

AD-A277 712

WR 140



STATION PAGE

Form Approved
OMB No. 0704-0188

Use to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering the data, reviewing the collection of information, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
4. TITLE AND SUBTITLE Laboratory transmission of Venezuelan equine encephalomyelitis virus by the tick <i>Hyalomma truncatum</i>			5. FUNDING NUMBERS
6. AUTHOR(S) Kenneth J. Linthicum and Thomas M. Logan			2
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Walter Reed Army Institute of Research Washington, DC 20307-5100			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research & Development Command Ft. Detrick, Frederick, MD 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION/AVAILABILITY STATEMENT APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) To assess the vector potential of <i>H. truncatum</i> ticks for Venezuelan equine encephalitis (VEE) virus, larval ticks were allowed to feed on a viremic guinea pig infected with an epizootic virus while feeding or a viremic guinea pig, transstadially transmitted the virus to subsequent nymphs and adults, and transmitted the virus to susceptible hosts.			
14. SUBJECT TERMS Alphavirus, Togaviridae, Venezuelan equine encephalomyelitis, tick, <i>H. truncatum</i> , Acari, Ixodae			15. NUMBER OF PAGES
17. SECURITY CLASSIFICATION OF REPORT			16. PRICE CODE
18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT		20. LIMITATION OF ABSTRACT

DTIC
S ELECTE D
APR 05 1994
F

Short Report

Laboratory transmission of Venezuelan equine encephalomyelitis virus by the tick *Hyalomma truncatum**

Kenneth J. Linthicum¹ and Thomas M. Logan²
¹Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Infectious Diseases, 315 6 Rajvith Road, Bangkok 10400, Thailand; ²Department of Epidemiology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701, USA

Epizootic strains of Venezuelan equine encephalomyelitis VEE virus (*Alphavirus*, family *Togaviridae*) cause serious disease in horses and humans throughout the 'New World' tropics and subtropics (WALTON & GRAYSON, 1989). Although various mosquito species serve as vectors of this virus during epizootics, recent experimental evidence has indicated that ticks may be involved in the maintenance cycle (LINTHICUM *et al.*, 1992). With the rapid expansion of air travel between the Americas and Africa and Europe the potential for importation of VEE virus into the 'Old World' poses a threat to immunologically naive human and equine populations. *Hyalomma truncatum* Koch is a tick species commonly found in Africa and south-west Asia, and is a known vector of Crimean-Congo haemorrhagic fever virus (*Nairovirus*, family *Bunyviridae*) (LOGAN *et al.*, 1989). To assess the vector potential of *H. truncatum* for VEE virus, larval ticks were allowed to feed on a viraemic guinea-pig infected with an epizootic VEE virus strain. Subsequently, ticks were evaluated to determine if virus replication occurred and if virus was transmitted.

H. truncatum ticks used in this study were maintained as described by LINTHICUM *et al.* (1991). All experiments were conducted in a B1.3+ laboratory specifically modified to contain ticks. Guinea-pigs used in this study had not been previously exposed to either VEE virus or ticks. The strain of VEE virus used (Trinidad donkey, variant 1-A) is almost always fatal to guinea-pigs. Virus content of sampled ticks was determined by plaque assay on Vero cell monolayers (LINTHICUM *et al.*, 1992).

Initially one guinea-pig was infested with approximately 2000 tick larvae. One day later, the guinea-pig was inoculated subcutaneously with $10^{6.2}$ plaque-forming units (PFU) of VEE virus. On day 4 after infestation the serum viral titre in the guinea-pig was $10^{5.5}$ PFU mL. The guinea-pig died 5 d after infestation.

More than 800 fed larvae dropped off the guinea-pig 4-5 d after infestation. All 10 fed larvae sampled after dropping off contained VEE virus (mean titre = $10^{2.6}$ PFU, range $10^{1.6}$ - $10^{3.3}$). Fed larvae started to moult 12 d after infestation, and at 14 d after infestation 3 of 21 pools of unfed nymphs [minimum infection rate = 3/105 (2.9%)] contained virus (mean titre = 10^4 PFU, range

$10^{4.0}$ - $10^{5.7}$). On day 14 after infestation about 100 of these unfed nymphs were placed on another guinea-pig, which died 6 d later; however, no virus was isolated from it or from 45 fed nymphs 2 d after they had dropped off the animal.

