

RIFT VALLEY FEVER ; TRANSMISSION OF THE VIRUS BY MOSQUITOES.

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A CONSIDERABLE amount of evidence was found by Daubney and Hudson (1931, 1933) which indicated that the virus of Rift Valley fever is transmitted by mosquitoes. Nevertheless, it was not until 1944 that the agent was first isolated from wild-caught mosquitoes (Smithburn, Haddow and Gillett, 1948) ; and prior to the present communication there has been no account of its successful transmission by hematophagous arthropods.

In 1944, while attempting to discover the vector responsible for the transmission of yellow fever among wild primates in uninhabited forest (Smithburn, Haddow and Gillett), Rift Valley fever virus was isolated 6 times from different lots of mosquitoes caught during a period of 39 days in a circumscribed area of the Semliki Forest in western Uganda. The agent was isolated once from *Aedes (Stegomyia) de-boeri* spp. *de-meilloni*, Edwards, twice from the *Aedes (Aedimorphus) tarsalis*, Newst. group, and 3 times in successive catches from *Eretmapodites* spp. The indications were that the former 2 contained little virus and may have been only casually infected, whereas the *Eretmapodites* not only were regularly infected during the period of virus activity, but apparently contained the virus in considerable quantity. These facts suggested that the local vector, if one was included in the species from which virus was isolated, was one of the *Eretmapodites* spp. Mosquitoes of the *Eretmapodites chrysogaster* Graham group were more numerous in the infected lots than were any of the other species of *Eretmapodites*. Moreover, members of this group were known to be capable of transmitting another virus disease—yellow fever (Bauer, 1928). Accordingly, it was decided to attempt the experimental transmission of Rift Valley fever virus with these mosquitoes, and with other species regarded as suspect vectors of the virus in Kenya. The tests with *Eretmapodites* were successful, and are described in this paper, which therefore records the first experimental transmission of Rift Valley fever virus by the bites of mosquitoes.

MATERIALS AND METHODS.

At the time these experiments were carried out we had no colony of mosquitoes of the *E. chrysogaster* Graham group, and we therefore were obliged to use wild insects, or adults reared from wild-caught larvae. These were captured at Kitinda, a forested locality near Entebbe, where they were present in good numbers, and where Rift Valley fever was not known to have occurred. Adult female mosquitoes were caught individually in glass tubes as they alighted to bite the catchers, and the tubes were then lightly plugged with cotton. The captured insects were transported the short distance to the laboratory in the

catching tubes ; there they were examined and sorted by one of us and released into Barraud cages.

The *Taeniorhynchus* employed in the experiments were also wild mosquitoes, which were caught in lake-shore localities near this Institute. The *Aedes aegypti* were laboratory-reared insects descended through several generations from material collected in Nigeria by Dr. J. C. Bugher, who supplied us with the mosquito eggs.

The animals used were mice and lambs. The lambs were European-native hybrid stock and were about 5 or 6 months old. None of them died as result of Rift Valley fever in these or other experiments. Whether their survival was due to age, or breed, or to characteristics of the virus is not known. Their clinical and immunological responses to infection, whether induced by bites of mosquitoes or by inoculation, were typical.

The mosquitoes received their infective feeds, in the first instance, from mice sick or moribund following inoculation with virus of Rift Valley fever. The inoculated mice were placed singly in close-fitting cylinders made of monel-metal gauze. Each end of the cylinder was closed with a cork to render the mouse relatively immobile without causing it discomfort. One of the corks was grooved to accommodate the animal's tail. In some instances moribund mice were exposed to mosquitoes without being confined in the cylinders. The infected mice in the cylinders (and the few not so confined) were placed in Barraud cages.

The mosquitoes which gorged were removed to other Barraud cages. All those which fed on a given day on mice of the same group were placed in the same cage and given the same lot number. The cages were kept in a controlled temperature cabinet. The cages and the floor of the cabinet were covered with moist cotton in order to provide a highly humid atmosphere for the insects. The temperature within the cabinet was 30° C. throughout the experiments. Mosquitoes of the genus *Taeniorhynchus* did not thrive well in the controlled temperature cabinet and, after a few preliminary trials, they were kept in large Barraud cages in the open air of the insectary, with only the tops of the cages covered with moist cotton. The temperature of the insectary varied between 22° C. and 26.6° C. during the experiments. The mosquitoes were given banana and water daily, but on the day before they were to be exposed to normal mice or a normal lamb for transmission attempts, the banana was removed from the cages.

The mosquitoes to be offered a transmission feed on a lamb were placed individually in wide-mouthed gauze-covered glass tubes, which were applied to the shaven skin of the animal. The transmission feeds on mice were given in the same manner as the infective feeds.

When the first transmission of the virus to a lamb was accomplished, fresh lots of mosquitoes were exposed to this animal. Those which gorged were thereafter handled in the same way as mosquitoes which received their infective feeds from mice.

An animal which served as source of virus for the mosquitoes was usually bled from the heart in the case of a mouse (for this and other procedures the animals were anaesthetized with ether), or the jugular vein in the case of a lamb, immediately after the infective feed, and the serum was tested for virus. In the case of the lamb sera, titrations of virus content were done.

