Onderstepoort J. vet. Res., 48, 119–122 (1981)

ATTEMPTS TO TRANSMIT *ANAPLASMA MARGINALE* WITH *HIPPOBOSCA RUFIPES* AND *STOMOXYS CALCITRANS*

F. T. POTGIETER(1), B. SUTHERLAND(1) and H. C. BIGGS(2)

ABSTRACT

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 (1981).

Three attempts to transmit anaplasmosis with field collections of *Hippobosca rufipes* were unsuccessful, despite the fact that the flies had been fed initially on splenectomized cattle acutely infected with *Anaplasma marginale*. However, 1 out of the 3 attempts, made concurrently with the others, to transmit this organism with *Stomoxys calcitrans* was successful. The prepatent period was 27 days.

Résumé

*TENTATIVES DE TRANSMISSION D'*ANAPLASMA MARGINALE *AVEC* HIPPOBOSCA RUFIPES *ET* STOMOXYS CALCITRANS

*Trois tentatives faites en vue de transmettre l'anaplasmose avec des collections champêtre d'*Hippobosca rufipes *se sont révélées infructueuses, malgré le fait que les mouches avaient initialement été alimentées sur du bétail sévèrement infecté avec l'*Anaplasma marginale *et splenectomisé. Cependant, 1 des 3 tentatives, faite en même temps avec les autres, pour transmettre l'organisme avec* Stomoxys calcitrans, *fut fructueuse. La période de prépatence fut de 27 jours.*

INTRODUCTION

The possible role that haematophagous insects may play in the epizootiology of bovine anaplasmosis has been the subject of speculation for some time in South Africa. Experimental and epizootiological evidence incriminates horse flies (*Tabanus* spp.) as the most significant insect vectors of anaplasmosis (Ristic, 1977). Experimental evidence of transmission was also produced with stable flies (*Stomoxys*), deer flies (*Chrysops*), horse flies (*Siphona*), and mosquitoes of the genus *Psorophora* (Ristic, 1968). The following trials were therefore undertaken, in which 2 of the most likely and most easily manageable insects available, *Hippobosca rufipes* (cattle louse fly) and *Stomoxys calcitrans* (stable fly), were used.

MATERIALS AND METHODS

Experimental animals

The cattle used were mainly of mixed European breeds, born and raised under strictly tick-free conditions at Onderstepoort, splenectomized, and susceptible to anaplasmosis. Rectal temperatures and thin blood smears were taken daily from all the animals. In addition, the haematocrits of all infected animals were monitored daily. Special precautions were taken to prevent mechanical transmission of the infection when the animals were being handled. The same 2 people worked together throughout this study, preparing blood smears with the utmost care and placing the scissors used for blood smear preparations in 96% alcohol each time after use.

Before being regarded as negative, possible reactors were closely observed and isolated in their stables for 3 months after the initial exposure. To test their susceptibility, all oxen that remained non-infected, as determined by blood smear examinations, were subsequently infected with *Anaplasma marginale*, either by subinoculation of infected blood or by infestation with infected ticks.

(1) Veterinary Research Institute, Onderstepoort 0110

(2) Central Veterinary Laboratory, Division of Veterinary Services, Windhoek, South West Africa (Namibia)

Received 20 March 1981—Editor

Strain of A. marginale

During an outbreak of anaplasmosis in the Barkly West district in the Northern Cape, adult ticks of 2 species, namely, *Hyalomma marginatum rufipes* and *Rhipicephalus evertsi evertsi*, were collected from clinical cases and brought to this laboratory. Each species was then fed separately on a susceptible ox. Both animals contracted *A. marginale* infections and blood stabilates were prepared from this isolate which was designated the BW-strain. To induce infection 2 mℓ of the stabilate was injected intravenously into the experimental animals during these trials.

Fly-proof facilities

Since no special insect-free stables were available, the animals were accommodated in tick-free units of an isolation stable complex. The 1st batch of *H. rufipes* was fed on animals kept in a stable which had been divided into 2 separate pens by means of plastered brick walls, 1,3 m high and 0,6 m apart. Both pens were surrounded by moats which effectively isolated the animals when tick feeding experiments were being carried out. Subsequent experiments were executed in a larger stable where the animals shared a single pen designed basically as described above.

Throughout the experiment the north-facing door was used for essential maintenance only, while the south-facing door remained locked. The upper half of the service door was a panel of wire fly-screening. All the windows were screened with the same wire and rubber strips were fitted to the bottom of both doors. In addition to this, electrocuting ultraviolet insect traps were installed in each stable and operated daily from 07h00 to 16h30. For 7 days prior to the installation of these traps, test runs were made with similar traps operating continuously in stables occupied by cattle.

S. calcitrans

Origin. All the stable flies used in these experiments were taken from a robust stock colony of *S. calcitrans* raised at this Institute. The adults of this colony were kept at 27 °C±1 °C with a relative humidity of 60%–80%, a photo-period with an 8-h light and 16-h dark cycle, and fed citrated cattle blood as nutrient (Sutherland, 1978).

