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Non-viraemic transmission of Thogoto virus: vector efficiency of *Rhipicephalus appendiculatus* and *Amblyomma variegatum*

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Abstract

Previous studies have demonstrated that Thogoto virus is transmitted from infected to uninfected ticks when co-feeding on uninfected guinea-pigs, even though the guinea-pigs do not develop a detectable viraemia. Furthermore, tick to tick transmission is potentiated by factors associated with the salivary glands of ticks (saliva activated transmission). The vector efficiency of 2 ixodid tick species, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, for Thogoto virus was assessed using this model. The number of uninfected recipient ticks that acquired Thogoto virus when co-feeding with virus-infected ticks (donors) on uninfected guinea-pigs was determined. When nymphs of either tick species were employed as donors, there was no significant difference in the number of infected recipient nymphs. In contrast, a significant difference in the vector efficiency of adults ticks was observed: 77·0% of recipient ticks which co-fed with *R. appendiculatus* donor adults acquired Thogoto virus compared to 44·7% of recipient ticks which co-fed with *A. variegatum* donors. No significant difference in susceptibility to Thogoto virus infection was observed between recipient ticks of the 2 species. Thus, adults of *R. appendiculatus* are more efficient than *A. variegatum* in mediating non-viraemic transmission.

Introduction

Vertebrates are considered important in the epidemiology of an arthropod-borne virus disease if they develop a viraemia that satisfies the threshold level considered necessary to infect the arthropod vector (HARDY *et al.*, 1983). In the laboratory we have demonstrated that transmission of Thogoto (THO) virus can occur without the requirement of a viraemic host (JONES *et al.*, 1987). Studies on the mechanism of this novel mode of arbovirus transmission indicate that a factor or factors associated with the salivary glands of ticks is involved, hence the term 'saliva activated transmission' (SAT) (JONES *et al.*, 1989).

THO virus is an orthomyxo-like virus (CLERX *et al.*, 1983; STAUNTON *et al.*, 1989) of medical and veterinary significance (DAVIES *et al.*, 1986). The virus has been isolated from 9 ixodid tick species from Central and southern Africa, the Middle East and southern Europe (reviewed by DAVIES *et al.*, 1986). Of these species, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* are proven biological vectors of THO virus (JONES *et al.*, 1989) and have similar infection thresholds for the virus when feeding on a viraemic host (DAVIES *et al.*, 1990). Both of these species overlap in their geographical distribution and their host ranges, and evidence of THO virus infection has been observed in a range of hosts on which both tick species feed (DAVIES *et al.*, 1986).

In this study we compare the vector efficiency of *R. appendiculatus* and *A. variegatum* for THO virus using a host that does not develop detectable viraemia. Uninfected ticks of both species (recipients) were allowed to co-feed on uninfected guinea-pigs with either *R. appendiculatus* or *A. variegatum* ticks infected with THO virus (donors). The vector efficiency of these species was assessed by determining the numbers of uninfected recipient ticks that became infected.

Materials and Methods

Cells and virus

BHK-21 and Vero cell cultures were propagated in modified Eagle's medium (EMEM) supplemented with 10% newborn bovine serum (NBS). The Sicilian (SiAr 126) isolate of THO virus was originally obtained from Dr R. E. Shope (Yale Arbovirus Research Unit, New Haven, Connecticut, USA) as an infected suckling mouse brain extract. The virus was plaque-cloned in Vero cells and virus stocks derived by passage in BHK-21 cells as previously described (DAVIES *et al.*, 1986).

Ticks

Laboratory colonies of *R. appendiculatus* and *A. variegatum* were established by feeding all three stages of *R. appendiculatus* and the larval and nymphal stages of *A. variegatum* on Dunkin Hartley guinea-pigs; *A. variegatum* adults were fed on New Zealand White rabbits (JONES *et al.*, 1988). During the interval between feeding the ticks were maintained in perforated tubes inside a dessicator at a temperature of 26°C and a relative humidity of 85%.

Infection of ticks by feeding on viraemic hamsters

Syrian hamsters were inoculated subcutaneously with 5000 plaque-forming units (pfu) of THO virus. Peak viraemias were obtained 3-4 d after inoculation (DAVIES *et al.*, 1986). In considering the duration of feeding by *R. appendiculatus* and *A. variegatum* (JONES *et al.*, 1988), and the time at which peak viraemia was reached, larvae were placed on hamsters 1 d, and nymphs 2–3 d, before inoculation of the hamsters.

