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# OBSERVATIONS ON THE TRANSMISSION OF *THEILERIA MUTANS* IN SOUTH AFRICA

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ABSTRACT

DE VOS, A. J. & ROOS, J. A., 1981. Observations on the transmission of *Theileria mutans* in South Africa. *Onderstepoort Journal of Veterinary Research*, 48, 1–6 (1981).

Four isolates of *Theileria mutans* obtained from geographically distant parts of South Africa by subinoculation of infected blood were found to be readily transmitted by the bont tick *Amblyomma hebraeum*. All attempts to transmit these isolates with *Rhipicephalus appendiculatus* were unsuccessful. Possible explanations for differences between these results and those reported by earlier workers in this country are discussed.

*Résumé*

*OBSERVATIONS SUR LA TRANSMISSION DE* THEILERIA MUTANS *EN AFRIQUE DU SUD*

*Quatre isolats de* Theileria mutans *obtenus de régions géographiquement distantes d'Afrique du Sud, par sub-inoculation de sang infecté ont pu être facilement transmis par le tique* Amblyomma hebraeum. *Toutes les tentatives faites pour transmettre ces isolats avec* Rhipicephalus appendiculatus *furent infructueuses. Des explications possibles des différences trouvées entre ces résultats et ceux rapportés antérieurement par d'autres chercheurs de ce pays, sont discutées.*

## INTRODUCTION

*Theileria mutans* was originally described from cattle in South Africa by Theiler in 1906. This description (Theiler, 1906) was based entirely on observations made on infections in animals inoculated with blood from infected animals. Theiler consequently classified this organism as "inoculable piroplasmosis" to distinguish it from what he called "non-inoculable" *Theileria parva*. This marked ability of the intra-erythrocytic piroplasms of *T. mutans* to multiply and persist in the blood is a characteristic used even today to differentiate this parasite from *T. parva* and *Theileria lawrencei* (Purnell, 1977). In his original description of *T. mutans*, Theiler made no mention of schizonts or ticks as possible vectors.

In subsequent observations, Theiler (1909a) observed the appearance near Nelspruit in the Eastern Transvaal of piroplasms of *T. mutans* in the blood of one susceptible animal on Day 18 and in a second on Day 19 after exposure to natural infection. The tick species present were not recorded but both *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* are known to be prevalent in that region (Howell, Walker & Nevill, 1978).

In another report, Theiler (1909b) mentioned the possibility that *A. hebraeum* and *R. appendiculatus* might be "responsible for this fever" but, to our knowledge, no attempts were made to study the ability of *A. hebraeum* to act as vector of *T. mutans*. During the same year, Theiler (1909c) reported the successful transmission of *T. mutans* to a single bovine with adults of *R. appendiculatus* fed earlier as nymphae on an infected animal. Piroplasms appeared in the erythrocytes 29 days after the last of 3 successive infestations. In the same paper, Theiler claimed to have successfully transmitted *T. mutans* with adult *Rhipicephalus evertsi* to a single animal. The prepatent period was also 29 days.

Schizonts, generally in low numbers, as well as piroplasms of *T. mutans* were later reported (De Kock, Van Heerden, Du Toit & Neitz, 1937) in animals exposed either naturally to *R. appendiculatus* infestation at Tzaneen or artificially at this Institute to ticks collected from the field at the same place. According to this report piroplasms persisted in the blood of some cases for several months after the initial exposure. It was also observed that this infection conferred no cross-immunity to the virulent form of *T. parva* present in South Africa at that time.

Further details of the ability of *R. appendiculatus* to act as vector of *T. mutans* are furnished by Neitz, Canham & Kluge (1955). They allowed the ensuing stages of *R. appendiculatus* ticks, collected as engorged larvae and nymphae from animals naturally affected with corridor disease (*T. lawrencei* infection), to feed on cattle at this Institute in an attempt to transmit this disease. The trial was unsuccessful, but 5 out of the 9 experimental animals developed a mild disease after an incubation period of 10–17 days. The disease was characterized by a mild fever, moderate swelling of the superficial lymph nodes and the presence of small numbers of schizonts in gland smears. These animals showed no immunity when challenged with *T. parva* and it was concluded that this was a form of "Tzaneen disease" (*T. mutans* infection). No mention was made of the presence or numbers of piroplasms in these animals, nor of subsequent transmission by blood inoculation.

