Beck, S. D. 1980. Insect photoperiodism, 2nd ed. Academic Press, New York. 387 pp.
Gallaway, W. J. and R. A. Brust. 1982. The occurrence of *Aedes hendersoni* Cockerell and *Aedes triseriatus* (Say) in Manitoba. Mosq. Syst. 14:262–264.
Holzapfel, C. M. and W. E. Bradshaw. 1981. Geography of larval dormancy in the tree-hole mosquito, *Aedes triseriatus* (Say). Can. J. Zool. 59:1014–1021.
Sims, S. R. 1982. Larval diapause in the eastern tree-hole mosquito, *Aedes triseriatus:* Latitudinal variation in induction and intensity. Ann. Entomol. Soc. Am. 75:195–200.
Wood, D. M., P. T. Dang and R. A. Ellis. 1979. The insects and arachnids of Canada. Part 6. The mosquitoes of Canada (Diptera: Culicidae). Agric. Can. Publ. 1686. 390 pp.
Zavortink, T. J. 1972. Mosquito studies (Diptera: Culicidae) XXVIII. The New World species formerly placed in *Aedes* (Finlaya). Contrib. Am. Entomol. Inst. (Ann Arbor) 8(3):1–206.

## A BLOOD MEAL ANALYSIS OF ENGORGED MOSQUITOES FOUND IN RIFT VALLEY FEVER EPIZOOTICS AREAS IN KENYA[1]

K. J. LINTHICUM, H. F. A. KABURIA,[2] F. G. DAVIES AND K. J. LINDQVIST[2]

U.S. Army Medical Research Unit (WRAIR) Kenya, Box 401, APO New York 09675, and Veterinary Research Laboratory, P.O. Kabete, Kenya

Epizootic Rift Valley fever (RVF) occurs in Kenya in grasslands adjacent to, within or derived from natural forest, and in the bushed and wooded grasslands with predominantly *Combretum* or *Acacia* tree cover (Davies 1975). These are in ecological zones II–IV described by Pratt et al. (1966), in their classification of East African habitat. Epizootics occur after periods of prolonged and heavy rainfall (Scott et al. 1956, Davies 1975, Davies and Highton 1980, Davies et al., unpublished data). The virus is transmitted by mosquitoes during RVF epizootics (Daubney and Hudson 1933, McIntosh 1972, Davies and Highton 1980). A knowledge of the mosquitoes found in such areas during epizootic and interepizootic periods is relevant to a greater understanding of the natural history of RVF. In this study we examined 739 blood-fed mosquitoes trapped during and following a period of particularly heavy rainfall (October–December 1982), which did not generate an epizootic of RVF. However, during this period the virus was isolated from mosquitoes at the trapping sites, and from one dead calf on a nearby farm; there were also 4 seroconversions in a group of 80 yearling cattle tested at one of the trapping sites (Davies, unpublished data). The emergence of mosquito species appeared to be similar to that occurring during the early stages of RVF epizootics (Linthicum et al. 1983, 1984a).

Mosquitoes were trapped with Solid State Army Miniature light traps (John W. Hock, Co., Gainesville, FL) at known RVF epizootic sites in ecological zones II (1°14′ 30″S, 36° 50′E; 1700 m) and III (1° 12′S, 37°E; 1500 m) in the vicinity of Nairobi, Kenya. Studies concerning the natural history of RVF during epizootics (Davies 1975, Davies and Highton 1980), the population biology of immature mosquitoes in dambos (Linthicum et al. 1983, 1984a) and the feeding habits of mosquitoes at human and calf bait (Linthicum et al. 1984b) have been conducted at these sites. The domestic animals present in these areas were predominantly cattle, with a few goats, horses and sheep. The common wild animals present were: bushbuck (*Tragelaphus scriptus*), eland (*Taurotragus oryx*), Grant's gazelle (*Gazella granti*) and Thomson's gazelle (*Gazella thomsonii*), giraffe (*Giraffa camelopardalis*), jackal (*Canis mesomelas*), ostrich (*Struthio camelus*), reedbuck (*Redunca redunca*) and zebra (*Equus burchelli*). Light traps were suspended 50–80 cm above the ground and baited with $CO_2$ (2 kg dry ice). The traps were generally placed adjacent to emergence sites (flooded dambo formations). Specimens were obtained in 380 trap night collections made during the period October 15, 1982 to February 15, 1983. One to eight traps were set 4–7 days a week at each study site. The catches were taken to the laboratory live, frozen at −70°C for 30 min and then examined. All blood-fed specimens were removed and their abdomens smeared onto filter paper, labelled and stored in a desiccator jar. The smeared area of the filter paper was later cut out and placed in 0.5 ml of a phosphate buffered saline which continued 0.1% M sodium azide as a preservative.