Infection of ticks by co-feeding on guinea-pigs

Eight virus-infected adult *R. appendiculatus* and *A. variegatum* (donors; equal sex ratio) were placed in one retaining chamber and allowed to co-feed with 40

uninfected *R. appendiculatus* and 40 uninfected *A. variegatum* nymphs (recipients; placed in separate retaining chambers) on uninfected guinea-pigs. In a second experiment the same protocol was employed except that 40 infected nymphs of either tick species were used as donors. Throughout the tick feeding period, blood samples were taken daily and the retaining chambers checked to ensure that there was no contact between ticks from adjacent cells.

Virus assay

Nymph and adult ticks were homogenized individually in 1 ml of EMEM containing 10% NBS and appropriate antibiotics to inhibit bacterial growth. Recipient nymphs were assayed for virus 12 d after engorgement (the time of maximum virus replication; DAVIES *et al.*, 1986). Donor ticks were assayed for virus at the completion of nymphal engorgement. Throughout the tick feeding period, daily blood samples were obtained from anaesthetized guinea-pigs. Titration of blood and tick derived material was performed in Vero cells incubated at 35°C for 4 d before fixation and staining (DAVIES *et al.*, 1986).

Table 1. Oral infection of *Rhipicephalus appendiculatus* and *Amblyomma variegatum* nymphs feeding with Thogoto virus-infected adults

Guinea-pig number	Chamber	Tick species[a]	Unfed tick numbers[a]	Virus[c]	Ticks after feeding No. infected/ no. tested	Percentage infected
GP1	1	A	4M	+	3/4	75
		A	4F	+	4/4	100
	2	A	40N	−	14/30	47
	3	R	40N	−	18/35	51
GP2	1	A	4M	+	1/2	50
		A	4F	+	4/4	100
	2	A	40N	−	21/38	55
	3	R	40N	−	17/35	49
GP3	1	A	4M	+	4/4	100
		A	4F	+	2/3	67
	2	A	40N	−	9/29	31
	3	R	40N	−	11/38	29
GP4	1	A	4M	+	4/4	100
		A	4F	+	4/4	100
	2	A	40N	−	12/34	35
	3	R	40N	−	8/29	28
GP5	1	R	4M	+	2/3	67
		R	4F	+	4/4	100
	2	A	40N	−	30/39	77
	3	R	40N	−	29/40	73
GP6	1	R	4M	+	1/3	33
		R	4F	+	4/4	100
	2	A	40N	−	25/33	76
	3	R	40N	−	26/30	87
GP7	1	R	4M	+	4/4	100
		R	4F	+	4/4	100
	2	A	40N	−	21/35	60
	3	R	40N	−	18/36	50
GP8	1	R	4M	+	4/4	100
		R	4F	+	2/3	67
	2	A	40N	−	30/39	77
	3	R	40N	−	30/34	88

[a]A=*Amblyomma variegatum*; R=*Rhipicephalus appendiculatus*.
[b]Numbers of adult males (M), females (F) and nymphs (N).
[c]+, indicates ticks were infected at the nymphal stage by feeding on viraemic hamsters inoculated with the Sicilian SiAr126 THO isolate, and then reared to the adult stage; −, indicates uninfected nymphs.

Statistical analysis

The results were analysed using a GLIM program (BAKER & NEDLER, 1978) which fits a series of generalized linear statistical models to the data. Data (which were binomially distributed) were not transformed as the GLIM program adjusts each model to fit the given data. Binomial errors were assessed with the *y*-variate being the number of ticks which became infected per replicate animal. Initially a null model was fitted to give total model deviance (total variation in data). The reduction in model deviance due to each parameter was then determined. The significance of each reduction in deviance was assessed by construction of the appropriate *F* table using values generated by the GLIM model. The GLIM program expressed the results of binomially distributed data as a logit function of a linear model (x) which could be transformed back to the natural state using the equation $r=1/(1+e^{-x})$. Mean transmission rates and their non-symmetrical standard errors were thus calculated.

