Medical and Veterinary Entomology (1992) **6**, 261–265

Saliva activated transmission (SAT) of Thogoto virus: relationship with vector potential of different haematophagous arthropods

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Abstract. Tick saliva (or salivary gland extract) potentiates the transmission of Thogoto (THO) virus to uninfected ticks feeding on a non-viraemic guinea-pig. This phenomenom has been named saliva activated transmission (SAT). To investigate the potential of different haematophagous arthropods to mediate SAT, guinea-pigs were infested with uninfected *R.appendiculatus* Neumann nymphs and inoculated with THO virus and salivary gland extract (SGE) derived from a range of ixodid (metastriate and prostriate) or argasid ticks, or mosquitoes; control guinea-pigs were inoculated with virus alone. Enhancement of THO virus transmission was observed only when SGE was derived from metastriate ticks. Comparison with the vector potential of these various arthropod species revealed that enhancement of THO virus transmission was specific for ticks which were competent vectors of the virus. The data indicate a correlation between vector competence and the ability of haematophagous arthropods to mediate SAT of THO virus.

Key words. Thogoto virus, saliva activated transmission, vector competence, haematophagous arthropods, *Aedes aegypti*, Argasidae, Ixodidae.

Introduction

Arthropod vectors of arboviruses become infected when they feed on the blood of a viraemic host (W.H.O., 1985). In addition, virus transmission from infected to uninfected ticks co-feeding on an uninfected vertebrate has been observed, even though the vertebrate host does not develop a detectable viraemia (Jones *et al.*, 1987). Investigations on the mechanism of non-viraemic transmission indicate that a factor(s) associated with the salivary glands of ticks and secreted in tick saliva potentiates this novel mode of arbovirus transmission, hence the term 'saliva activated transmission' (SAT) (Jones *et al.*, 1989b, 1992a).

Experiments on SAT were conducted with Thogoto (THO) virus, an arbovirus recently classified in the family Orthomyxoviridae (Davies *et al.*, 1986; Francki *et al.*, 1991). The virus was originally isolated from a mixed pool of *Boophilus* and *Rhipicephalus* ticks removed from sentinel cattle in Thogoto forest near Nairobi, Kenya (Haig *et al.*, 1965), and has been isolated subsequently from various ixodid ticks: in sub-Saharan Africa from *Amblyomma variegatum* Fabricius, *B.decoloratus* Koch, *B.annulatus* Say, *Hyalomma truncatum* Koch, *R.evertsi* Neumann and *R.appendiculatus* Neumann; in Egypt from *H.anatolicum anoticulum* Koch; in Sicily from *R.bursa* Canestrini & Fanzago; in Portugal from *R.sanguineus* Latreille; and in Iran from *H.a.anatolicum* (reviewed by Davies *et al.*, 1986). The frequent isolation of THO virus from these tick species indicates that they are probably competent natural vectors of the virus. Experimentally, *R.appendiculatus* and *Am.variegatum* are proven biological vectors (Davies *et al.*, 1986; Jones *et al.*, 1989a) with similar infection thresholds when feeding on a viraemic host (Davies *et al.*, 1990).

The natural transmission cycle of THO virus involves various species of small mammals, with the adult ticks transmitting the virus to larger mammals, including domestic animals. The virus has been isolated from cattle and

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camels (Kemp *et al.*, 1973), sheep (Davies *et al.*, 1984) and man (Moore *et al.*, 1975), and neutralizing antibodies have been demonstrated in the sera of cattle, sheep, donkeys, buffaloes, camels, rats and man (Davies *et al.*, 1986). In sheep, the virus causes pyrexia (Haig *et al.*, 1965) and abortions (Davies *et al.*, 1984), and in the two reported human cases, THO virus infection was associated with optic neuritis and a fatal meningitis (Moore *et al.*, 1975).

The significance of SAT in nature and the relative importance of different tick species in the epizootiology of THO virus are unknown. To date, potentiation of SAT of THO virus has been demonstrated only with salivary glands derived from partially fed female *R.appendiculatus*, *Am. variegatum* and *B.microplus* ticks (Jones *et al.*, 1992b). In this paper we assess the potential of salivary glands derived from a range of haematophagous arthropods to mediate SAT and compare this with their vector potential for THO virus.

