RESEARCH COMMUNICATION

Rhipicephalus zambeziensis unlikely to transmit foot-and-mouth disease virus

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ABSTRACT

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The potential of the ixodid tick, *Rhipicephalus zambeziensis*, was investigated as a vector in the transstadial transmission of the foot-and-mouth disease virus by feeding nymphae on viraemic (log 1,0–4,0 $TCID_{50}/m\ell$) cattle. Suspensions were prepared, at various intervals after detachment, from pools of engorged nymphae—some of which were allowed to moult first. Suspensions were inoculated into sucking mice, cell cultures and, in some cases, cattle to detect the FMD virus. Newly moulted adult ticks, derived from nymphae which had fed on viraemic cattle, were also allowed to feed on susceptible cattle. The pattern of virus detection indicated that the FMD virus was capable of surviving at least 3 d in engorged nymphae, but less than 7 d following repletion. It was concluded that *R. zambeziensis* is unlikely to transmit the FMD virus.

A rich variety and abundance of arthropods occur in the Kruger National Park (KNP) in South Africa where foot-and-mouth disease (FMD) is enzootic, but there is little evidence to suggest that arthropods are important in the epidemiology of the disease (Bachrach 1968; Greenberg 1973; Thomson, Doube, Braack, Gainaru & Bengis 1988). Nevertheless, the FMD virus is able to persist up to 48 h in infected arthropods and there are reports of transmission of the virus, amongst others, by *Rhipicephalus* and *Hyalomma* spp., so the possibility of tick-borne transmission of the FMD virus remains (Hyslop 1970).

African buffalo, *Syncerus caffer*, the only known free-living maintenance hosts for the SAT types of FMD virus (Hedger, 1976; Thomson & Bengis 1990, unpublished observations), have been shown to be important hosts of ixodid ticks and at least 5 sp. of *Rhipicephalus* have been identified on buffalo (Horak, Potgieter, Walker, De Vos & Boomker 1983). Experimental infection of buffalo with SAT-1 or SAT-2 virus resulted in the development of viraemias which persisted up to 4 d and reached levels of $10^{5,2}$ $MLD_{50}/m\ell$. These levels and duration of viraemia are very similar to those obtained in cattle inoculated with the same virus strains (Gainaru, Thomson, Bengis, Esterhuysen, Bruce & Pini 1986).

In order to investigate the possible role of ixodid ticks in the epidemiology of FMD in the KNP and to develop a strategy for conducting biologically safe research on *Theileria parva lawrencei* (the causative agent of Corridor disease, obtained from *R. zambeziensis* collected in the KNP) an experiment was devised to establish the duration that the FMD virus, ingested by these ticks, survives in ticks and whether transstadial transmission is possible.

Ticks infected with FMD virus were obtained as follows. Three healthy steers, housed in a high

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TABLE 1 Viraemias in cattle on which *R. zambeziensis* nymphae were fed

Animal No.	Detection system	Days after virus inoculation					
		1	2	3	4	5	6
3	M	[1]	—	—	—	—	—
	CC	—	—	—	—	—	—
4	M	2,8[2]	5,0	—	—	—	—
	CC	3,5[3]	4,0	—	—	—	—
6	M	2,8	2,1	—	—	—	—
	CC	3,2	1,0	—	—	—	—

M — sucking mice
CC — cell culture (IB-RS-2)
[1] — no virus detected
[2] — $\log_{10}$ MLD/mℓ
[3] — $\log_{10}$ $TCID_{50}$/mℓ

TABLE 2 Virus recovery from *R. zambeziensis* nymphae and adults fed on viraemic cattle

Detection system	Number of days after tick infestation									
	6		7		11		13		36	
	TPT	NT	TPT	NT	TPT	NT	TPT	NT	TPT	NT
Sucking mice	2,5	400	1,7	50	—	50	—	50	NA	ND
IB-RS-2 cells	1,2	400	1,0	50	—	50	—	50	NA	ND
Cattle	+	400	NA	ND	NA	ND	NA	ND	—	300

TPT — titre ($\log MLD_{50}$ or $TCID_{50}$) per tick
NT — number of ticks tested
— — no virus detected
NA — not applicable
ND — not done
+ — virus recovered but not titrated

security stable, were bled and screened for antibodies to SAT-1, -2 and -3 viruses. Thereafter approximately 600 *R. zambeziensis* nymphae were allowed to feed on the ears of the steers, according to the method described by Neitz, Boughton & Walters (1971). After allowing a 2 d period for the nymphae to attach, the steers were inoculated intradermalingually with the BOT 1/77 strain of FMD virus (SAT-1). An inoculum of 0,2 mℓ containing $10^{5,8}$ cattle infective doses was injected in 2 sites on the tongue of each steer. The steers were bled daily for 6 d after virus inoculation to monitor levels of viraemia which are shown in Table 1.

