diminazene has long been used to block-treat the unfrozen redwater vaccine reactions on day 7 with no known adverse effects to the organisms or to the development of protective immunity in the animal (De Waal 1996). The attenuated organisms used in the unfrozen redwater vaccine are susceptible for longer periods to the residual effects of diminazene and imidocarb dipropionate than the virulent field strains (F.T. Potgieter & M.P. Combrink, unpublished observations 1982). In addition, the inhibitory effect, especially of imidocarb, is more pronounced in reactions following the administr-
tion of the frozen South African *B. bovis* and *B. bigemina* vaccines than in those of the unfrozen vaccine (Combrink, Troskie & De Waal 2002). In a recent study on 19 different brands of diminazene obtained from 11 African countries, it was found that approximately one out of every three samples tested fell outside the ± 10 % tolerance limit of the manufacturer’s label claim on the content of diminazene aceturate (Tettey, Atsriku, Chizyuka & Slingenberg 2002).

Based on these findings and reports of vaccine failures in some animals in which diminazene was used for the block treatment of vaccine reactions (Combrink, personal observations 2001), it was decided to reinvestigate the duration of the interval between vaccination and treatment as well as the optimum dosage rate of diminazene necessary for successful treatment and development of immunity.

**MATERIALS AND METHODS**

**Animals**

Fifty-four fully susceptible 6- to 12-month-old intact Ayershire and Friesian cattle, procured as calves and raised in quarantine stables, were used in a trial to determine the waiting period required before administering diminazene after inoculation with the frozen vaccine. The animals were selected randomly and were divided into two groups (Table 1).

The efficacy of using reduced dosage rates of diminazene to treat frozen vaccine reactions was determined in eight fully susceptible 20- to 30-month-old splenectomized Hereford cattle born and bred in the stables of the Parasitology Division of the Onderstepoort Veterinary Institute. They were divided into four groups of two animals each (Table 2).

The effect of using reduced dosage levels of diminazene to block-treat frozen vaccine reactions was assessed using twenty-four fully susceptible 12- to 22-month-old intact Hereford and Friesian cattle born and bred in the stables of the Parasitology Division. The animals were placed into six experimental, two treated and two untreated control groups (Table 3).

All the animals were housed under tick-free conditions before and for the duration of the experiments.

**Drug administration, vaccination and heterologous challenge**

Accurately determined reduced doses of commercially available diminazene (Berenil RTU, Intervet SA) were administered to those experimental animals indicated in Tables 2 and 3.

The frozen *Babesia* vaccines that were used contained the *B. bovis* South African “S” strain (De Vos 1978; Callow, Mellors & McGreggor 1979) and the *B. bigemina* Australian “G” strain (Dalgleish, Callow, Mellors & McGreggor 1981; De Vos, Combrink & Bessenger 1982). The vaccines were thawed by placing them directly from liquid nitrogen storage onto melting ice in which they were kept for 4 h before intramuscular inoculation of 1 ml volumes into those experimental animals indicated in Tables 1, 2 and 3.

The frozen heterologous blood stabilates that were used to challenge the cattle were the *B. bovis* South African “F” strain (De Vos 1978) and the *B. bigemina* South African unmodified “P” strain (De Vos et al. 1982). At 120 days post vaccination, these frozen stabilates were thawed directly in a container with water at 37 °C and administered intramuscularly, in quantities that contained 5 x 10^7 parasites before freezing, into those animals indicated in Table 3.

**Monitoring redwater reactions**

Rectal temperatures, haematocrit levels and blood smears were monitored daily (De Waal & Potgieter 1987). Antibodies against *Babesia* were determined in sera collected before and 30 days after vaccination or challenge, using the indirect fluorescent antibody test technique (Gray & De Vos 1981).