Blood meal identification was carried out by an enzyme immunoassay system developed by Lindqvist, Gathuma and Kaburia (1982) for use in East Africa, and many conjugates had been prepared against the domestic and wild ruminants found in this region. The antispecies—antisera were produced in rabbits, goats or sheep by weekly inoculations of the different

[1] This work was supported by Research Grant No. DAMD17–83–G–9517 from the U.S. Army Medical Research and Development Command, Ft. Detrick, MD 21701 and by a Research Grant for Project No. 3792 by the Overseas Development Administration, United Kingdom.

[2] Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, P. O. Box 29053 Nairobi, Kenya.

species globulin with Freud's adjuvant, these continued for several months. The sera were subjected to repeated absorption cycles to achieve species specificity. This was considered complete when serum samples from other species gave reactions similar to the buffer controls in double immunodiffusion tests. Enzyme conjugates were prepared from either IgG or immunoglobulins with horse-radish peroxidase. Tests were carried out directly with species specific conjugates, or indirectly using antisera produced in rabbits and a conjugated anti-rabbit globulin prepared in a goat. This method regularly detected dilutions of test blood beyond $10^{-6}$. The test thus gives a high degree of sensitivity and specificity, and is particularly suitable for this type of investigation. The eluants were first examined for the presence of haemoglobin by oxidizing reduced phenophthalein in the presence of $H_2O_2$ in alkaline solution. Those positive for haemoglobin were then tested against the following enzyme conjugates: goat anti-cattle, -human, -sheep, -rabbit (*Oryctogalus*), -bushbuck, -reedbuck, -Grant's and Thomson's gazelle IgG; sheep anti-goat, -dog (jackal) IgG; and rabbit anti-ostrich, -chicken, -horse (donkey, zebra), -mouse (rat), -giraffe whole serum; and an anti-bovidae conjugate which detects all members of this family.

The results of the haemoglobin screening and blood meal identification tests are shown in Table 1. Of 800 specimens examined, 61 did not give a reaction for haemoglobin. The most common mosquito species collected over the trapping period was *Aedes lineatopennis* (Ludlow), which comprised more than half the blood-fed specimens examined. Of the 389 specimens shown to contain haemoglobin 333 (85.6%) had taken cattle blood meals. Three other common *Aedes* species, *Ae. dentatus* (Theobald), *Ae. cumminsii* (Theobald), and *Ae. sudanensis* (Theobald) were 90.7%, 79.2% and 92.3%, respectively, positive to the specific anti-cattle conjugate (Table 1). Comparatively few *Culex* specimens were obtained, but of these 93.3% of the haemoglobin positive *Culex antennatus* (Becker) had taken cattle blood meals. One specimen of *Ae. lineatopennis* had taken a human and one a rabbit blood meal. A single *Ae. dentatus* had fed upon a giraffe and one *Ae. cumminsii* upon a dog or jackal and 2 on a rabbit. The anti-rabbit reactions were weak, suggesting that they were not due to the closely related *Lepus* or *Pronolagus* species. *Pedetes capensis* (Rodentia, Pedetidae), the jumping hare, was commonly encountered at the sites and may have been the host causing the weak reactions. Four specimens of *Ae. circumluteolus* (Theobald) and one *Ae. quasiunivittatus* (Theobald) were strongly positive with the anti-horse or -zebra conjugate. Of the 104 specimens which contained haemoglobin but were not identified by the 13 specific conjugates, 8 reacted strongly when tested later with the broad spectrum anti-bovidae conjugate. These 8 may have fed on a less abundant wild ruminant at these trapping sites, most likely impala (*Aepyceros melampus*) or kongoni (*Alcelaphus buselaphus cokii*). There was a residue of 96 (13%) blood-fed mosquitoes that have not been identified. Over 50% of the mosquitoes containing unidentified haemoglobin were *Ae. lineatopennis*. *Aedes lineatopennis* does feed on birds (Chandler et al. 1976). The anti-chicken conjugate may have failed to react with the many diverse bird species encountered at the site. It remains possible that some of the unidentified specimens were avian feeds.

