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TICK TRANSMISSION OF *ANAPLASMA CENTRALE*

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ABSTRACT

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Anaplasma centrale was isolated from a field collection of *Rhipicephalus simus*. Transstadial transmission of *A. centrale* with adult ticks was demonstrated, but the infection was not carried transovarially. Ticks from this collection were subsequently reared as a non-infected, laboratory strain. It was proved that the Onderstepoort live blood vaccine strain of *A. centrale*, isolated by Theiler in 1911, is still tick transmissible after more than 75 years of needle passage through cattle in the laboratory. Attempts to demonstrate transstadial transmission of the vaccine strain with *Boophilus decoloratus* and *Boophilus microplus* failed.

INTRODUCTION

Anaplasmosis is an economically important, tick-borne, rickettsial disease of cattle in the Republic of South Africa. So far it has been shown in the laboratory that 5 different important cattle ticks can transmit *Anaplasma marginale* infections in this country (Potgieter, 1979; 1981). *Stomoxys calcitrans* can transmit *A. marginale* mechanically (Potgieter, Sutherland & Biggs, 1981), and recent laboratory investigations have indicated that transplacental infections of both *A. marginale* and *A. centrale* probably occur more frequently than was previously thought (Potgieter & Van Rensburg, unpublished observations 1986).

Anaplasma marginale was first described by Theiler (1910) as the causative organism of anaplasmosis in cattle in South Africa.

In an attempt to isolate pure infections of *Anaplasma marginale*, Theiler (1911) found an organism in a heifer from Aliwal North in the Cape province that was smaller and more centrally placed in the erythrocyte than *A. marginale*. This organism caused less severe reactions in cattle than *A. marginale* and he called it *Anaplasma marginale* var. *centrale*. It was also demonstrated that animals infected with *A. centrale* had sufficient immunity and were protected against *A. marginale* challenge. Having demonstrated this cross-immunity, Theiler (1912) produced a live blood vaccine containing *A. centrale* organisms. The isolate has been exported to other countries, where it is used as a live blood vaccine.

Apparently this was the only isolate of *A. centrale* to have been made in this country until recently, when an *A. centrale* infection was transmitted to a splenectomized ox in the laboratory with a field collection of unfed adult *Rhipicephalus simus*.

Theiler (1911) reported the transovarial transmission of anaplasmosis by *Boophilus decoloratus* and *R. simus*, the former apparently transmitting both *A. marginale* and *A. centrale*. *R. simus* was reported to have transmitted anaplasmosis, but the specific parasite involved was not mentioned.

The purpose of this study was to determine whether the *A. centrale* vaccine strain, originally isolated by Theiler more than 75 years ago and still being used in the current live blood vaccine, is still tick transmissible after having been maintained in splenectomized vaccine donor cattle through needle passage in the laboratory all these years.

MATERIALS AND METHODS

Cattle

All the cattle used in these transmission experiments were born and reared under strict, tick-free conditions at this laboratory. They were mainly mixed *Bos taurus* breeds, splenectomized at approximately 6 months of age and fully susceptible to anaplasmosis.

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Certain precautions were taken to prevent possible mechanical transmission of the *Anaplasma* infections in a particular stable complex, as described by Potgieter *et al.* (1981).

All the cattle were monitored on a regular basis. Rectal temperatures were taken daily and thin blood smears, prepared from the tip of the tail, were stained with Giemsa's stain and examined daily for *Anaplasma* bodies. Haematocrits were determined 3 times a week during active infections. Animals showing severe reactions were treated daily intravenously with oxytetracyclines at a dosage rate of 10 mg/kg live body mass for 3 consecutive days.

Cattle that remained negative on blood smear examination were tested serologically after 90 days with a card agglutination test (Potgieter & Van Rensburg, 1983) to confirm unsuccessful transmission.