On day 21 after infestation of the first guinea-pig, none of 95 unfed nymphs sampled contained virus; however, when 100 unfed nymphs were placed on a guinea-pig the animal died 6 d later with a serum viral titre of $10^{7.1}$ PFU mL. At drop-off, 6 of 7 (86%) fed nymphs contained virus (mean titre = $10^{3.6}$ PFU, range $10^{2.8}$ - $10^{4.3}$). About 80 partially fed nymphs were transferred to another guinea-pig, which died 4 d later, with a serum viral titre of $10^{6.1}$ PFU mL, and 28/31 (90%) fed nymphs contained virus (mean titre = $10^{4.3}$ PFU, range $10^{1.9}$ - $10^{5.3}$) when they dropped off. At 56 days after infestation 3 of 14 (21.4%) subsequent adults contained virus (mean titre = $10^{3.9}$ PFU, range $10^{2.5}$ - $10^{4.6}$).

On day 28 after infestation, 2 of 40 pools of unfed nymphs [minimum infection rate = 2/200 (1%)] contained virus (mean titre = $10^{2.1}$ PFU). About 200 unfed nymphs were placed on a guinea-pig at this time, and the guinea-pig survived. Only 1 of 124 (0.8%) fed nymphs contained virus (titre = $10^{5.0}$ PFU) when they dropped off.

The ability of *H. truncatum* larvae to become infected with VEE virus while feeding on a viraemic guinea-pig, trans-stadially transmit the virus to subsequent nymphs and adults, and transmit the virus to susceptible hosts, indicates that this species is a competent laboratory vector of the virus. Infection and transmission rates, and the viral titres observed for *H. truncatum*, are equal to or greater than those observed previously for *Amblyomma cajennense* infected with the same strain of virus (LINTHICUM *et al.*, 1992). Thus if VEE virus were introduced into south-west Asia or Africa it could be maintained in, and transmitted by, an indigenous tick species.

Acknowledgements

We thank J. Kondig for his invaluable professional and technical contributions to this study and R. E. Coleman, S. Frances, K. Kenyon, R. Rosenberg, and M. J. Turell for their critical review of the manuscript. Research was conducted in compliance with the Animal Welfare Act and other US Federal statutes and regulations relating to animals and experiments involving animals and adhered to the *Guide For the Use of Laboratory Animals*, NIH Publication 86-23, 1985 edition. The views of the authors do not necessarily reflect the position of the Department of the Army or the Department of Defense.

References

- Linthicum, K. J., Logan, T. M., Kondig, J. P., Gordon, S. W. & Bailev, C. L. 1991. Laboratory biology of *Hyalomma truncatum* Acari: Ixodidae. *Journal of Medical Entomology*, **28**, 280-283.
- Linthicum, K. J., Gordon, S. W. & Monath, T. P. 1992. Comparative infections of epizootic and enzootic strains of Venezuelan equine encephalomyelitis virus in *Amblyomma cajennense* Acari: Ixodidae. *Journal of Medical Entomology*, **29**, 827-831.
- Logan, T. M., Linthicum, K. J., Bailev, C. L., Watts, D. M. and Dohm, D. J. 1989. Experimental transmission of Crimean-Congo haemorrhagic fever virus (family *Bunyviridae*, genus *Nairovirus*). *American Journal of Tropical Medicine and Hygiene*, **40**, 207-212.
- Walton, T. E. & Grayson, M. A. 1989. Venezuelan equine encephalomyelitis. In: *The Arboviruses: Epidemiology and Ecology*, vol. 4, Monath, T. P., editor. Boca Raton, Florida: CRC Press, pp. 203-231.

Received 14 May 1993; accepted for publication 23 June 1993

*This work was prepared by an employee of the US government as part of official duties and therefore cannot be copyrighted.

†Author for correspondence alternative address within the USA: Department of Entomology, USAMC, AFRIMS, APO AP 96546.

