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WORK SHEET
The Carrier Status of Sheep, Cattle and African Buffalo Recovered from Heartwater

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ABSTRACT


Sheep, cattle and the African buffalo (*Syncerus caffer*) were shown to remain carriers of heartwater (caused by *Cowdria ruminantium*) for long periods after recovery; 223, 246 and 161 days, respectively. Transmission was achieved using adults of the southern African bont tick (*Amblyomma hebraeum*) that had fed as nymphs on recovered animals. Our findings differ from those of other workers who attempted transmission using nymphs that had fed as larvae on recovered animals or with blood from recovered animals.

INTRODUCTION

The question of the existence of a carrier state in ruminants that have recovered from heartwater (*Cowdria ruminantium*) has been examined by heartwater researchers over the years. In most cases, these studies have involved inoculating blood from heartwater recovered animals into susceptible ones (Barré and Camus, 1987). Alexander (1931) reported that blood (animal unspecified) was infective for a maximum of 35 days. Neitz (1939) and Neitz et al. (1941) found the period of infectivity in sheep to be a maximum of 2 months. Ilemobade (1978) found sheep blood to be infective up to 50 days after recovery in one case.

Few investigators have attempted transstadial transmission with natural vectors of heartwater, such as the southern African bont tick (*Amblyomma hebraeum*), when investigating the reservoir status of ruminants (Barré and Camus, 1987). Alexander (1931) fed two batches of *A. hebraeum* larvae on recovered animals and found the resulting nymphs to remain uninfected. Barré

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and Camus (1987) fed 88 batches of *A. hebraeum* larvae on Creole goats around and just after the temperature reaction and concluded that these goats were not reservoirs of *Cowdria* for more than 5 days after recovery. Nymphs, fed as larvae over a 42-day period on a heartwater-immune sheep which was challenged with virulent blood, were reported by Bezuidenhout (1987) to be unable to transmit the disease. Bezuidenhout also reported that he and Olivier (unpublished data, 1986) fed nymphs at regular intervals on two sheep experimentally infected with heartwater before and during the febrile reaction, and up to 20 days after treatment. None of the ticks that dropped before the start of the febrile reaction or 3 days after treatment transmitted the disease in the adult stage. According to Uilenberg (1981, 1983), blood is infective for ticks only from the start of the febrile reaction until a few weeks after recovery.

To determine whether or not a carrier state exists in ruminants which have recovered from heartwater, we carried out numerous transstadial transmission trials using uninfected nymphs of *A. hebraeum* and various heartwater-recovered sheep, cattle and African buffalo (*Syncerus caffer*).

**MATERIALS AND METHODS**

Heartwater-susceptible sheep, cattle and weaner buffalo, raised at the Veterinary Research (VRL) Field Station in an area free of heartwater and its vectors on the Zimbabwe highveld near Harare (Norval, 1973), were used. These animals were infected with various Zimbabwe and South African isolates of heartwater (see Table 1) and monitored for febrile reactions. Brain biopsies were taken from sheep on the third day of fever, and, if positive for heartwater, the sheep were treated intravenously with oxytetracycline hydrochloride (8 mg kg⁻¹). Cattle were either treated or left untreated. Buffalo were monitored, but as they showed no febrile reactions or clinical signs, they were left untreated. Five milliliters of blood was collected in heparin from each buffalo 16 days after their initial inoculation with heartwater. This blood was combined and inoculated into a heartwater-susceptible sheep which was then monitored for a febrile reaction.

All recovered animals were kept at the VRL Field Station when not required for tick feeding.

All ticks used in the experiments were confined in cloth bags that were glued to the backs of the animals.

Five hundred uninfected Malipati stock (established at the VRL in 1978) nymphs of *A. hebraeum*, the larvae of which had fed on rabbits, were placed on each of the heartwater-recovered sheep, cattle and buffalo at various times after recovery. These nymphs engorged, dropped off, and were placed in an incubator at a relative humidity of 80% and 27.0°C where they moulted to adults.

Surviving adults were placed on heartwater-susceptible sheep (Table 1).
which were then monitored for febrile reactions. Brain biopsies were taken on the third day of temperature and, if the brain smears were positive, the animals were treated with oxytetracycline. In some cases the disease was allowed to take its course to either death or recovery. Non-reactors were fully susceptible to heartwater when challenged by intravenous inoculation of heartwater-infected sheep blood.