Even more convincing evidence was brought forward by Neitz (1957). He fed nymphae on the ears of 3 splenectomized calves previously infected iatrogenically by injection of infected blood. When the ensuing adult ticks were fed on the same animals, a thermal reaction, swelling of the lymph glands and the appearance of schizonts in these glands resulted. This led Neitz to conclude that the erythrocytic stage of *T. mutans* had the ability to maintain itself in the vertebrate host in the complete absence of schizonts. It also points to successful transmission with *R. appendiculatus* of a *Theileria* transmissible by subinoculation of infected blood.

Later, during an attempt to reproduce cerebral theileriosis (turning sickness), Neitz (1962) also successfully transmitted a mild form of *T. mutans* infections with adult *R. appendiculatus* fed as nymphae on an infected animal. In addition, Ishihara (cited by Uilenberg, Robson & Pedersen, 1974) apparently had no trouble in transmitting a South African isolate of *T. mutans* with this tick species.

In East Africa, Brocklesby (1969) succeeded in serially passaging what he regarded as *T. mutans* with *R. appendiculatus*. Other work from Kenya (Barnett & Brocklesby, 1966; Purnell, Branagan & Brown, 1970; Irvin, Brown, Burridge, Cunningham, Musoke,

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Peirce, Purnell & Radley, 1972), Uganda (Uilenberg *et al.* 1974) and Tanzania (Uilenberg, Schreuder & Mpangala, 1976), however, does not support these results. *Amblyomma variegatum* has, on the contrary, been shown to be an efficient vector of what was considered to be *T. mutans* in these countries and Nigeria (Uilenberg *et al.*, 1974, 1976; Purnell, Young, Payne & Mwangi, 1975; Young, Purnell, Payne, Brown & Kanhai, 1978; Perié, Uilenberg & Schreuder, 1979).

In addition, a theilerial parasite serologically very similar to *T. mutans* was transmitted successfully to cattle with *Amblyomma cohaerens* ticks collected as engorged larvae off buffalo in Kenya (Young, Burridge & Payne, 1977).

These observations have led some workers (Uilenberg, *et al.* 1976) to conclude that Brocklesby (1969) "was, in fact, dealing with a strain of *T. parva*" and that "*R. appendiculatus* is not a vector of *T. mutans* in East Africa". It was further suggested that what is known as *T. mutans* in East Africa may be a different species from the parasite originally described in South Africa. More recently, however, serological studies have demonstrated significant cross-reactions between East and South African strains of *T. mutans* (Morzaria, Young, Kimber & Brocklesby, 1977; Kimber & Young, 1977; Uilenberg, McGregor, Mpangala, Callow & De Vos, 1978). As far as can be ascertained, Theiler's original observation on the ability of *R. evertsi* to transmit *T. mutans* (see above) has not been confirmed. Barnett & Brocklesby (1966) failed to transmit *T. mutans* with *R. evertsi* from a buffalo to cattle and concluded that the parasite involved was not able to infect *R. evertsi*.

The purpose of this study was to determine the ability of *R. appendiculatus* and *A. hebraeum* to transmit 4 isolates of *T. mutans* recently obtained from geographically different parts of South Africa by subinoculation of blood. One of these isolates was obtained from African buffalo (*Syncerus caffer*).

## Materials and Methods

### *Animals used*

Herefords and Hereford crosses born and raised at this Institute under strict tick-free conditions were used in these trials. All the animals were splenectomized with the exception of 3 cattle that were used to determine the infectivity of *T. mutans* infected blood when inoculated subcutaneously.

