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THE ISOLATION OF *THEILERIA*? *TAUROTRAGI* IN SOUTH AFRICA

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ABSTRACT

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In 3 out of 4 attempts strains of a *Theileria* sp. of low virulence were isolated in the laboratory by feeding adult *Rhipicephalus appendiculatus* collected from the field on susceptible cattle. One of the strains, previously identified as *Theileria*? *taurotragi* (Tzaneen), was found to be serologically cross-reactive with the other 2 strains. It was concluded that *T.*? *taurotragi* is prevalent in South Africa in those parts where the vector exists.

Infection was characterized by a transient fever and small numbers of macroschizonts and piroplasms. Subinoculation of the infection with small volumes of blood proved to be difficult.

Résumé

L'ISOLEMENT DE THEILERIA? TAUROTRAGI *EN AFRIQUE DU SUD*

Dans trois sur quatre tentatives, des souches de Theileria *sp. de faible virulence ont été isolées au laboratoire en permettant l'adulte de* Rhipicephalus appendiculatus *récolté dans la nature de se nourrir sur bovins susceptible. Une des souches, antérieurement identifiée comme* Theileria? taurotragi (*Tzaneen*) *a mis en evidence une reaction sérologique croisée avec les deux autres souches. Il fut déduit que* T.? taurotragi *est present en Afrique du Sud dans les endroits où le vecteur existe.*

L'infection fut caractérisée par une fièvre passagère et de petits nombres de macroschizontes et piroplasmes. La transmission de l'infection avec de petits volumes de sang s'avéra être difficile.

INTRODUCTION

Theileria mutans has long been regarded as the only benign *Theileria* sp. in South Africa (Neitz, 1957). Recently, however, De Vos & Roos (1981) showed that the vector of this species is *Amblyomma herbraeum* and not *Rhipicephalus appendiculatus,* as was thought before. Reports, reviewed by De Vos & Roos (1981), of a *Theileria* sp. present in South Africa that is readily transmitted by *R. appendiculatus* and of low pathogenicity are, however, too numerous to be ignored.

A *Theileria* sp., found to be readily transmitted to cattle by *R. appendiculatus*, was described in Kenya by Burridge, Brown, Crawford, Kirimi, Morzaria, Payne & Newson (1974). Macroschizonts were present in the local parotid lymph nodes of these infected cattle, but the percentage of parasitized lymphoid cells was low. No microschizonts and few or no piroplasms were seen.

This parasite, *Theileria* sp. (Githunguri), caused a transient febrile response and the animals that recovered were fully susceptible when challenged with *Theileria parva*. Serological studies using the indirect fluorescent antibody test indicated that it was antigenically distinct from *T. parva, T. lawrencei* and *T. mutans,* but had some antigens in common with *T. parva* and *T. lawrencei*.

In 1977, Uilenberg, Schreuder, Mpangala & Tondeur reported 2 non-pathogenic Theileriae, designated *Theileria* sp. (Idobogo) and *Theileria* sp. (Mwanza), from Tanzania. Features of these 2 strains included low numbers of macroschizonts, low pathogenicity, the full susceptibility of recovered animals to challenge with *T. parva* and low indirect fluorescent antibody (IFA) titres to *T. parva* antigen. Serological comparison with the Githunguri strain gave inconclusive results.

Also in 1977, Young, Grootenhuis, Kimber, Kanhai & Stagg reported the isolation of a *Theileria* sp. from eland (*Taurotragus oryx*) in Kenya that is infective for cattle and, like the Githunguri strain, is of low virulence. Using the IFA test, they found that sera of cattle which had recovered from the eland parasite showed significant antibody titres only against antigens prepared from the *Theileria* sp. (eland) and the Githunguri strain. They concluded that it was possible that these 2 parasites could represent a new species of *Theileria* infective to cattle in Kenya. Subsequently, Grootenhuis, Young, Dolan & Stagg (1979) suggested that *Theileria* sp. (eland) be called *Theileria taurotragi*.