Transmission experiments

Three of the *A. marginale*-infected cattle used in the *H. rufipes* trials (Oxen 2542, 1820 & 794, *vide infra*) were also used in these experiments.

Sixteen hours before being used, approximately 50–80 *S. calcitrans* flies, both males and females, 4–6 days old, were collected from the adult rearing cages and transferred to a wooden frame cage (200 mm × 120 mm × 120 mm) covered with dark-coloured nylon fly screening. During the pre-exposure periods, the experimental flies were kept under room conditions and given access to a water-soaked cotton wool pad until 2,5 h before exposure. The cage containing the flies was then pressed down firmly onto an area on the back of the animal from which the hair had been closely clipped with an electric razor, and the flies were allowed to feed through the nylon fly screen for 3–10 min. The cage was then transferred to the susceptible animal. This process was repeated a number of times during each experiment at 09h30 and again at 14h30.

H. rufipes

Origin. All the flies for this experiment were collected off cattle and a few horses on farms in the Windhoek district of South West Africa.

The flies were caught and subsequently transported in "funnel traps" made out of 750 mℓ transparent plastic bottles. The top third of each bottle had been removed and replaced with a plastic funnel of the same diameter affixed to the brim. The trap was inverted over a fly on the animal and, as the fly flew up through the funnel, it became trapped in the bottle.

Initially, the funnels of the traps containing the flies were plugged with paper wads and the traps were then packed in cardboard boxes lined for insulation with polystyrene sheets. Frozen freezer packs were included in the boxes to keep the temperature down. This method caused high mortalities because the smooth plastic surface was not a suitable resting place for the flies. In subsequent collections, tissue paper, which provided suitable resting surfaces, was placed in the traps and virtually no losses occurred in transit. The flies were air-freighted from Windhoek to South Africa on the day of collection and released onto the experimental animals the following day.

Exposure to A. marginale infection

First trial. Ox 2542 was infected with the BW-strain of *A. marginale,* as described above, and housed in a closed stable divided into 2 pens.

Approximately 250 flies were released onto this animal when the *A. marginale* parasitaemia had reached a level of 3%. The following day Ox 2378 was brought into the adjacent pen in the same stable.

Since very few flies had moved from the infested to the susceptible animal after 6 h, 50–100 flies were transferred manually back and forth between the 2 animals daily for 5 days.

On Day 7 after the release of the flies, the infected ox, which was showing an *A. marginale* parasitaemia of 90%, was euthanized. The remainder of the flies were removed from the carcass and placed onto the susceptible animal.

Second trial. Another batch of approximately 450 flies was released onto infected Ox 1820 showing a parasitaemia of 11% at the time. After approximately 2 h, Ox 2519 was brought into the same pen. Some flies were immediately attracted to this animal and the following day they were almost evenly distributed between the 2 oxen. The flies remained thus distributed until 5 days later, when the infected animal showed a parasitaemia of 88% and was euthanized. It is not known to what extent the flies had changed hosts during this time. All the flies were transferred from the dead animal to the susceptible animal before the carcass was removed.

Third trial. Essentially the same procedure as in the 2nd trial was followed in the 3rd and last attempt to transmit *A. marginale* mechanically with *H. rufipes*, except that, to demonstrate that no other vector could have played a role in the possible transmission of an infection, a control experiment was conducted simultaneously in similar fly-proof facilities.

Approximately 350 flies were released onto Ox 794 on Day 0 when the animal had an *A. marginale* parasitaemia of 12%. The non-infected animal, Ox 1969, was brought into the same pen a few hours later. As in the previous experiment, in which the animals shared the same pen, the flies appeared to be more or less evenly distributed between the animals the next day. The infected animal was euthanized on Day 6, and the flies were transferred to the susceptible animal. At the time of death the parasitaemia in the infected animals was 91% and the haematocrit 9%.

The control experiment was conducted in an identical stable next door. Ox 3167 was infected with *A. marginale* and the susceptible Ox 9416 was brought into the stable when Ox 3167 showed a parasitaemia of approximately 1%. They shared the stable for 8 days until Ox 3167 died as a result of the *A. marginale* infection. It was showing a parasitaemia of 90% the day before it died.

RESULTS

The only blood-sucking insects of possible significance to be attracted to the pen and to cross the fly-screen barrier included *Culex* spp. and *Culicoides* spp. A few *S. calcitrans* and *Musca domestica* also entered the stables when the doors were opened, but appear to have been eliminated very rapidly by the electrocuting ultraviolet light traps.

S. calcitrans

Only 1 out of 3 animals, namely, Ox 2276 (Table 1), contracted an *A. marginale* infection after *S. calcitrans* were allowed to feed repeatedly on it, just after they had been fed on an infected animal. The infection had a prepatent period of 27 days, counting the 1st day of exposure to feeding flies as Day 1. This animal was euthanized on Day 37 when it showed a parasitaemia of 90%, while the control (Ox 9416), stabled with an infected animal in a similar stable, remained non-infected for the duration of the 3-month observation period.

H. rufipes

Hardly any *H. rufipes* were caught in electrocuting ultraviolet light traps during this investigation. Only when the flies were disturbed during their release onto or transference between hosts did a few apparently accidentally land in the traps. Once they had settled on their hosts these flies were apparently not attracted at all by the ultraviolet light.