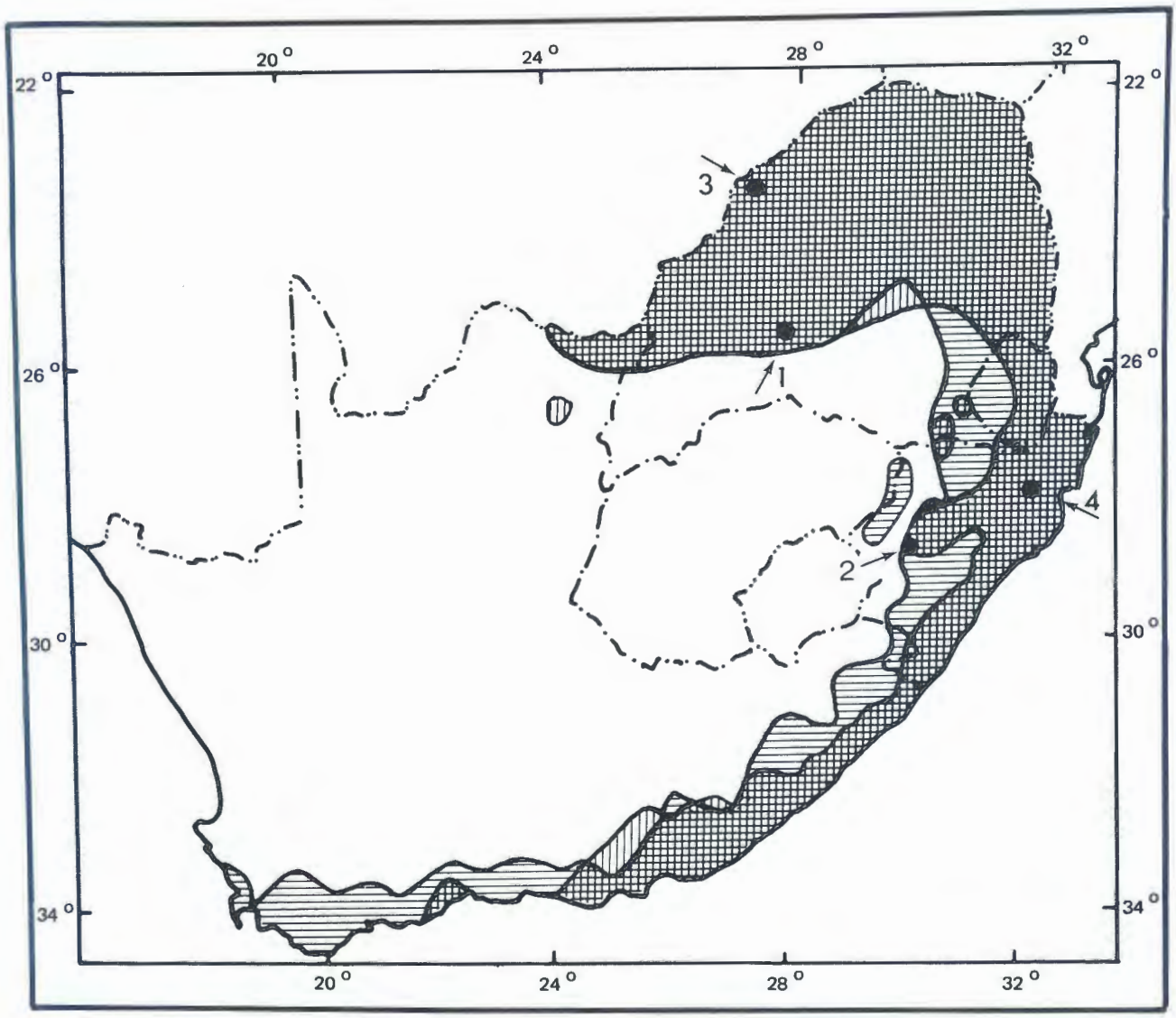


FIG. 1 Distribution of *R. appendiculatus* (horizontal lines) and *A. hebraeum* (vertical lines) in South Africa (adapted from Howell *et al.*, 1978). The sites where the 4 isolates of *T. mutans* were made are indicated: 1=MSD isolate: 2=Estcourt isolate; 3=Steenbokpan isolate; 4=Buffalo isolate

### T. mutans *isolates*

Four attempts were made to isolate *T. mutans* by sub-inoculation of blood, as described by Theiler (1906). Localities were selected (Fig. 1) where both *R. appendiculatus* and *A. hebraeum* were known to occur (Howell *et al.* 1978).

In 2 cases, later designated MSD and Estcourt isolates of *T. mutans* (Fig. 1), 50 mℓ of blood from single cattle harbouring detectable numbers of theilerial piroplasms in their blood was inoculated into susceptible animals in the laboratory. In the 3rd case (Steenbokpan isolate) 50 mℓ of blood of each of 4 bovines was pooled and inoculated into a susceptible animal. In the 4th case, 50 mℓ of blood of each of 2 African buffalo (*Syncerus caffer*) from the Hluhluwe Game Reserve, Natal, was pooled and inoculated into a bovine (Buffalo isolate). Blood smears were made daily of the recipients, stained with Giemsa's stain and examined for the presence of blood parasites.