The most abundant mosquitoes obtained in this study after heavy rains in RVF epizootic areas were *Aedes* spp. Cattle were the blood meal source for most of the *Aedes* spp. which were collected. They were also the most common large animal species in the study areas. Cattle are considered to be the major amplify-

Table 1. Results of haemoglobin screening and blood meal identification tests of engorged mosquitoes collected from October 15, 1982 to February 15, 1983 in Rift Valley fever epizootic areas in Kenya.

| Species | Unknown Hb+ | Unidentified Bovidae | Cattle | Human | Canine | Rabbit (*Oryctolagus*) | Giraffe | Horse/Zebra | Total |
|---|---|---|---|---|---|---|---|---|---|
| *Ae. lineatopennis* | 50 | 4 | 333 (85.6%)* | 1 | | 1 | | | 389 |
| *Ae. circumluteolus* | 8 | 1 | 27 (67.5%) | | | | | 4 | 40 |
| *Ae. cumminsii* | 25 | 2 | 114 (79.2%) | | 1 | 2 | | | 144 |
| *Ae. sudanensis* | 3 | | 36 (92.3%) | | | | | | 39 |
| *Ae. dentatus* | 7 | 1 | 88 (90.7%) | | | | 1 | | 97 |
| *Ae. quasiunivittatus* | 2 | | 6 | | | | | 1 | 9 |
| *Cx. antennatus* | 1 | | 14 | | | | | | 15 |
| *Cx.* spp. | | | 6 | | | | | | 6 |
| Total | 96 | 8 | 624 | 1 | 1 | 3 | 1 | 5 | 739 |

* Percentage of total number of blood fed specimens.

ing and disease hosts for RVF in many parts of Africa (Davies 1975), although local farming practices often dictate whether sheep assume this role in some areas (Davies, unpublished data). These results suggest that *Aedes* mosquitoes, in this ecosystem *Ae. lineatopennis*, could play some role in the generation of epizootics of RVF. Rift Valley Fever virus has been isolated from *Ae. lineatopennis* in epizootics of RVF in Africa (McIntosh 1972, Davies and Highton 1980) and from *Ae. circumluteolus* (Gear et al. 1955). McIntosh et al. (1980) have demonstrated that *Ae. lineatopennis* transmitted RVF virus under laboratory conditions.

The authors with to thank Drs. C. L. Bailey, L. D. Hendricks, M. J. Reardon, D. R. Roberts and R. A. Ward for review of the manuscript and to the staff of the Veterinary Research Laboratory, Kabete for technical assistance. The results are published with the approval of Dr. A. R. Njogu, Director, Kenya Trypanosomiasis Institute, Government of Kenya and the Director of Veterinary Services, Kenya.

*References Cited*

Chandler, J. A., P. F. L. Boreham, R. B. Highton and M. N. Hill. 1976. A study of the host selection patterns of the mosquitoes of the Kisumu area of Kenya. Trans. R. Soc. Trop. Med. Hyg. 64:415–425.

Daubney, R. and J. R. Hudson. 1933. Rift Valley Fever. E. Afr. Med. J. 10:2–19.

Davies, F. G. 1975. Observations on the epidemiology of Rift Valley fever in Kenya. J. Hyg. 75:219–230.