**Evaluation of infectivity and reactions**

The criteria used to evaluate successful infectivity were either a positive blood smear diagnosis or positive seroconversion following vaccination or challenge. Evaluation of the vaccine or challenge reactions was based on a total reaction index score determined during the reaction period for each animal, by adding one point scored for every 1 % parasitaemia, 1 % decline in packed red blood cell volume (De Vos 1978) and 1 °C in total temperature rise above the mean pre-inoculation normal temperature of the animal (Combrink, De Waal & Troskie 1997). Five points were scored for other clinical signs and ten points for every antibabesial drug treatment, blood transfusion and death.

The unpaired *t*-test was used to determine whether results obtained for the mean total reaction indexes of the vaccinated and challenged groups differed significantly (*P* < 0.05).
RESULTS AND DISCUSSION

Start of vaccine temperature reactions

Results from febrile responses in 12 of the 28 B. bi-
gemina vaccinated animals of group 1 (Table 1),
indicated day 7 as the mean starting time of tem-
perature rise > 39.5°C. As some of the temperature
reactions to B. bovis in group 2 animals began as
early as day 6 and due to the fact that the two vac-
cines are generally inoculated simultaneously, it was
decided that the waiting period required before block
treating of vaccine reactions should remain at 7
days. None of the animals in the two groups showed
any clinical signs of disease.

Treatment efficacy of reduced diminazene doses

Results obtained in this study using 0.5 and 0.35
mg/kg doses of diminazene to treat B. bigemina
and B. bovis vaccine reactions in the splenecto-
mized cattle proved to be quite effective at para-
sitaemia levels higher than those which normally
prevail in intact animals on day 7 after vaccination.
None of the animals in the four groups required any
additional treatment (Table 2).

Effect of block treatment on the development
of immunity

Babesia bigemina

No parasites could be demonstrated in the blood
smears of the experimental animals in groups 1, 2
and 3 after treatment of vaccine reactions on day 7
with 1.16, 0.58 or 0.35 mg/kg doses of diminazene
and none of the animals seroconverted (Table 3).
Attempts to detect the organisms by subinoculating
100 ml blood from each individual animal into sus-
ceptible splenectomized animals also proved nega-
tive.

Heterologous challenge of experimental animals
produced clinical disease requiring treatment in
50 % of the animals. This contributed to significant
differences between challenge and vaccination re-
action indices, indicating no or inadequate immune
response to the killed vaccine parasites. Both the
vaccinated untreated control animals (group 4) were
positive on blood smear examination and serology,
and showed no difference between mean challenge
and vaccination reaction index results.

None of the heterologous strain-infected animals of
the unvaccinated treated control group 5 developed
clinical signs that required treatment and the result-
ning mean reaction index also showed no significant
differences when compared to those obtained for
the vaccinated control and experimental groups.
Nevertheless, all other results clearly indicate that
block treatment with diminazene on day 7 of the B.
bigemina vaccine reaction cannot be recommended.

Babesia bovis

Parasites could still be demonstrated in the blood
smears of all of the experimental animals in groups

TABLE 1 Start of temperature reactions in cattle vaccinated with the live frozen Babesia bigemina and Babesia bovis blood vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Group No.</th>
<th>No. of animals</th>
<th>No. of animals with blood smear positive showing temperature reactions &gt; 39.5°C</th>
<th>Mean start time of temperature reactions &gt; 39.5°C (day ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia bigemina</td>
<td>1</td>
<td>28</td>
<td>28/28</td>
<td>7.08 ± 3.26</td>
</tr>
<tr>
<td>Babesia bovis</td>
<td>2</td>
<td>26</td>
<td>26/26</td>
<td>10.55 ± 4.87</td>
</tr>
</tbody>
</table>

TABLE 2 Efficacy of 0.50 and 0.35 mg/kg diminazene as treatment dose of Babesia bigemina and Babesia bovis frozen vaccine reactions in splenectomized cattle