*T. mutans* reactions occurred in all 4 recipients and were evaluated on the basis of percentage erythrocytic infection. Unwanted *B. bigemina* reactions in 3 animals were controlled with diminazene* (3,5 mg/kg) and a *Borellia theileri* reaction in one animal was suppressed with benethamine penicillin** (10 000 iu/kg). *Anaplasma marginale* was seen in 3 of the animals after the primary *T. mutans* reactions.

### *Subinoculation of* T. mutans

To determine the infectivity of the MSD isolate when small amounts of infected blood are inoculated subcutaneously into non-splenectomized animals, as described by Theiler in his original studies on *T. mutans* (Theiler, 1906, 1907), 3 animals were inoculated as summarized in Table 1. Blood smears of the recipients were examined daily for the presence of parasites.

### *Tick transmission of* T. mutans

*Ticks used:* The strain of *R. appendiculatus* used in these transmission studies is the progeny from crossing females of a strain (Rietvlei) maintained in the laboratory since 1953 with the male progeny of an engorged female collected from the field in 1975. This strain is known to be capable of transmitting the virulent (Schoonspruit) strain of *T. parva* (De Vos, unpublished observations) maintained at this Institute.

The *A. hebraeum* and *R. evertsi* ticks were obtained as the larval progeny of several engorged females of strains maintained by the Section of Entomology at this Institute.

*Tick feeding:* The procedures employed for rearing these ticks accorded with the methods of Neitz, Boughton & Walters (1971). All the ticks were fed on the ears of cattle with the exception of 1 batch of larvae of *A. hebraeum* which were fed on the ears of rabbits.

Ticks were fed on animals harbouring microscopically detectable infections of *T. mutans* as summarized in Table 2. The ensuing stages were fed on fully susceptible spenectomized cattle. Regional lymph nodes were palpated daily for 14 days after engorgement of the ticks and smears were prepared from swollen glands. Blood smears were also prepared daily from these animals for at least 8 weeks after infestation. The smears were stained and examined for the presence of schizonts and piroplasms.

* Berenil, Hoechst
** Compropen, Glaxo

## Results

*T. mutans* (MSD) was readily transmitted to 3 animals by subcutaneous inoculation of small amounts of blood with a low-level piroplasm infection (Table 1). All 3 animals became infected with a mean prepatent period for piroplasms of 29 days. Schizonts were not detected in these animals. The piroplasms reached maximum parasitaemias of 2,8%–4,5% in the different animals (Table 1) 38, 43 and 44 days after infection respectively. No anaemic changes or other signs of disease were observed in these animals.

TABLE 1 Infectivity of *T. mutans* (MSD isolate) after subcutaneous inoculation of 10 mℓ of infected blood into non-splenectomized cattle

| Donor No. | Parasitaemia of donor | Recipient No. | Prepatent period | Maximum parasitaemia |
|---|---|---|---|---|
| 1821 | 2% | 3861 | 28 | 4,0% |
| 1821 | 2% | 5034 | 28 | 4,5% |
| 1821 | 2% | 6725 | 30 | 2,8% |

Attempts to transmit the 4 isolates of *T. mutans* with *R. appendiculatus, A. hebraeum* and a single attempt with *R. evertsi* are summarized in Table 2.

All attempts to transmit the 4 different isolates of *T. mutans* with *R. appendiculatus* transstadially from larvae to nymphae and nymphae to adults, as indicated by the failure to infect cattle, were unsuccessful (Table 2) despite the fact that in 3 out of the 6 cases repletion of the ticks coincided with high parasitaemias and that large numbers of ticks were used that engorged satisfactorily during the attempted transmission.

The single attempt to transmit *T. mutans* (MSD) with *R. evertsi* was likewise unsuccessful.

*A. hebraeum* readily transmitted the 4 isolates transstadially from larvae to nymphae, as indicated by the successful infection of cattle in all 6 attempts (Table 2). *T. mutans* (MSD) was also transmitted successfully from nymphae to adults (1 attempt).