Results

Number of recipient nymphs that became infected when co-feeding with infected adult ticks

Eight guinea-pigs (GP1–8) were infested with uninfected *R. appendiculatus* and *A. variegatum* nymphs (recipients in chambers 2 and 3). Guinea-pigs GP1–4 were also infested with THO virus-infected *R. appendiculatus* adults and GP 5–8 with THO virus-infected *A. variegatum* adults (donors in chamber 1; Table 1). A significant difference in the vector efficiency of donor *R. appendiculatus* and *A. variegatum* for THO virus was observed ($F_{1,12}=19{\cdot}56$, $P<0{\cdot}005$). Following engorgement, 77% (±standard

Table 2. Oral infection of *Rhipicephalus appendiculatus* and *Amblyomma variegatum* nymphs feeding with Thogoto virus-infected nymphs

Guinea-pig number	Chamber	Tick species[a]	Virus[b]	Ticks after feeding No. infected/ No. tested	Percentage infected
GP9	1	A	+	30/36	83
	2	A	−	25/30	83
	3	R	−	22/29	76
GP10	1	A	+	24/29	83
	2	A	−	25/33	76
	3	R	−	30/37	81
GP11	1	A	+	20/31	65
	2	A	−	17/19	89
	3	R	−	21/25	84
GP12	1	R	+	19/35	54
	2	A	−	35/39	90
	3	R	−	36/38	95
GP13	1	R	+	27/31	87
	2	A	−	21/28	71
	3	R	−	24/29	83
GP14	1	R	+	28/29	97
	2	A	−	25/35	71
	3	R	−	30/38	79

[a]=*Ambylomma variegatum*; R=*Rhipicephalus appendiculatus*.
[b]+, indicates ticks were infected at the larval stage by feeding on viraemic hamsters inoculated with the Sicilian SiAr126 THO isolate, and then reared to the nymphal stage; −, indicates uninfected nymphs.

error [SE], 80·0–73·6) of all recipient nymphs which co-fed with *R. appendiculatus* donor adult ticks on guinea-pigs (GP5–8) acquired THO virus. In contrast, only 44·8% (±SE, 56·2–33·8) of recipient nymphs which co-fed with *A. variegatum* donor ticks (GP1–4) became infected. No significant difference in susceptibility to THO virus infection was observed between recipient tick species (GP1–8; $F_{1,12}=2·58$).

Numbers of recipient ticks which became infected when co-feeding with infected nymphs

The experiment described above was repeated using *R. appendiculatus* and *A. variegatum* nymphs as donor ticks (Table 2). The numbers of infected recipient nymphs were the same irrespective of donor tick species (GP9–14; $F_{1,8}=0·01$). Following engorgement, 79·1% (±SE, 83·7–73·7) of recipient ticks which co-fed with *R. appendiculatus* donor ticks acquired virus (GP12–14), compared to 80·9% (±SE, 84·1–77·3) which co-fed with *A. variegatum* donor ticks (GP9–11). No significant difference in susceptibility to THO virus infection was observed between recipient tick species (GP9–14, $F_{1,8}=0·08$).

Virus was not detected in the blood of any of the test animals (GP1–14) during the tick feeding period (<20 pfu/ml blood).

Discussion

In considering the importance of vectors in the epidemiology of arboviruses, the species with the lowest infection threshold for that virus is assumed to be its most important vector. Previous studies with THO virus have demonstrated that the infection thresholds for *R. appendiculatus* and *A. variegatum* are similar. However, in terms of number of plaque forming units of virus ingested, *R. appendiculatus* is more susceptible to infection than *A. variegatum* (DAVIES *et al.*, 1990).

As SAT does not rely on detectable viraemia the relative importance of different vector species cannot be determined on the basis of infection thresholds. Thus, to assess the vector efficiency of *R. appendiculatus* and *A. variegatum* for THO virus, we compared the number of recipient ticks of both species which acquired virus whilst co-feeding with donor ticks on non-viraemic guinea-pigs. No significant difference in vector efficiency was observed when nymphs of either species were employed as donors. However, when virus-infected adults were allowed to co-feed with uninfected nymphs, the infection prevalence in recipient nymphs which co-fed with adult *A. variegatum* ticks was significantly reduced. These results indicate that *R. appendiculatus* is a more efficient donor of THO virus for SAT than *A. variegatum*. No significant difference in susceptibility to THO virus infection was observed between recipient nymphs of either species irrespective of donor tick species.

Studies with *R. appendiculatus* have demonstrated that the most important determinant of infection prevalence in recipient ticks is the duration of co-feeding (JONES & NUTTALL, 1989). Furthermore, in terms of potentiation by salivary glands, maximum enhancement of virus transmission was achieved when salivary glands were derived from ticks which had fed for a period of 6 d. Adult *A. variegatum* have a prolonged feeding period in comparison to *R. appendiculatus* (12–14 d and 8–12 d respectively), whereas the nymphal stages of both species feed for similar periods of time (6–7 d; JONES *et al.*, 1988). Thus, the natural feeding behaviour of ticks may be an important factor in determining their efficiency as SAT vectors. The significance of SAT in nature and the relative importance of different tick species in the epidemiology of other arthropod-borne viruses need to be explored.

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