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 (obtained as an infected suckling mouse brain extract) was plaque cloned in Vero cells and virus stocks derived by passage in BHK-21 cells (Davies *et al.*, 1986).

Ticks. Laboratory colonies of the three-host ixodid tick species, *R.appendiculatus*, *Am.hebraeum* Koch and *Am. cajennense* Fabricius, were established by feeding all three stages of *R.appendiculatus* and the larval and nymphal stages of the *Amblyomma* tick species on Dunkin Hartley guinea-pigs. Adult *Amblyomma* ticks were fed on New Zealand White (NZW) rabbits (Jones *et al.*, 1988). Similar feeding protocols were undertaken for the maintenance of *H.dromedarii* Koch, *H.m.rufipes* Koch, *R.evertsi*, *Ixodes ricinus* Linnaeus and *I.hexagonus* Leach. However, the larval and nymphal stages of these species were fed on hamsters and the adults on NZW rabbits. During the interval between feeding, ticks of each species were maintained in perforated tubes, held inside a desiccator, at a temperature of 21–26°C and at 85% relative humidity (r.h.).

Colonies of the soft tick species *Ornithodoros maritimus* Vermeil & Marguet and *Argas monolakensis* Schwan, Corwin & Brown were maintained as previously described. Briefly, the larval stage was fed on a hamster and the nymphal and adult stages on a membrane feeding system which consisted of a glass reservoir of 2–3 ml volume around which a circulatory system of warm water (28°C) was constructed. A membrane prepared from a 2-day-old chick skin was cut to size and stretched over the lip of the reservoir and secured with a rubber band. Defribinated goose blood (4 ml) was introduced into the reservoir using a 5 ml syringe. Ticks were then placed on the membrane and a retaining gauze cap clamped over the reservoir to contain the ticks (Jones *et al.*, 1988).

Mosquitoes. Aedes aegypti (L.) was maintained in our insectary by standard procedures. *Ae.aegypti* females were fed on an adult Pathology Oxford (PO) strain mouse (12 weeks of age) using standard procedures. The mouse was anaesthetized with pentabarbitone sodium (at the recommended dose) and placed on top of a gauze-covered jar containing the unfed mosquitoes. Both unfed and fed mosquitoes were maintained in gauze covered jars at a temperature of 21°C and a r.h. of 55–65%. Healthy adult *Anopheles stephensi* Giles mosquitoes were kindly supplied by Dr C. R. Davies, London School of Hygiene and Tropical Medicine.

Virus assay. Ticks and mosquitoes were homogenized individually in 1 ml and 0.2 ml respectively, of EMEM containing 10% NBS and appropriate antibiotics to inhibit bacterial growth. Blood samples were obtained on selected days post attachment of ticks or mosquitoes, by cardiac puncture from anaesthetized animals. Titration of material derived from blood or ticks or mosquitoes, and plaque neutralization assays, were undertaken as described by Davies *et al.* (1986).

Per os infection of ixodid and argasid tick species and the mosquito species Ae.aegypti. Hamsters inoculated with THO virus developed a high titre viraemia of up to 8.2 $\log_{10}$ PFU/ml blood, 3 days post-inoculation of THO virus (Davies *et al.*, 1986). For *per os* infection, the ticks and mosquitoes were fed on viraemic hamsters. The timing of tick and mosquito application, with respect to virus inoculation of the host, was determined by the tick and mosquito feeding behaviour. The nymphal stages of *Am. hebraeum* and *Am.cajennense* were allowed to attach 3–4 days prior to inoculation of the hamsters with 5000 PFU THO virus, *R.evertsi*, *H.m.rufipes*, *H.dromedarii*, *I.ricinus* and *I.hexagonus* 1 day prior to inoculation, and *O.maritimus* and adult *Ae.aegypti* at the time of peak viraemia, i.e. 3 days post inoculation with THO virus.