Using *T. taurotragi* piroplasm antigen in the IFA test, Grootenhuis, Young & Uilenberg (1981) found a high degree of cross-reaction between antisera of *T. taurotragi* and *Theileria* sp. (Idobogo). These authors also showed that the Idobogo strain was infective for eland. It would therefore appear that these 2 parasites are strains of the same species which are adapted to different hosts. It was felt that *Theileria* sp. (Idobogo) might be called *T. taurotragi* but that it required further studies on the biology and antigenic nature of these parasites before firm conclusions could be made. Consequently, Uilenberg, Perié, Lawrence, De Vos, Paling & Spanjer (1981) preferred to call it *Theileria*? *taurotragi* (Idobogo strain.)

Recently, a benign *Theileria* sp., transmitted by *R. appendiculatus* and designated *Theileria* sp. (Tzaneen), was isolated in South Africa. This strain and 3 similar, benign strains were compared with *T.*? *taurotragi* (Idobogo) (Uilenberg *et al.*, 1981). This study showed that these strains are closely related and probably identical with the Idobogo strain. It was therefore proposed to use the name *T.*? *taurotragi* for the Tzaneen strain as well.

The purpose of this paper is to record the original isolation of *T.*? *taurotragi* (Tzaneen) and to compare it with 2 other theilerial strains isolated from other localities in South Africa.

MATERIALS AND METHODS

Animals used

The animals used in this study were similar to those used in earlier work (De Vos & Roos, 1981).

Attempted isolation of theilerial infections

Isolation of theilerial parasites was attempted by feeding adult *R. appendiculatus* collected from different localities in South Africa on susceptible cattle at this Institute.

1. Tzaneen, Transvaal. Unfed adult ticks were collected manually off the grass on a very heavily infested farm.

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2. Vaalwater, Transvaal. Unfed adult ticks were collected by dragging a white linen cloth, 1,5×1,5 m in size, through infested pastures.

3. Hluhluwe, Natal. Unengorged adult ticks were removed manually from the ears of a heavily infested cow.

4. Louis Trichardt, Transvaal. Unengorged adults were removed from an African buffalo (*Syncerus caffer*) shot near this town. The conditions under which this animal was killed made it extremely unlikely that the previous instar of these ticks had fed on a buffalo.

The procedures employed for the feeding of these ticks on the ears of cattle were carried out according to the method of Neitz, Boughton & Walters (1971). After infestation of 4 animals with these tick collections, blood smears of the animals were prepared at regular intervals, stained with Giemsa's stain and examined for blood parasites. Rectal temperatures of these animals were recorded and subparotid lymph nodes palpated. Biopsy material was collected by needle puncture from noticeably enlarged regional parotid lymph nodes and smears were prepared and examined after the procedure for blood smears. All smears were examined with the aid of a Leitz Orthoplan microscope and measurements were taken with an ocular micrometer.

Tick transmission

Theilerial infections were transmitted by 3 out of the 4 collections of *R. appendiculatus* and were designated *T.? taurotragi* (Tzaneen), *Theileria* sp. (Vaalwater) and *Theileria* sp. (Hluhluwe) (Table 1).

Immature, uninfected *R. appendiculatus* and *A. hebraeum* were fed on animals harbouring microscopically detectable infections of the 3 isolates (Table 2). In addition, immatures of *R. evertsi* were fed on an animal infected with *Theileria* sp. (Hluhluwe). The ticks used were the same laboratory-maintained strains as were used by De Vos & Roos (1981) and the prodecures employed for rearing these ticks were those of Neitz *et al.* (1971). All ticks were fed on the ears of cattle.

The ensuing stages of these ticks after moulting were allowed to feed on susceptible splenectomized animals as outlined in Table 2. The animals were observed in the same way as those used for the primary isolation of the infections.

Transmission by blood inoculation

Several attempts were made to passage these 3 isolates by the intravenous subinoculation of 100 mℓ of blood with detectable piroplasm parasitaemias into susceptible splenectomized animals (Table 3). The blood was collected in ACD (citric acid, sodium citrate, dextrose) anticoagulant. Blood smears of the recipients were examined regularly for the presence of schizonts and piroplasms.