RESULTS

The carrier state was demonstrated in the three ruminant species tested (Table 1). Heartwater was transmitted with relatively low numbers of adult

<p>| TABLE 1 |
|---|---|---|---|---|---|
| Transmission of <em>Cowdria ruminantium</em> from heartwater-recovered ruminants to nymphal ticks, <em>Amblyomma hebraeum</em> |</p>
<table>
<thead>
<tr>
<th>I.D. of recovered animal</th>
<th>Ruminant species</th>
<th><em>Cowdria</em> stock</th>
<th>No. of days after infection to placement of nymphs on recovered animals</th>
<th>I.D. of sheep receiving adults moulted from nymphs fed on recovered animals</th>
<th>No. adults (M = males, F = females)</th>
<th>Heartwater diagnosis</th>
<th>I.P.¹ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>051</td>
<td>Ovine²</td>
<td>Nyats²</td>
<td>114</td>
<td>1398</td>
<td>18 M</td>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>053</td>
<td>Ovine²</td>
<td>Lemco</td>
<td>119</td>
<td>1395</td>
<td>18 M</td>
<td>Positive²</td>
<td>12</td>
</tr>
<tr>
<td>1659</td>
<td>Ovine²</td>
<td>Mbizi</td>
<td>201</td>
<td>10</td>
<td>7 M, 6 F</td>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>215</td>
<td>Ovine²</td>
<td>Welge</td>
<td>90</td>
<td>4381</td>
<td>1 M, 8 F</td>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>89</td>
<td>Ovine²</td>
<td>Welge</td>
<td>166⁴</td>
<td>4382</td>
<td>10 M, 5 F</td>
<td>Positive⁵</td>
<td>12</td>
</tr>
<tr>
<td>91</td>
<td>Ovine²</td>
<td>Lemco</td>
<td>183⁴</td>
<td>4382</td>
<td>10 M, 5 F</td>
<td>Positive⁵</td>
<td>12</td>
</tr>
<tr>
<td>1764</td>
<td>Ovine²</td>
<td>Welge</td>
<td>179⁴</td>
<td>4382</td>
<td>10 M, 5 F</td>
<td>Positive⁵</td>
<td>12</td>
</tr>
<tr>
<td>1621</td>
<td>Ovine²</td>
<td>Lemco</td>
<td>223</td>
<td>4387</td>
<td>6 M, 10 F</td>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>1433</td>
<td>Ovine²</td>
<td>Ball</td>
<td>245</td>
<td>4388</td>
<td>6 M, 5 F</td>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>1404</td>
<td>Ovine⁴</td>
<td>Lemco</td>
<td>102</td>
<td>4392</td>
<td>20 M, 15 F</td>
<td>Positive</td>
<td>17</td>
</tr>
<tr>
<td>1410</td>
<td>Ovine⁴</td>
<td>Lemco</td>
<td>102</td>
<td>4392</td>
<td>20 M, 15 F</td>
<td>Positive</td>
<td>17</td>
</tr>
<tr>
<td>1342</td>
<td>Bovine</td>
<td>Lemco</td>
<td>98</td>
<td>1454</td>
<td>7 M</td>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>1322</td>
<td>Bovine</td>
<td>Lemco</td>
<td>98</td>
<td>1393</td>
<td>10 M</td>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>1342</td>
<td>Bovine⁴</td>
<td>Lemco</td>
<td>102</td>
<td>4392</td>
<td>20 M, 15 F</td>
<td>Positive</td>
<td>17</td>
</tr>
<tr>
<td>1322</td>
<td>Bovine⁴</td>
<td>Lemco</td>
<td>246</td>
<td>4394</td>
<td>10 M, 10 F</td>
<td>Positive</td>
<td>13</td>
</tr>
<tr>
<td>Tobe</td>
<td>Buffalo⁴</td>
<td>Lemco</td>
<td>17⁵</td>
<td>4383</td>
<td>10 M, 2 F</td>
<td>Positive</td>
<td>15</td>
</tr>
<tr>
<td>Tobe</td>
<td>Buffalo</td>
<td>Lemco</td>
<td>98</td>
<td>10 M, 15 F</td>
<td>Positive</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sany</td>
<td>Buffalo</td>
<td>Lemco</td>
<td>98</td>
<td>1393</td>
<td>10 M, 15 F</td>
<td>Positive</td>
<td>15</td>
</tr>
<tr>
<td>Tobe</td>
<td>Buffalo</td>
<td>Lemco</td>
<td>161</td>
<td>1453</td>
<td>30 M</td>
<td>Positive</td>
<td>33</td>
</tr>
<tr>
<td>Sany</td>
<td>Buffalo</td>
<td>Lemco</td>
<td>161</td>
<td>1455</td>
<td>27 M</td>
<td>Negative</td>
<td>-</td>
</tr>
</tbody>
</table>