Anaplasma *isolates*

The original *A. centrale* isolate made by Theiler (1911) is still used to produce a live blood vaccine, as described by Potgieter (1979). Almost 60 years later, a second *A. centrale* isolate from a tick-transmitted infection was made by Potgieter (1979). Adult *R. simus* ticks, amongst other species, were collected from the field during a Corridor disease outbreak in the Louis Trichardt district of the Northern Transvaal in an attempt to isolate *Theileria p. lawrencei* in the laboratory. These ticks were identified and fed on susceptible splenectomized cattle under controlled conditions. An ox infested with 80 adult *R. simus* contracted a pure *A. centrale* infection, showing a prepatent period of 55 days after tick infestation. Further transmission experiments indicated that the *A. centrale* infection was not carried transovarially and it was decided to maintain these ticks, designated the LT-strain (Potgieter & Van Rensburg, 1982), as a non-infected laboratory strain.

Infected blood stabilates of both these isolates, stored in liquid nitrogen, were used to infect the experimental animals intravenously.

Ticks

Non-infected, laboratory-maintained batches of 3 tick species, the LT-strain of *R. simus* (see above) and 2 laboratory strains of *B. decoloratus* and *Boophilus microplus*, were used in these transmission trials. All 3 tick strains have previously been shown to transmit *Anaplasma marginale* infections transstadially (Potgieter, 1979; 1981).

Non-feeding stages were kept in the acaridarium at 26 °C and 85 % RH.

Attempts to infect ticks with A. centrale

R. simus: In 2 separate experiments non-infected nymphae were used to infest the ears of 2 oxen undergoing primary *A. centrale* reactions, according to the method of Neitz, Boughton & Walters (1971), as indicated in

TABLE 1 Infection and transmission of *A. centrale* by *R. simus*

Infection of nymphae (N)

Animal No.	Infection (i.v.) with *A. centrale* blood stabilate	Prepatent period of infection (days)	Infestation with non-infected nymphae	Collection of engorged nymphae	*A. centrale* parasitaemia during feed (%)
9211-8	9 mℓ	19	± 800	±537	2-37
9117-4	9 mℓ	17	±1 200	±528	1–41
9630-6	*in utero* infection	relapse after splenectomy	±1 600	±816	18–26

Transmission by adults (A)

Animal No.	Tick infestation and i.v. inoculation of tick suspensions	Age post moult (days)	Engorged females collected	Prepatent period of *A. centrale* infection (days)
9287-4	120 (A) from 9211-8	42	38	41
9438-6	130 (A) from 9117-4	42	27	45
9513-7	2 mℓ (10 ticks/mℓ) from 9117-4	42	—	36
9508-0	2 mℓ (5,4 ticks/mℓ) from 9630-6	30	—	48

TABLE 2 Attempts to demonstrate transstadial transmission of *A. centrale* by *B. decoloratus*

Infection of larvae (L) and nymphae (N)

Animal No.	Infection (i.v.) with *A. centrale* blood stabilate	Prepatent period (days)	Tick infestation	Removal of engorged immatures	*A. centrale* parasitaemia during feed (%)
9386-7	9 mℓ	13	± 8 000 (L)	± 50 (L) ±1 030 (N)	17–36
9436-0	9 mℓ	15	±28 000 (L)	±3 000 (N)	20–42

Transmission by nymphae and adults (A)

Animal No.	Tick infestation	Engorged females collected	Blood smear examination	Serology CAT
9511-7	45 (L) from 9386-7	26	—	—
9472-2	± 1 025 (N & A) from 9386-7	30	—	—
9533-8	± 2 300 (N & A) from 9436-0	326	—	—

TABLE 3 Attempts to demonstrate transstadial transmission of *A. centrale* by *B. microplus*

Infection of larvae (L) and nymphae (N)

Animal No.	Infection (i.v.) with *A. centrale* blood stabilate	Prepatent period (days)	Tick infestation	Removal of engorged immatures	*A. centrale* parasitaemia during feed (%)
9669-4	9 mℓ	17	±20 000 (L)	±8 500 (N)	23–29
9551-3	9 mℓ	14	±26 000 (L)	±3 500 (N)	31–46

Transmission by adults (A)*

Animal No.	Tick infestation	Engorged females collected	Blood smear examination	Serology CAT
9533-8	± 3 000 (N & A) from 9669-4	48	—	—
9475-5	± 3 000 (N & A) from 9551-3	274	—	—

* Nymphal transmission not done

Table 1. The engorged nymphae were collected and allowed to moult in the acaridarium and the ensuing adults were used to infest the ears of 2 oxen in an attempt to demonstrate transstadial transmission from nymph to adult.