To determine the infectivity of 10 mℓ amounts of blood inoculated subcutaneously [the route used by Theiler (1906, 1907) in his original work on *T. mutans*], 3 non-splenectomized animals were inoculated with *Theileria* sp. (Vaalwater) as outlined in Table 4. Blood smears of the recipients were examined regularly for the presence of parasites.

Serology

The indirect fluorescent antibody test was performed, following the technique used by Gray & De Vos (1981). Piroplasm antigen was used in all cases. The low level parasitaemias of animals infected with the strains of *Theileria* sp. used in this study necessitated the use of thick blood smears fixed in cold acetone as antigen.

Results and Discussion

Isolation

The results of attempts to transmit theilerial parasites with 4 field collections of adult *R. appendiculatus* are summarized in Table 1. Benign *Theileria* infections were successfully transmitted by 3 of the collections and, for the purpose of this paper, these isolates will be referred to as the Tzaneen, Vaalwater and Hluhluwe strains.

In all 3 primary isolations, schizonts were first seen in biopsy smears of the regional lymph nodes 12, 13 and 15 days after infestation respectively, (Table 1). The schizonts were present in very low numbers (less than 10 per 1 000 lymphocytes) and were detected for 4–12 days in lymph node smears of the different animals. The schizonts of the 3 strains were morphologically identical. Only macroschizonts were seen and 50 of these were 2–4 (mean 3) μm in longest diameter and contained 1–8 (mean 4) compact, well-stained nuclei.

The intra-erythrocytic piroplasms were small and round to oval but, less frequently, elongated. Very few dividing forms ("Maltese crosses") were seen. The prepatent period for piroplasm parasitaemias observed in the 3 animals ranged from 14–42 days, while the maximum parasitaemias ranged from 0,2%–7,4% (Table 1).

Transmission

The results of the transmission experiments with the 3 *Theileria* isolates, using different tick species in the laboratory, are summarized in Table 2. All 7 attempts to transmit these infections with *R. appendiculatus* stage to stage from nymphae to adults were successful as were the 2 attempts to transmit the Tzaneen strain from larvae to nymphae. Schizonts were first seen 9–13 days after infestation, while the prepatent

TABLE 1 Attempted transmission to splenectomized cattle of theilerial parasites with adult *Rhipicephalus appendiculatus* collected from the field

Origin of ticks	Animal No.	No. of ticks used	No. of ticks engorged	Prepatent period (days)		Maximum piroplasm parasitaemia (%)
				Macroschizonts	Piroplasms	
Tzaneen	1645	700	209	13	14	0,2
Vaalwater	784	90	14	15	42	3,0
Hluhluwe	1830	200	24	12	25	7,4
Louis Trichardt	4304	110	8	—	—	—

TABLE 2 Attempted transmission of 3 theilerial isolates with ticks

Strain	Attempted infection of ticks				Attempted transmission with next stage						Maximum parasitaemia (%)
	Animal No.	Tick species	Tick stage	Parasitaemia* (%)	Animal No.	No. of ticks used (approx)	Days post-repletion	No. of ticks engorged	Prepatent period		
									Schizont	Piroplasms	
Tzaneen.....	1645	*R. appendiculatus*.....	N	0,1	1307	140	26	56	9	62**	1,0
	1307	*R. appendiculatus*.....	L	0,2	1861	600	14	336	12	29	1,0
	1307	*R. appendiculatus*.....	L	0,5	795	600	148	460	9	25	1,0
	1307	*A. hebraeum*.....	N	0,2	1660	55	30	26	—	—	—
	1645	*A. hebraeum*.....	L	0,1	1308	80	22	44	—	—	—
Vaalwater.....	784	*R. appendiculatus*.....	N	2,0	1405	120	23	19	12	47	7,4
	2638	*R. appendiculatus*.....	N	1,0	2682	130	43	37	13	28	0,7
	2638	*R. appendiculatus*.....	N	1,0	2749	130	143	34	12	19	2,0
	2749	*R. appendiculatus*.....	N	2,0	3347	130	87	67	10	16	4,4
	2749	*R. appendiculatus*.....	N	2,0	3349	130	87	62	10	23	1,1
	784	*A. hebraeum*.....	L	2,0	1380	300	21	197	—	—	—
Hluhluwe.....	1830	*R. appendiculatus*.....	N	0,2	1584	150	70	57	11	21	1,0
	1830	*A. hebraeum*.....	L	0,2	1816	400	67	361	—	—	—
	1584	*R. evertsi*.....	L	0,2	2009	150	88	41	—	—	—