Macroschizonts were first seen in very low numbers in smears of the regional lymph nodes and blood of 5 out of the 7 animals from Days 10–13 post-infestation, and these persisted for 1–5 days (Table 2). Thirty undistorted intra- and extracellular schizonts had a longest diameter of 5–15 $\mu$m (mean 12,5 $\mu$m) and contained 8–50 (mean 32) nuclei. The nuclei of these schizonts were generally large, poorly stained, and irregular in outline. No microschizonts were seen.

Intra-erythrocytic piroplasms were first detected from Day 12–29 and persisted in readily detectable numbers for the duration of this experiment (at least 8 weeks). All the animals used in the tick transmission experiments were splenectomized and peak parasitaemias of 28–50% were observed. Parasitaemic reactions of animals infected with the various isolates are represented in Fig. 2.

Marked anaemic changes, punctate basophilia and anisocytosis in particular coincided with the peak parasitaemias, but all the animals recovered without therapy. No febrile reactions were observed.

TABLE 2 Attempted transmission of *Theileria mutans* with *Amblyomma hebraeum*, *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi*

| Isolate | Attempted infection of ticks | | | | Attempted transmission with next tick stage | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Animal No. | Tick species | Tick stage or origin | Parasitaemia* | Animal No. | No. of ticks used (approx.) | Days post-repletion | No. of ticks engorged | Prepatent period | |
| | | | | | | | | | Schizont | Piroplasms |
| MSD | 9453 | *A. hebraeum* | N | 30 | 217 | 130 | 76 | 32 | — | 19 |
| | 9453 | *A. hebraeum* | L | 30 | 9317 | 500 | 78 | 497 | 11 | 13 |
| | 9453 | *A. hebraeum* | L | 30 | 431 | 500 | 96 | 477 | 10 | 12 |
| | 1404 | *A. hebraeum* | L | 10 | 2060 | 600 | 98 | 83 | 13 | 13 |
| | F–1 | *R. appendiculatus* | N | 10 | 284 | 300 | 28 | 138 | — | — |
| | 9453 | *R. appendiculatus* | L | 30 | 9539 | 1 200 | 72 | 1 168 | — | — |
| | 9453 | *R. evertsi* | L-N | 30 | 199 | 180 | 56 | 62 | — | — |
| Steenbokpan | 1553 | *A. hebraeum* | L | 30 | 1311 | 300 | 36 | 118 | 13 | 13 |
| | 1553 | *R. appendiculatus* | L | 20 | 1132 | 1 200 | 48 | 1 115 | — | — |
| Estcourt | 475 | *A. hebraeum* | L | 15 | 605 | 100 | 45 | 58 | — | 19 |
| | 475 | *R. appendiculatus* | L | 1 | 1235 | 1 200 | 20 | 1 150 | — | — |
| | 475 | *R. appendiculatus* | L | 15 | 1272 | 250 | 46 | 24 | — | — |
| Buffalo | 1957 | *A. hebraeum* | L | 1 | 2008 | 240 | 43 | 199 | 10 | 29 |
| | 1957 | *R. appendiculatus* | L | 1 | 2204 | 150 | 43 | 56 | — | — |

* Maximum parasitaemia (%) at the time of engorgement

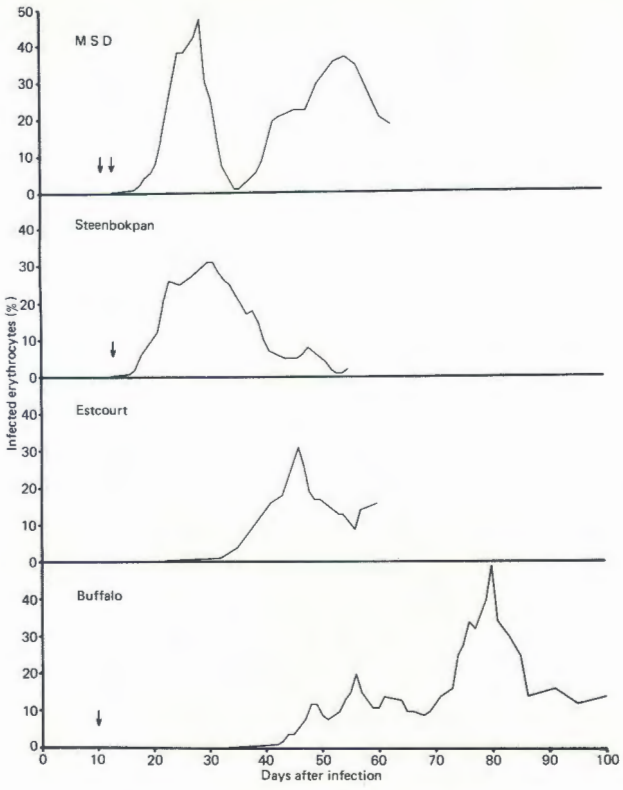


FIG. 2 Piroplasm parasitaemias observed in 4 spenectomized cattle infected with the 4 different isolates. Arrows indicate the days when macroschizonts were seen