Assay of SAT factor activity. Salivary glands were dissected out from uninfected adult female *Am.cajennense*, *Am.hebraeum*, *H.dromedarii*, *H.m.rufipes* and *R.evertsi* ticks which had fed for a period of 6 days, *I.ricinus* and *I.hexagonus* female ticks which had fed for a period of 5 days, and from partially replete females of *O.maritimus* and *Ar.monolakensis*, bloodfed *Ae.aegypti* and unfed *An.stephensi*. The dissected salivary glands were placed in phosphate buffered saline (PBS) pH 7.2, extracted by homogenization and low-speed centrifugation, and frozen at −20°C.

Previous studies have demonstrated that there is no significant difference in the vector efficiency of nymphs as recipients of SAT, at least in the case of *Am.variegatum* and *R.appendiculatus* (Jones *et al.*, 1990). Thus, experiments were standardized by infesting guinea-pigs (two per salivary gland sample) with approximately fifty uninfected *R.appendiculatus* nymphs. Each guinea-pig was inoculated subcutaneously with 5000 PFU THO virus mixed with salivary gland extract SGE (40 μg protein per animal) derived from partially fed ticks or mosquitoes; control guinea-pigs were inoculated with virus alone. For assay of SAT factor activity, recipient nymphs were titrated

for virus 12 days post-engorgement (the time of maximum virus titre; Davies *et al.*, 1986). Virus transmission was measured by the number of uninfected ticks that acquired virus.

Statistical analysis. Virus titres in ticks which had fed on viraemic hamsters were analysed using Student's *t*-test (Table 1). No significant difference was observed in virus titres in ticks which fed on duplicate hamsters. Thus, all titres were pooled for each tick species. Further statistical analyses were undertaken to determine if a relationship existed between virus titres in ticks and viraemic titres in the hamster (Table 2). Data were analysed by fitting a series of generalized linear models, using a GLIM program (Baker & Nedler, 1978).

Results

Uptake of THO virus in a bloodmeal

To determine the vector potential of different arthropod species for THO virus, approximately thirty nymphs of the ticks *Am.hebraeum*, *Am.cajennense*, *R.evertsi*, *H.m.rufipes*, *H.dromedarii*, *I.hexagonous*, *I.ricinus* and *O.maritimus*, and adult female *Ae.aegypti* mosquitoes, were fed on viraemic hamsters. Vector competence of *Ar.monolakensis* and *An.stephensi* was not assessed due to the limited numbers of ticks and mosquitoes which were available for testing. Blood titres of 6.7–8.3 $\log_{10}$ PFU/ml blood were recorded on day 3 post-inoculation of hamsters. Virus was detected in a high proportion of nymphs, when assayed for virus immediately after feeding (Table 1). Statistical analysis of virus titres in ticks following engorgement revealed no significant differences between species except for *I.ricinus* nymphs which had lower titres than the majority of the other ixodid tick species; *O.maritimus* titres were not tested due to the limited number of ticks.

Replication and transmission of THO virus

Transtadial persistence and virus transmission for the above tick species was assessed. Virus transmission was examined by placing groups of ten adults (with an equal sex ratio) on sixteen hamsters, 42 days following *per os* infection at the nymphal stage (Table 2). Viraemia was detected in all of the hamsters on which *R.evertsi*, *Am. cajennense*, *Am.hebraeum*, *H.m.rufipes* and *H.dromedarii* adult ticks fed (H 16–25; viraemic hamsters were found dead or sick and killed on days 4–8 following tick attachment). Maximum blood titres ranged from 6.5 to 8.3 $\log_{10}$ PFU/ml blood, and were highest on the day before death; no significant relationship was observed between virus titres in ticks and viraemia titres in the hamsters.

Thogoto virus transmission was not observed when *I.ricinus*, *I.hexagonus* or *O.maritimus* adults were fed on hamsters (Table 2; H 26–30). Following engorgement none of the ticks contained virus, and there was no evidence of viraemia (<100 PFU/ml blood) or seroconversion in the hamsters.