H. rufipes was never seen flying around or resting on any surface other than the host during the day, and it remained out of reach of any grooming actions by the host. Stables were hosed out daily and all deposited immatures were thus removed. The number of flies gradually diminished, but some flies were still alive and well on the host 6 weeks after their initial release.

TABLE 1 Summary of 3 attempts to transmit *A. marginale* mechanically with *S. calcitrans*

Exposure to infection								Attempted transmission							
Animal No.	*A. marginale* parasitaemia at time of exposure (%)	Average number of flies per batch	Number and duration of feeding periods (minutes)					Animal No.	Number and duration of feeding periods (minutes)					Prepatent period (days)
			Day 1	Day 2	Day 3	Day 4	Day 5		Day 1	Day 2	Day 3	Day 4	Day 5	
2542	49–90	±60	2×5	4×8	4×10*	8×8	10×5*	1660	2×5	4×8	4×10	8×8	10×5	.
1820	54	±50	4×10	.	.	.	.	2589	8×8	.	.	.	.	.
794	75–91	±70	10×3	8×3*	8×3*	.	.	2276	8×3	8×3	10×3	.	.	27

* New batch of flies

All 3 attempts to transmit *A. marginale* mechanically with *H. rufipes* failed.

It was finally demonstrated after the 3-month observation period of this investigation had elapsed, that all 6 of the oxen, namely, No. 2378, 2519, 1969, 1660, 2589 and 9416, that remained non-infected during this investigation, were susceptible to the BW-strain of *A. marginale*.

Discussion

There are conflicting opinions on the importance of mechanical transmission of anaplasmosis. *S. calcitrans* has been known to transmit *A. marginale* mechanically under laboratory conditions since 1927 (Dikmans, 1950). Interspecies transmission of *A. marginale* was reported when a large population of *S. calcitrans* was allowed to feed at will upon a susceptible white-tailed deer and an acutely infected calf (Lancaster, Roberts, Lewis, Dinkins & De Varey, 1968). In several reviews of anaplasmosis in the USA, however, horse flies (*Tabanus* spp.) are regarded as the most significant vectors of the disease (Piercy, 1956; Ristic, 1968; Roberts, Pund, McCrory, Scales & Collins, 1968; Thompson, 1977). In Zimbabwe, Lawrence (1977) found that 4 splenectomized calves contracted *Anaplasma centrale* infections when they were reared in contact with infected cows under tick-free conditions. The cattle were exposed to *Tabanus* spp. and *Stomoxys* spp. by day and to mosquitoes and *Culicoides* spp. by night. The results of a serological survey of this laboratory herd suggested that mechanical transmission of anaplasmosis after its introduction by infected ticks may be a significant factor in the epizootiology of this desease.

Other observations in the USA indicate that *S. calcitrans* plays no role in the natural transmission of anaplasmosis (Roberts *et al.*, 1968; Peterson, Raleigh, Stroud & Goulding, 1977). Epizootiological evidence from Australia suggests that insect transmission does not occur (Leatch, 1973) and it appears to be generally accepted that *B. microplus* is the main if not the only vector (Rogers, Blight & Knott, 1978).

The few mosquitoes (*Culex* spp.) that were trapped in the stables during the course of this study are regarded as unimportant, since they are considered insignificant vectors, especially if they are present in small numbers (Ristic, 1968). This is supported by Bram & Roby (1970), who failed to transmit anaplasmosis with *Anopheles quadrimaculatus*, and by Roberts *et al.* (1968), who concluded after a 3-year field study that Culicidae did not appear to be involved in the transmission of anaplasmosis.

The animals used in this study were acutely infected, splenectomized oxen, and the transfer feeds were executed under what were believed to be optimum conditions for transmission. It seems, however, that the vector potential of *S. calcitrans* under these apparently ideal conditions was not very high, as only 1 out of 3 animals became infected. It is possible that the small number of flies used in these experiments could have played a role in this result. This aspect should be borne in mind before definite conclusions are drawn as to the relative importance of *S. calcitrans* during outbreaks of anaplasmosis.

The practical implications of these findings are that, when cattle are farmed under intensive conditions, the stable fly must be regarded as a potential mechanical vector of *A. marginale*. In conditions where cattle are kept in close contact, for example, in feedlots and in most dairy herds, it is conceivable that *S. calcitrans* could spread the disease from clinical reactors to susceptible cattle during an outbreak. Field observations in South West Africa (Namibia) suggest that *H. rufipes* might play a role in the transmission of anaplasmosis (Biggs, personal communication, 1979), but this hypothesis could not be proved in this investigation.

Acknowledgements

We wish to thank Mr H. Langenhoven from the Central Veterinary Laboratory, Windhoek, for his participation in collecting *Hippobosca rufipes*, Mr O. Matthee for his assistance during the absence of Dr B. Sutherland and Mr E. Nevill for the identification of the light trap catches.

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Printed by and obtainable from the Government Printer, Private Bag X85, Pretoria, 0001