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Group No.</th>
<th>No. of animals</th>
<th>Diminazene dosage mg/kg (fraction of normal dose)</th>
<th>Mean parasitaemia at treatment (% ± sd)</th>
<th>Recovered animals not requiring additional treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia bigemina</td>
<td>1</td>
<td>2</td>
<td>0.50 (1/7)</td>
<td>1.6 ± 1.13</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>0.35 (1/10)</td>
<td>0.8 ± 0.14</td>
<td>2/2</td>
</tr>
<tr>
<td>Babesia bovis</td>
<td>3</td>
<td>2</td>
<td>0.50 (1/7)</td>
<td>3.2 ± 3.11</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>0.35 (1/10)</td>
<td>0.9 ± 0.14</td>
<td>2/2</td>
</tr>
</tbody>
</table>
### TABLE 3 Effect of 1.16, 0.58 and 0.35 mg/kg doses of diminazene administered to intact cattle on day 7 after vaccination with the live frozen *Babesia bigemina* and *Babesia bovis* vaccines

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Group</th>
<th>No. of animals</th>
<th>Diminazene dosage mg/kg (fraction of normal dose)</th>
<th>Vaccine strain</th>
<th>Blood smear positive on day 7</th>
<th>Blood smear and serology positive post day 7</th>
<th>Group reaction index Blood smear and serology mean ± sd **</th>
<th>Blood smear Group reaction index mean ± sd **</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Babesia bigemina</em></td>
<td>1</td>
<td>2</td>
<td>1.16 (1/3)</td>
<td>1/2</td>
<td>0/2 *</td>
<td>0/2 *</td>
<td>18.75 ± 2.62 ab</td>
<td>62.25 ± 48.59 ab</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>0.58 (1/6)</td>
<td>0/2</td>
<td>0/2 *</td>
<td>0/2 *</td>
<td>20.45 ± 10.25 ab</td>
<td>118.30 ± 27.72 ab</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>0.35 (1/10)</td>
<td>0/2</td>
<td>1/2</td>
<td>0/6 *</td>
<td>11.73 ± 7.05 b</td>
<td>42.07 ± 10.68 ab</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>Untreated</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>21.60 ± 2.26 ab</td>
<td>13.42 ± 5.69 ab</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>1.16 (1/3)</td>
<td>Unvaccinated</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33.70 ± 13.01 ab</td>
</tr>
<tr>
<td><em>Babesia bovis</em></td>
<td>6</td>
<td>2</td>
<td>1.16 (1/3)</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>22.50 ± 4.74 a</td>
<td>25.38 ± 3.34 a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2</td>
<td>0.58 (1/6)</td>
<td>1/2</td>
<td>2/2</td>
<td>2/2</td>
<td>23.05 ± 6.15 a</td>
<td>30.90 ± 7.85 a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2</td>
<td>0.35 (1/10)</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>24.02 ± 15.69 ab</td>
<td>54.82 ± 26.13 a</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2</td>
<td>Untreated</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>23.35 ± 2.47 a</td>
<td>9.79 ± 4.29 a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>1.16 (1/3)</td>
<td>Unvaccinated</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>48.66 ± 1.77 bc</td>
</tr>
</tbody>
</table>

* Negative for subinoculation of blood to susceptible splenectomized cattle
** Means with different superscripts within a group are significantly (*P* < 0.05) different
* Number of animals in group showing clinical disease that required treatment
6, 7 and 8 after treatment of vaccine reactions on day 7 with 1.16, 0.58 or 0.35 mg/kg doses of diminazene and all seroconverted (Table 3). None of these, nor the animals in control group 9 required any treatment during the heterologous challenge reaction and there were no significant differences to be found between the respective challenge and vaccination reaction index results, indicating adequate protection. However, various factors, such as the degree of natural resistance of different cattle breeds and individual animals to Babesia parasites, the infectivity of frozen vaccine being less predictable than that of unfrozen vaccine (due to the death of parasites during freezing and thawing), different procedures of vaccine administration, the accuracy of diminazene content according manufacturer’s label claim and the accuracy of the drug dose administered, all influence the successful immunization of animals. Consequently the block treating of B. bovis on day 7 of the vaccine reaction is also not recommended.

REFERENCES