The experiment was repeated using *Ae.aegypti* mosquitoes. Forty uninfected *Ae.aegypti* females were allowed to feed on a viraemic hamster (maximum titre 8.3 $\log_{10}$ PFU/ml blood). Owing to the limited number of mosquitoes which fed on the hamster (a total of fourteen), none were assayed for virus (following engorgement). Fourteen days post-feeding, mosquitoes were re-fed on an uninfected hamster (Table 2, H31). Following engorgement, none of the mosquitoes were found to contain THO virus (<10 PFU/mosquito) and there was no evidence of viraemia or seroconversion in the hamster.

These results indicate that, of the species tested, *R.evertsi*, *Am.hebraeum*, *Am.cajennense*, *H.m.rufipes* and *H.dromedarii* are competent vectors of THO virus.

Table 1. *Per os* infection of ixodid and argasid tick species with THO virus.

Hamster	Titre (PFU/ml blood)*	Tick species	No. infected/ no. tested	Mean titre $\log_{10}$ PFU/tick†
H1	7.2	*R.evertsi*	7/10	3.2 (2.1–4.5)
H2	6.9	*R.evertsi*	8/10	3.5 (2.9–4.7)
H3	8.0	*A.hebraeum*	9/10	3.4 (3.1–3.8)
H4	7.6	*A.hebraeum*	7/10	3.1 (2.5–4.2)
H5	6.7	*A.cajennense*	6/10	3.3 (2.2–3.9)
H6	7.6	*A.cajennense*	9/10	3.8 (3.1–4.9)
H7	8.0	*H.m.rufipes*	10/10	3.7 (3.2–4.3)
H8	7.4	*H.m.rufipes*	8/10	4.0 (3.3–5.1)
H9	7.9	*H.dromedarii*	7/10	3.6 (2.9–4.2)
H10	8.1	*H.dromedarii*	9/10	3.9 (3.1–4.9)
H11	7.7	*I.ricinus*	7/10	3.1 (2.6–3.5)
H12	8.1	*I.ricinus*	9/10	3.3 (2.9–3.6)
H13	6.9	*I.hexagonus*	6/10	3.8 (2.9–5.1)
H14	7.3	*I.hexagonus*	5/10	3.6 (3.0–4.2)
H15	8.0	*O.maritimus*	3/5	2.9 (2.1–3.4)

* Blood taken on day 3 post inoculation of hamsters on which the indicated uninfected tick species fed.

† Individual ticks were assayed for virus on day 0 post-engorgement. The means and range were calculated from those ticks found to contain virus.

Table 2. Transmission of THO virus by selected tick and mosquito species to hamsters. Titres less than 1.0 $\log_{10}$ PFU/ml blood are scored negative; those that were moribund are scored positive.

		Virus titre ($\log_{10}$ PFU/ml blood) at days:				
Hamster	Species	4	5	6	7	8
H16	*R.evertsi*	–	3.3	6.8	+	
H17	*R.evertsi*	2.9	5.7	8.0	+	
H18	*A.cajennense*	–	4.2	7.3	+	
H19	*A.cajennense*	–	–	3.6	5.9	+
H20	*A.hebraeum*	–	4.6	7.5	+	
H21	*A.hebraeum*	5.5	7.2	+		
H22	*H.m.rufipes*	4.9	7.3	+		
H23	*H.m.rufipes*	–	5.6	7.6		
H24	*H.dromedarii*	5.5	8.3	+		
H25	*H.dromedarii*	–	3.7	6.5	+	
H26	*I.ricinus*	–	–	–	–	–
H27	*I.ricinus*	–	–	–	–	–
H28	*I.hexagonus*	–	–	–	–	–
H29	*I.hexagonus*	–	–	–	–	–
H30	*O.maritimus*	–	–	–	–	–
H31	*Ae.aegypti*	–	–	–	–	–

Assay for SAT factor activity in salivary glands

A mixture of THO virus and SGE derived from either partially fed uninfected female *R.evertsi*, *Am.cajennense*, *Am.hebraeum*, *H.m.rufipes* or *H.dromedarii* was inoculated into uninfected guinea-pigs infested with uninfected *R.appendiculatus* nymphs (Table 3; GP 1–10); control guinea-pigs were inoculated with virus alone (GP 22–24). Enhancement of virus transmission was observed with all of the above tick species, i.e. a 7–10-fold increase in the number of recipient ticks which became infected compared with the control ticks which fed on guinea-pigs inoculated with virus alone. In contrast, SAT factor activity was not observed when the inoculum included SGE derived from *I.ricinus*, *I.hexagonus*, *O.maritimus* or *Ar.monolakensis* ticks, or the mosquito species, *Ae.aegypti* or *An.stephensi* (Table 3; GP 11–21). Virus was not detected in the blood of any of the guinea-pigs on day 5 post attachment of ticks (GP 1–24; <10 PFU/ml blood).