* Maximum parasitaemia at the time of engorgement
** Animal 1307 was treated with tetracycline (10 mg/kg) on Days 28 and 30 after infection

TABLE 3 Transmission of 3 theilerial isolates by intravenous inoculation of 100 mℓ of blood into splenectomized animals

Strain	Donor animal No.	Parasitaemia of donor (%)	Recipient No.	Prepatent period		Maximum parasitaemia in recipient %
				Schizont (days)	Piroplasms (days)	
Tzaneen	1645	0,1	4800	—	39	0,6
	795	0,4	2259	—	35	1,0
Vaalwater	784	<0,1	1644	—	46	0,6
	790	0,1	615	—	24	1,0
	615	0,1	2638	—	19	0,9
	1405	<0,1	790	—	70	2,0
Hluhluwe	1830	5,0	1286	—	20	<0,1
	1830	0,2	1310	31	40	6,0
	1310	1,0	1680	—	11	0,8
	1830	1,0	1398	—	30	1,0

periods for piroplasm parasitaemia ranged from 16–62 days. It should be noted that the animal (1307) with a prepatent period of 62 days was treated on Days 28 and 30 with tetracycline (10 mg/kg) to control an *Eperythrozoon* infection.

All 3 attempts to transmit these isolates with *A. hebraeum* stage to stage from larvae to nymphae were unsuccessful, as was a single attempt to transmit the Hluhluwe strain with *R. evertsi* (Table 2).

These results are similar to those recorded by Uilenberg *et al.* (1981) for the Tzaneen strain except that they found the prepatent periods for piroplasm parasitaemia to be slightly shorter, i.e. 15–19 days.

Pathogenicity

Infected animals showed a transient enlargement of the regional parotid lymph nodes and an increase in body temperature (39,0–40,8) for 1–3 days at the time when schizonts were present in detectable numbers. The primary reactions of both the Tzaneen and Vaalwater strains in Animals 1645 and 784 respectively were followed by a second temperature rise associated with the presence of *Ehrlichia bovis* in the leucocytes. All 3 strains must therefore be considered at best to be only mildly pathogenic. Even splenectomy did not noticeably affect the course of the reaction, since the highest piroplasm parasitaemia recorded was 7,4%.

The mild nature of these strains is similar to that reported for similar theilerial parasites transmitted by *R. appendiculatus* in other parts of Africa (Burridge *et al.*, 1974; Grootenhuis *et al.*, 1979; Uilenberg *et al.*, 1977; Uilenberg *et al.*, 1981).

Inoculability

The 3 theilerial isolates were transmitted successfully by the intravenous inoculation of 100 mℓ of infected blood into susceptible splenectomized animals (Table 3). The prepatent periods for piroplasm parasitaemia ranged from 11–70 days with a maximum parasitaemia ranging from less than 0,1%–6,0%. The subcutaneous inoculation into non-splenectomized animals of 10 mℓ of blood with a 5% parasitaemia, however, resulted in a detectable parasitaemia in only 1 out of 3 animals, with a prepatent period of 204 days (Table 4).