94-10157



DTIC QUALITY INSPECTED 3

94 4 4 064

- H. W. & Meuwissen, J. H. E. T. 1987. Transmission blockade of *Plasmodium falciparum*: its variability with gametocyte numbers and concentration of antibody. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 491-493.
- Ponnudurai, T., Lensen, A. H. W., van Gemert, G. J. A., Bensink, M. P. E., Bolmer, M. & Meuwissen, J. H. E. T. 1989. Infectivity of cultured *Plasmodium falciparum* gametocytes to mosquitoes. *Parasitology*, **98**, 165-173.
- Ranawaka, M. B., Munesinghe, Y. D., Silva, de D. M. R., Carter, R. & Mendis, K. N. 1988. Boosting of transmission-blocking immunity during natural *Plasmodium vivax* infections in humans depends upon frequent reinfection. *Infection and Immunity*, **56**, 1820-1824.
- Rener, J., Graves, P. M., Carter, R., Williams, J. & Burkot, T. R. 1983. Target antigens of transmission-blocking immunity on gametes of *Plasmodium falciparum*. *Journal of Experimental Medicine*, **158**, 976-981.
- Sinden, R. E. & Smalley, M. E. 1976. Gametocytes of *Plasmodium falciparum*: phagocytosis by leucocytes *in vivo* and *in vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 344-345.
- Smalley, M. E. 1977. *Plasmodium falciparum* gametocytes: the effect of chloroquine on their development. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 526-529.
- Tchuinkam, T., Mulder, L., Dechering, K., Stoffels, H., Verhave, J. P., Carnevale, P., Meuwissen, J. H. E. Th. & Robert, V. in press. Experimental infections of *Anopheles gambiae* with *Plasmodium falciparum* in Cameroon: infectivity of gametocytes of naturally infected gametocyte carriers. *Tropical Medicine and Parasitology*.
- Vermeulen, A. N., Ponnudurai, T., Beckers, P. J. A., Verhave, J. P., Smits, M. & Meuwissen, J. H. E. T. 1985. Sequential expression of antigens on sexual stages of *Plasmodium falciparum* accessible to transmission-blocking antibodies in the mosquito. *Journal of Experimental Medicine*, **162**, 1460-1476.
- Wilkinson, R. N., Noepatmanondh, S. & Gould, D. J. 1976. Infectivity of falciparum malaria patients for anopheline mosquitoes before and after chloroquine treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 306-307.
- Witte, A. M. C., Klever, H. J. H., Brabin, B. J., Eggelte, T. A., van der Kaay, H. J. & Alpers, M. P. 1990. Field evaluation of the use of an ELISA to detect chloroquine and its metabolites in blood, urine and breast-milk. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 521-525.
- Zovva, A. P. K., Herath, P. R. J., Abhayawardana, T. A., Padmalal, U. K. G. K. & Mendis, K. N. 1988. Modulation of human malaria by anti-gamete transmission-blocking immunity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**, 548-553.

Received 28 October 1992; revised 5 April 1993; accepted for publication 13 May 1993

Accession For		
NTIS	CRA&I	<input checked="" type="checkbox"/>
DIIC	TAB	<input type="checkbox"/>
Univ. of Calif		<input type="checkbox"/>
Justification		
By		
Distribution /		
Availability Codes		
Dist	Avail and/or Special	
A-1	20	

Corrections

F. Pratloug et al. 1993. Characterization of *Leishmania* isolates from two AIDS patients originating from Valencia, Spain. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 705-706.

The international code numbers of 2 of the strains of *Leishmania infantum* isolated from these patients were incorrectly printed on p. 705 (3 lines from the bottom of column 2) and p. 706 (line 13 of column 1); the correct numbers are MHOM ES 91 LEM2298 and MHOM ES 91 LEM2361, respectively. The editor apologizes for these errors.

M. Corcos and C. Corcos 1993. A transposon in Hansen's bacillus? [Correspondence]. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 708.

The authors have pointed out that the word 'its', in line 4 of paragraph 5 of their letter, appeared as 'whose' in the original typescript, and that this more clearly indicates their meaning, that it is the replication of the *plasmid* which is an epiphenomenal self-perpetuating feedback process.