Table 3. The effect of SGE derived from haematophagous arthropod vectors on the ability of *R.appendiculatus* nymphs to acquire THO virus.

Guinea-pig	Species	No. infected/ no. tested*	% infected
GP1–2	*R.evertsi*	31/60	52
GP3–4	*A.cajennense*	36/70	51
GP5–6	*A.hebraeum*	25/60	42
GP7–8	*H.m.rufipes*	40/70	57
GP9–10	*H.dromedarii*	29/50	58
GP11–12	*I.ricinus*	2/60	3
GP13–14	*I.hexagonus*	5/59	8
GP15–16	*O.maritimus*	4/40	10
GP17–18	*Ar.monolakensis*	3/40	7
GP19	*An.stephensi*	2/20	10
GP20–21	*Ae.aegypti*	5/80	6
GP22–24	Control†	5/80	6

* Individuals were assayed for virus on day 12 post-engorgement.
† Control guinea-pigs were inoculated with virus alone.

Discussion

The ability of an arthropod to sustain an infection and subsequently deliver the virus during feeding is a measure of vector competence, 'the combined effect of all the physiological and ecological factors of vector, host, pathogen, and environment that determine the vector status of a given arthropod population' (McKelvey *et al.*, 1981). Comparison of the vector potential of a variety of arthropod species for THO virus indicated that only metastriate ixodid ticks were susceptible to the virus. In general, the natural vector species of an arbovirus are more competent for that virus than are potential vector species, which do not overlap in their geographic range with the virus. However, in this study, there was no evidence to suggest differences in the vector efficiency of competent tick species.

The feeding preferences of vectors also have significance for the transmission of arboviruses in nature, in that different hosts will vary in their susceptibility to a given arbovirus. Furthermore, as in the case of SAT of THO virus, there is evidence to suggest that the salivary glands (and their secretions) can play an integral role in disease transmission with the parasite utilizing the physiological activities of the vector's feeding mechanism to enhance its

own relative infectivity (Jones *et al.*, 1992a). Assessment of the potential of other haematophagous arthropods to mediate SAT of THO virus indicate that the SAT factor synthesis is specific to the salivary glands of metastriate ticks and is not synthesized in the salivary glands of prostriate and argasid ticks, or mosquitoes (at least those species tested). Thus, an intriguing correlation has emerged in the apparent relatedness of vector competence and the synthesis of the SAT factor.

The main difference between metastriate and prostriate ticks is in the female's reproductive physiology (Diehl *et al.*, 1982). In addition, of the family, Ixodidae, prostriate ticks have the most primitive form of attachment and are considered to be intermediate in complexity between that of argasids and the metastriate ixodids (Kemp, 1982). The observation that the SAT factor is not synthesized in the salivary glands of prostriate ticks may be related to their simpler mechanisms of attachment and feeding.

In order to evaluate fully the role of prostriate and argasid tick species in the epizootiology of THO virus, these studies need to be extended to tick species that are sympatric in their geographical distribution with the virus, e.g. *O.moubata* Murray and various *Ixodes* species (Hoogstraal, 1956). Furthermore, in view of the potential impact of climatic change on the distribution and prevalence of vector populations (Sutherst & Maywald, 1985), other tick species — competent vectors of THO virus but not prevalent in areas from which the virus has been isolated — may in the future be of significance in the epizootiology of the virus.

Acknowledgments

The authors thank Ms Pauline Henbest for the rearing of the tick colonies and Professor D. H. L. Bishop for his support.

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Accepted 1 April 1992