In his original work on *T. mutans,* Theiler (1906, 1907) found this parasite to be readily transmissible when 10 mℓ volumes of infected blood were inoculated subcutaneously. It must therefore be concluded that he was dealing with the *Theileria* now known to be transmitted by *A. hebraeum* and not with the *Theileria* sp. reported here, although Theiler (1909) claimed that *R. appendiculatus* was the vector.

TABLE 4 Infectivity of *Theileria* sp. (Vaalwater) after inoculation of 10 mℓ of infected blood subcutaneously into non-splenectomized animals

Donor No.	Parasitaemia of donor (%)	Recipient No.	Prepatent period (days)	Maximum parasitaemia (%)
1405	5	9467	—	—
1405	5	9478	204	<0,01
1405	5	9482	—	—

Piroplasm parasitaemias of tick-transmitted infections as well as of blood-induced infections remained patent for the duration of this study. One splenectomized animal, infected with the Vaalwater strain, remained positive for 2 years, while another infected with the Hluhluwe strain still had detectable piroplasms in its blood 18 months after infection.

Serology

The observations made on *Theileria* sp. (Vaalwater), *Theileria* sp. (Hluhluwe), *T.*? *taurotragi* (Tzaneen), *T. p. parva, T. p. bovis* and *T. p. lawrencei* are summarized in Table 5. *Theileria* sp. (Vaalwater) and *Theileria* sp. (Hluhluwe) were found to be cross-reactive with *T.*? *taurotragi* (Tzaneen), but the sera of all 3 isolates gave very weak reactions with the *T. parva* piroplasm antigen. In the opposite test, however, sera of the *T. p. parva* group gave strong reactions with the heterologous antigens since, in some cases, the titres obtained were the same as those obtained with homologous antisera.

The cross-reactivity between *T.*? *taurotragi* (Tzaneen) and *Theileria sp.* (Vaalwater and Hluhluwe) confirms that the 3 isolates are of the same species, namely, *T.*? *taurotragi.* As stated by Uilenberg *et al.* (1981), the question mark expresses the uncertainty still persisting on the complete identity of this species with *T. taurotragi* of the eland.

The primarily one-direction cross-reactivity seen in this study between *T.*? *taurotragi* and the *T. parva* group is similar to that reported by Uilenberg *et al.* (1981) with the use of piroplasm antigens for the Tzaneen strain as well as for 3 strains of the same species from Zimbabwe. The titres determined by them were higher, however, than those seen in this work.

TABLE 5 Reciprocal titres of sera of animals infected with theilerial infections against homologous and heterologous antigens, using the indirect fluorescent antibody test

Sera		Antigens				
Species	Animal No.	*T.? taurotragi* (Tzaneen)	*Theileria* sp. (Vaalwater)	*Theileria* sp. (Hluhluwe)	*T.p. parva*	*T. mutans*
T.? taurotragi (Tzaneen).....	2259	160	160	80	<40	<40
Theileria sp. (Vaalwater).....	2638	320	320	320	40	<40
	615	80	160	160	<40	ND
	615	160	640	320	40	<40
Theileria sp. (Hluhluwe).....	1830	80	80	80	<40	ND
	1830	160	80	160	<40	<40
T. parva parva.....	4806	160	160	320	320	<40
T. parva bovis.....	3991	160	320	320	320	<40
T. parva lawrencei.....	4033	80	160	80	320	<40
	2519	160	320	320	1280	<40
T. mutans.....	3861	<40	<40	ND	<40	5120

ND=Not done

Conclussion

The presence in South Africa of a benign *Theileria* sp. transmitted by *R. appendiculatus* has been known for a long time (Theiler, 1909; Neitz, 1957). This study confirms the presence of *T.? taurotragi* in this country. It can be differentiated from *T. mutans* (with which it was confused in the past) by the difficulty with which it is transmitted by the sub-inoculation of small volumes of blood (Uilenberg *et al.*, 1981; De Vos & Roos, 1981).

The report of *T. mutans* (De Vos & Roos, 1981) and *Theileria velifera* (Berger, 1979) brings the total number of benign bovine *Theileria* spp. present in South Africa to 3.

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