The majority of the piroplasms were oval or round in shape, although comma-shaped, rod-shaped and irregular forms were also common. Multiplication of the piroplasms appeared to be by either binary fission or the formation of 4 daughter cells ("Maltese crosses").

## Discussion

This report adds *A. hebraeum* to the list of *Amblyomma* spp. known to be capable of transmitting *Theileria* spp. in Africa.

Morphologically, the macroschizonts resembled the ones reported for *T. mutans* in East Africa (Uilenberg *et al.* 1974; Young *et al.* 1978) both with regard to appearance and size of the schizonts. In this study, macroschizonts were seen in very small numbers in 5 out of 7 animals, but no microschizonts were detected. Uilenberg *et al.* (1974) apparently also had difficulty in finding schizonts and reported these from only 3 out of 8 animals. In reporting work on *T. mutans* (Aitong), however, Young *et al.* (1978) found macroschizonts in all cases with macroschizont-indexes as high as 9,8% (98 schizonts in 1 000 lymphoid cells). They also found microschizonts in some animals.

Although the splenectomized animals used in the present study developed a severe anaemia following tick-transmitted infections, all of them recovered without treatment. The 3 non-splenectomized animals were not clinically affected after infection with the second blood-passage and showed peak parasitaemias ranging from 2,8–4,5%. This is in striking contrast to observations made in East Africa on *T. mutans* (Aitong strain) by Young *et al.* (1978). This strain caused severe disease and even mortality associated with parasitaemias of up to 45% in non-splenectomized animals. These had been artificially infected with the first few blood-passages after isolation from the field.

It is significant that the original description of *T. mutans* by Theiler (1906, 1907) was based entirely on observations made on infections in animals inoculated with infected blood. Since piroplasms appeared in the blood of the recipients 25–41 (mean 31) days after subcutaneous inoculation, Theiler classified this organism as "inoculable piroplasmosis" (see above). The volume of blood used, namely, 10 mℓ, was only specified in the case of 3 out of the 15 animals observed with a mean prepatent period of 26 days. In our observations, also made after the subcutaneous inoculation of 10 mℓ of blood, the mean prepatent period was 29 days. Theiler (1906, 1907) also described the presence of "small rosettes similar to those described for *Piroplasma equi*" in the erythrocytes and considered these to be the multiplying forms of the parasite. With regard to both prepatent period and mode of multiplication, the isolates used in this study all fitted Theiler's original description. Because of this and the fact that Theiler made no mention of a possible vector in his original description, these isolates which are transmissible by *A. hebraeum* but not by *R. appendiculatus,* must be identified as *T. mutans.*

However, earlier reports, as outlined above, of the successful transmission of *T. mutans* by *R. appendiculatus* in South Africa are too numerous to be ignored. It is therefore possible that further studies will demonstrate the presence of a readily inoculable *Theileria* transmissible with *R. appendiculatus.* Another more likely explanation is that confusion may have existed in the past between *T. mutans* (inoculable and mildly pathogenic) and a *Theileria* transmitted by *R. appendiculatus* similar to *Theileria* sp. (Githunguri strain) (Burridge, Brown, Crawford, Kirimi, Morzaria & Payne, 1974) and *Theileria* sp. (Idobogo and Mwanza strains) (Uilenberg, Schreuder, Mpangala & Tondeur, 1977). Such a *Theileria* (not readily inoculable, but also mildly pathogenic) is known to be present in South Africa and has been identified as *T. ?taurotragi* (Uilenberg, Perié, Lawrence, De Vos, Paling & Spanjer, 1980).

No conclusions can be drawn from the single unsuccessful attempt reported above to transmit *T. mutans* (MSD) with *R. evertsi.* The difference between this result and that reported by Theiler (1909c), however, emphasizes the need for more work to be done on the vectors of the less pathogenic theilerias of Africa.

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