The engorged nymphae of *R. zambeziensis* detached irregularly and were collected daily for 4 d, starting the day after inoculation of the steers with the virus (Fig. 1). When all the engorged nymphae had been collected they were pooled and divided randomly into batches of 50 ticks, each of which was placed in a glass vial and maintained in an acaridium at 26 °C and 75 % relative humidity.

The level of infectivity in the pooled nymphae after they had all detached was established by grinding up 400 nymphae with a pestle and mortar in phosphate buffered saline, pH 7,4, to produce a 10% suspension. This suspension was filtered and titrated in sucking mice and IB-RS-2 cell cultures. Titres of $10^{2,5}$ MLD_{50} and $10^{1,2}$ $TCID_{50}$ per tick were obtained (Table 2, Day 6).

Two cattle were inoculated intradermalingually in 2 sites with 0,2 mℓ of the tick suspension prepared on Day 6 and a further 10 mℓ of the suspension administered intramuscularly in each animal in divided doses. Typical FMD lesions were observed in both animals 48 h later and the SAT-1 virus was recovered from lesion material collected from both cattle.

A suspension was prepared from 50 engorged nymphae 7 d after infestation and titrated in sucking mice and IB-RS-2 cells. Virus titres of $10^{1,7}$ MLD_{50} and $10^{1,0}$ $TCID_{50}$ were obtained. This procedure was repeated on Days 11 and 13 after infestation, but no virus could be detected in either group of ticks (Table 2).

A pool of engorged nymphae was maintained in the acaridium for a period of 30 d, during which time they moulted. Two hundred of these newly moulted adults ticks were used to prepare at 10 % suspension as previously described. Two cattle were inoculated intradermalingually in 2 sites with 0,2 mℓ of this suspension with an additional 10 mℓ injected subcutaneously into each animal. Daily clinical examination of the cattle over a 2-week period showed no evidence of FMD infection, nor could a serological response to SAT-1 virus be detected 3 weeks later.

Finally, newly moulted adult ticks were assessed for their ability to transmit FMD to cattle 30 d after detachment by placing 100 newly moulted individuals into each of 2 ear bags which were attached to the ears of 2 cattle. Most of these adult ticks had fed

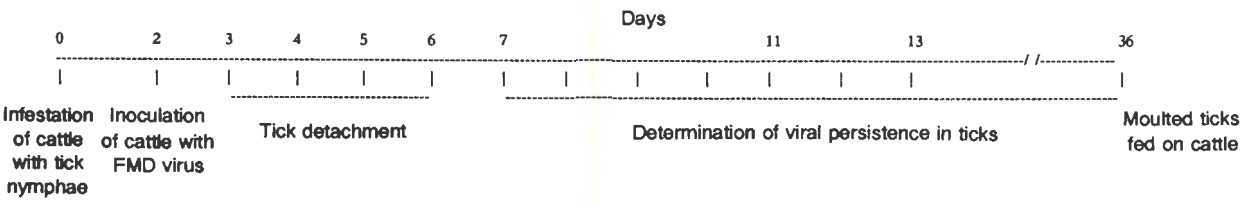

FIG. 1 Flow diagram depicting the sequence of events in this investigation

when they were examined 1 week later. Daily clinical examination for 14 d revealed no evidence of FMD infection and no serological response was detected by virus neutralization tests conducted 3 weeks after attaching the ear bags to the cattle.

Results from this experiment indicated that FMD virus survived for at least 3 d, but less than 7 d in *R. zambeziensis* nymphae. Although the epidemiology of FMD in southern Africa has not yet been completely elucidated, we conclude that it is unlikely that *R. zambeziensis* is able to transmit FMD virus.

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