A 3rd batch of nymphae was fed on splenectomized Calf 9630-6, which initially contracted an intra-uterine *A. centrale* infection and consequently suffered a severe relapse after splenectomy. The dam was an ex-*A. centrale* vaccine donor used in the tick-free breeding herd.

B. decoloratus: Two attempts with different batches of the same tick strain were used to demonstrate transstadial transmission, larva to nymph-adult and larva-nymph to adult.

Ox 9386-7 was infested with larvae twice, at a 7-day interval. On Days 7 and 8 after the larval infestations, the life cycle of this one-host tick was interrupted when engorged larvae and nymphae were removed by hand and transferred to 2 calves respectively, over a period of 3–5 days, as indicated in Table 2. During this time some of the engorged nymphae moulted in the acaridarium before they were used to infest Calf 9472-2. The ticks were placed into cloth containers (back pockets), glued to the calves' backs.

When this experiment was repeated, only engorged nymphae (no larvae) were removed from the reacting Ox 9436-0 and transferred to Ox 9533-8 (Table 2).

B. microplus: Two experiments were executed to demonstrate transstadial, larva-nymph to adult transmission. Engorged nymphae were manually removed from the reacting cattle and transferred over a period of 5 days (Table 3) to the susceptible animals, mainly as newly moulted adults.

Tick suspension

Unfed *R. simus* adult ticks, which had become infected as nymphae (Table 1), were triturated in a tissue

homogenizer as described by Potgieter & Van Rensburg (1980). Freshly prepared tick suspensions were used to inoculate cattle to demonstrate infectivity.

RESULTS

The *A. centrale* Onderstepoort live blood vaccine strain was successfully transmitted transstadially by *R. simus* adult ticks in 4 separate experiments (Table 1).

All attempts to transmit the infection transstadially with the 2 one-host blue tick species failed (Tables 2 & 3).

DISCUSSION

The fact that the original isolate of *A. centrale* (Theiler, 1911) retained its infectivity to *R. simus*, after being artificially maintained by needle passage in splenectomized cattle in this laboratory for more than 75 years, is quite remarkable. At this stage it is not known how many tick species are involved in the transmission of this parasite, and limited attempts have been made to compare the pathogenicity of the original isolate made by Theiler with the recent *R. simus*-transmitted field isolate mentioned above. No significant differences were observed in the clinical response between 2 groups of 4 animals each, infected with these 2 isolates respectively. On challenge with *A. marginale* the reactions in all 8 animals were comparable (Potgieter, unpublished data, 1980).

The natural host of *A. centrale* and the prevalence of natural infections in cattle are now obscured by the fact that the vaccine-strain is transmitted by ticks. During the past 10 years an average of 454 446 doses of vaccine per annum have been issued from this Institute. Mechanical as well as *in utero* transmission of the infection probably also make a significant contribution to spreading the infection.

It is known that vaccination with *A. centrale* can cause severe clinical reactions and even mortality in older cattle (Bigalke, 1980; Pipano, Mayer & Frank, 1985). However, no evidence is forthcoming that incriminates *A. centrale* as the causative organism of natural anaplasmosis outbreaks in this country except for a few isolated cases in which older lactating Friesian dairy cows were involved and some unconfirmed field reports (Potgieter, unpublished observations, 1981).

It is believed that this mild *A. centrale* vaccine organism could have been spread in enzootic anaplasmosis areas were the vaccine is used regularly.