Davies, F. G. and R. B. Highton. 1980. Possible vectors of Rift Valley fever in Kenya. Trans. R. Soc. Trop. Med. Hyg. 74:815–816.

Gear, J., B. de Meillon, A. F. Le Roux, R. Kofsky, R. Rose Innes, J. J. Steyn, W. D. Oliff and K. H. Schulz. 1955. Rift Valley Fever in South Africa. A study of the 1953 outbreak in the Orange Free State, with special reference to the vectors and possible reservoir hosts. S. Afr. Med. J. 29:514–518.

Lindqvist, K. J., J. M. Gathuma and H. F. A. Kaburia. 1982. Analysis of blood meals of haematophagous insects by haemagglutination inhibition and enzyme immunoassay. pp. 122–133. *In:* Current medical research in eastern Africa with emphasis on zoonoses and waterborne diseases. Proceedings of the third annual medical scientific conference, Nairobi, Kenya, 1982. P. M. Tukei and A. R. Njogu (Ed.) Africascience International, Nairobi.

Linthicum, K. J., F. G .Davies, C. L. Bailey and A. Kairo. 1983. Mosquito species succession in a dambo in an East African forest. Mosq. News 43:464–470.

Linthicum, K. J., F. G. Davies, C. L. Bailey and A. Kairo. 1984a. Mosquito species encountered in a flooded grassland dambo in Kenya. Mosq. News 44:228–232.

Linthicum, K. J., F. G. Davies and A. Kairo 1984b. Observations of the biting activity of mosquitoes at a flooded dambo in Kenya. Mosq. News 44:595–598.

McIntosh, B. M. 1972. Rift Valley fever I. Vector studies in the field. J. S. Afr. Vet. Assoc. 43:391–395.

McIntosh, B. M., P. G. Jupp, I. dos Santos and B. J. H. Barnard, 1980. Vector studies on Rift Valley fever virus in South Africa. S. Afr. Med. J. 58:127–132.

Pratt, D. J., P. J. Greenway and M. D. Gwynne. 1966. A classification of East African rangeland, with an appendix on terminology. J. Appl. Ecol. 3:369–382.

Scott, G. R., I. W. Weddel and D. Reid. 1956. Preliminary findings on the prevalence of Rift Valley fever in Kenya cattle, Bull. Epizoot. Dis. Afr. 4:17–26.

## GENETIC ANALYSIS OF A LINKAGE GROUP III MUTANT IN *ANOPHELES STEPHENSI*

KANEEZ AKHTAR[1] AND R. K. SAKAI[1, 2]

A new mutant short, curved palpus/proboscis (*scp*) was discovered during routine handling of a translocation strain, *T(2R;3R)4* (Sakai et al. 1983), of *Anopheles stephensi* Liston. The mutant is characterized by shortened (2/3–3/4 normal length) palpi and proboscis, both of which curve upward toward the dorsum of the adult. There is some variability in expression but penetrance is complete and both sexes express the mutation. A true-breeding *scp* strain, free of the translocation, was isolated and crosses were made to study the mode of inheritance of this mutation.

Preliminary crosses indicated that the mutation was recessive and autosomal as reciprocal crosses with a wild type (+) strain gave all + $F_1$ progeny whereas a sex-linked mutation in this species in which females are XX and males XY (Aslamkhan 1973) would have resulted in + ♀ and *scp* ♂ $F_1$ progeny in one of the crosses (*scp* ♀ x + ♂). Moreover, crosses with the chromosome 2 mutant marker, *mar* (Mahmood and Sakai 1982), indicated that *mar* and *scp* segregated independently of each other. Therefore, a series of crosses were initiated between the *scp* and *Bl* (black larva, a chromosome 3 mutant, Akhtar et al. 1982) strains. Table 1 summarizes the results and also contains the observed recombination frequencies between *scp* and *Bl*.

[1] International Center for Medical Research and Training, 6, A. R. Chughtai Road, Lahore 3, Pakistan.

[2] International Health Program, University of Maryland School of Medicine, 10 S. Pine Street, Baltimore, MD 21201.