¹I.P., Incubation period.
²Nyats = Nyatsanga; Welge = Welgevonden; Lemco, Nyatsanga and Mbizi stocks are Zimbabwean field isolates. Ball and Welgevonden stocks originate in South Africa.
³Animal died despite treatment.
⁴Indicates that ticks from the same host species were combined and placed on the receiver animal.
⁵Blood taken from the two buffalo at this time was combined and injected into a sheep causing a fatal heartwater infection.
ticks (15–35) from two of five individual sheep, from two pools of three sheep, from one individual bovine, from a pool of two bovines, from one of two buffalo (tested on two occasions) and from a pool of two buffalo. With one buffalo, transmission was not achieved using ticks fed on it 98 days post-infection, but it was achieved using ticks fed on it 161 days post-infection. Sheep were shown to remain carriers for 223 days, cattle for 246 days and buffalo for 161 days. Some individual animals may have remained infected for longer than these periods, but the maximum duration of the carrier state was not determined.

The sheep inoculated with blood collected 16 days after infection from buffalo developed a febrile reaction after 14 days and died of heartwater.

DISCUSSION

We have shown that sheep, cattle and African buffalo remain carriers of heartwater for long periods and therefore serve as reservoirs of infection. These findings contrast with previous accounts of the carrier state in heartwater (Alexander, 1931; Uilenberg, 1981, 1983; Barré and Camus, 1987). It is possible that in recovered animals, low numbers of organisms are reproducing in capillary cells and periodically being released into the bloodstream. This would explain why ticks are able to pick up infections from individual animals on some occasions and not on others. In our attempts to detect the carrier state, we used nymphs, rather than larvae (Alexander, 1931; Barré and Camus, 1987) to pick up infection. As nymphs ingest considerably greater amounts of blood than do larvae, they would be more likely to ingest organisms. It is also possible that the organism multiplies more effectively in nymphs than in larvae.

Cowdria ruminantium multiplies in the epithelial cells of the tick gut (Kocan et al., 1987); thus, if even low numbers of organisms are ingested, the ticks are likely to become infected. This concentrating effect could explain why transmission through nymphs is also more likely to succeed than transmission by inoculation of blood from carrier animals. The number of circulating organisms is probably high during the febrile reaction and for a short period thereafter, corresponding to the period when it is easy to transmit infection by blood inoculation.

Further evidence for the existence of the carrier state in heartwater is provided by Jongejan et al. (1988) who isolated heartwater from Lutale in Zambia by feeding uninfected laboratory-reared nymphs of Amblyomma variegatum on apparently healthy indigenous cattle.

Our findings indicate that the risk of spreading heartwater with recovered animals is significant. The results may also provide an explanation for the occurrence of relapses as reported by Neitz (1968), Neitz et al. (1947), du Plessis (1981), Uilenberg (1983) and Uilenberg et al. (1985).

Although the buffalo in this experiment were born and raised in an area free of heartwater and its vectors, they remained symptomless when infected with
heartwater. This concurs with the observations of Keffen (1985), who inoculated two fifth generation captive African buffalo with heartwater-infected blood both failed to develop clinical disease. Blood collected from our two buffalo, 16 days after injection, was infectious to sheep. Ticks fed on the buffalo at the same time also became infected. This is proof that buffalo can play a role in the epidemiology of the disease. The occurrence of a carrier state in African buffalo indicates that wild ruminants are probably also symptomless reservoirs of heartwater. This is supported by the finding of Mackenzie and Norval (1980) that losses due to heartwater occurred in cattle taken to Rekomitjie Research Station, located in Mana Pools National Park, Zimbabwe, where no other cattle were present.

In South Africa, du Plessis and Malan (1987) have reported that enzootic stability for heartwater can occur where the numbers of A. hebraeum on cattle are low. In these circumstances, losses due to heartwater are common in introduced susceptible cattle, but rare in those born in the areas (Bezuidenhout and Bigalke, 1987). Similar observations have been made regarding cattle infested with A. variegatum in Kenya (Barrett and Bailey, 1955). The existence of the carrier state, together with high infection rates in adult ticks, provides a likely explanation for these observations.

ACKNOWLEDGEMENTS

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