Differences between these parasites and the host's response to the specific infections have been described by Theiler, 1911; Kuttler, 1966; Schindler, Ristic & Wokatsch, 1966; Wokatsch, 1968; Kuttler, 1972. It may also be true that they do not share the same tick vectors, because the same strains of the 2 *Boophilus* spp. used in the present negative transmission experiments readily transmitted *A. marginale* infections transstadially under the same laboratory conditions (Potgieter, 1979; 1981).

We conclude that natural infection of calves with *A. centrale* probably enhances the development of enzootic stability of anaplasmosis in many areas in this country.

REFERENCES

BIGALKE, R. D., 1980. Laboratory and field observations on the use of *Anaplasma centrale* as a vaccine against anaplasmosis. *Zimbabwe Veterinary Journal*, 11, 21–22.

KUTTLER, K. L., 1966. Clinical and hematologic comparison of *Anaplasma marginale* and *Anaplasma centrale* infections in cattle. *American Journal of Veterinary Research*, 27, 741–946.

KUTTLER, K. L., 1972. Comparative response to premunization using attenuated *Anaplasma marginale* virulent *A. marginale* and *A. centrale* in different age groups. *Tropical Animal Health and Production*, 4, 197–203.

NEITZ, W. O., BOUGHTON, F. & WALTERS, H. S., 1971. Laboratory investigation on the life cycle of the Karoo paralysis (*Ixodes rubicundus* Neumann, 1904). *Onderstepoort Journal of Veterinary Research*, 38, 215–223.

PIPANO, E., MAYER, E. & FRANK, M., 1985. Comparative response of Friesian milking cows and calves to *Anaplasma centrale* vaccine. *British Veterinary Journal*, 141, 174–178.

POTGIETER, F. T., 1979. Epizootiology and control of anaplasmosis in South Africa. *Journal of the South African Veterinary Association*, 50, 367–372.

POTGIETER, F. T. & VAN RENSBURG, L., 1980. Isolation of *Anaplasma marginale* from *Rhipicephalus simus* males. *Onderstepoort Journal of Veterinary Research*, 47, 285–286.

POTGIETER, F. T., 1981. Tick transmission of anaplasmosis in South Africa. *Proceedings of the International Conference on Tick Biology and Control, Grahamstown*, 27–29 January 1981. pp. 53–56.

POTGIETER, F. T., SUTHERLAND, B. & BIGGS, H. C., 1981. Attempts to transmit *Anaplasma marginale* with *Hippobosca rufipes* and *Stomoxys calcitrans*. *Onderstepoort Journal of Veterinary Research*, 48, 119–122.

POTGIETER, F. T. & VAN RENSBURG, L., 1982. The effect of incubation and prefeeding of infected *Rhipicephalus simus* nymphae and adults on the transmission of *Anaplasma marginale*. *Onderstepoort Journal of Veterinary Research*, 49, 99–101.

POTGIETER, F. T. & VAN RENSBURG, L., 1983. Infectivity virulence and immunogenicity of *Anaplasma centrale* live blood vaccine. *Onderstepoort Journal of Veterinary Research*, 50, 29–31.

SCHINDLER, VON R., RISTIC, M. & WOKATSCH, R., 1966. Vergleichende Untersuchugen mit *Anaplasma marginale* und *A. centrale*. *Zeitschrift für Tropenmedizin und Parasitologie*, 17, 337–360.

THEILER, A., 1910. *Anaplasma marginale* (gen. spec. nov.). The marginal points in the blood of cattle suffering from a specific disease. *Report of the Government Veterinary Bacteriologist*, 1908–9, 7–64.

THEILER, A., 1911. Further investigations into anaplasmosis of South African cattle. *1st Report of the Director of Veterinary Research, Union of South Africa*, 7–46.

THEILER, A., 1912. Gallsickness of imported cattle and the protective-inoculation against this disease. *Agricultural Journal of the Union of South Africa*, 3, 1–2.

WOKATSCH, R., 1968. Serologische Untersuchungen über das Vorkommen der Anaplasmose bei Hauswiederkäuren in Tanzania/Ostafrica. *Zeitschrift für Tropenmedizin und Parasitologie*, 19, 210–215.