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TABLE I.—*Mosquitoes of the Better Infected Lots of each Species, showing Number which Probed or Gorged on Normal Animals at Various Intervals after their Infective Feeds.*

Days after infective feeds.	No. mosquitoes which probed or gorged on mice.							No. mosquitoes which probed or gorged on lambs.							
	<i>Aedes aegypti</i> .		<i>Eretmapodites chrysogaster</i> group.				<i>Taeniorhynchus fuscopennatus</i> .		<i>Aedes aegypti</i> .		<i>Eretmapodites chrysogaster</i> group.			<i>Taeniorhynchus fuscopennatus</i> .	
	Lot 5	Lot 1	Lot 3	Lot 7	Lot 11	Lot 15	Lot 17	Lot 5	Lot 1	Lot 3	Lot 7	Lot 11	Lot 15	Lot 17	
3	13	
5	4	Many	
6	3	2	
7	7	11	
8	Several	
9	Several	
10	2	4	7	..	4	
11	1	5	2	
12	1	2	3	
13	4	..	2	1	4†	
14	5	
15	3	
16	1	..	
17	7	..	1	
18	5	
19	2*	
20	4*	3*†	..	3*	1	10	
21	1	
22	1	2	6	
23	1	..	
24	1	2	4	
25	1	1	1	
27	1	..	5	
29	1	3	
31	1	1	
32	1	

* Transmission occurred.

† Mosquitoes of Lots 1 and 7, which received their infective feeds 6 days apart, bit Lamb 3 on the same day. It is probable that the transmission was effected by Lot 1.

Transmission 1.—Twelve *E. chrysogaster* group mosquitoes, comprising Lot 1, received their infective feeds from mice on May 12. Although they were afterward repeatedly offered feedings on normal mice in attempts to secure transmission of the virus, none of them bit these animals. Two of the lot bit normal Lamb 3 on the 11th day after their infection without results. Three of the lot bit the same animal on the 20th day after their infection (June 1), together with 4 *E. chrysogaster* group mosquitoes of Lot 7, which had received their infective feeds on May 18, 6 days after the mosquitoes of Lot 1 (Table I). On June 4 the lamb's temperature rose sharply to 107.2° F. (Fig. 1), and remained elevated throughout the day, but it was within normal limits by the following morning.

Six tests for virus were made with mosquitoes of Lot 1 from the 15th to the 26th day after their infective feeds. Five of the tests were made with individual mosquitoes, and the 6th with a pool of 2. The pool of 2 mosquitoes and 3 of

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	Lot 5	Lot 1	Lot 3	Lot 7	Lot 11	Lot 15	Lot 17	Lot 5	Lot 1	Lot 3	Lot 7	Lot 11	Lot 15	Lot 17
3	13
5	..	4	Many
6	3	2
7	..	7	11
8	Several
9	Several
10	2	4	7	..	4
11	1	5	2
12	..	1	2	3
13	4	..	2	1	4†
14	5
15	3
16	1	..
17	7	..	1
18	5
19	2*
20	4*	3*†	..	3*	1	10	..
21	1
22	1	2	..	6	..
23	1	..
24	1	2	4
25	1	1	1
27	1	5
29	1	..	3	..
31	1	..	1	..
32	1

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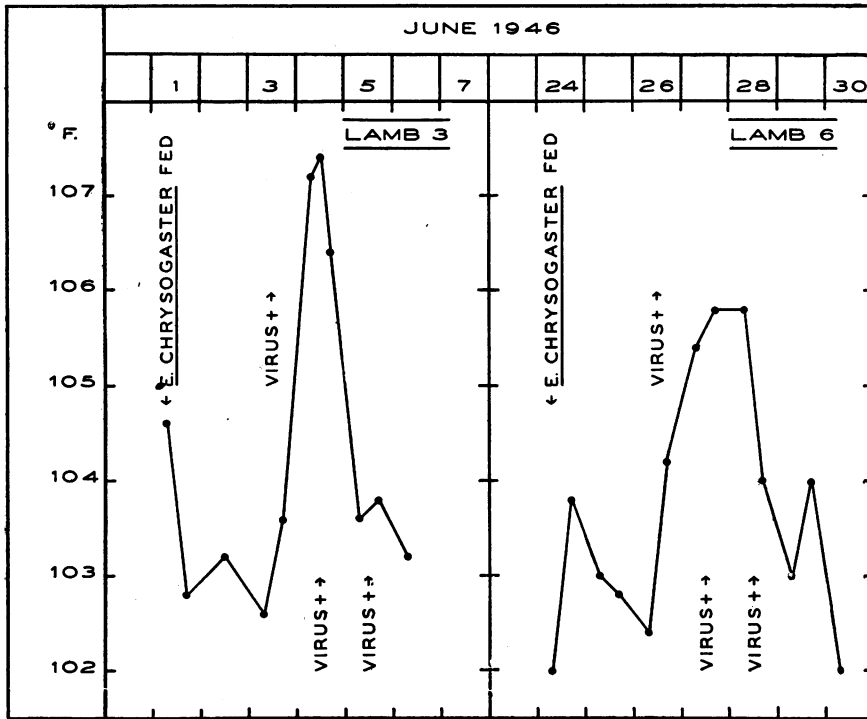


Fig. 1.—Temperature charts of Lamb 3 and Lamb 6.

the individual mosquitoes contained virus, so that either 4 or 5 of the 7 mosquitoes tested were shown to have retained the virus, all of them for more than 2 weeks.

Lamb 3 was bled daily for 10 days, and groups of mice were inoculated intraperitoneally with its serum to test this for the presence of circulating virus. Serum specimens taken on June 4 (the day of fever) and on June 5 were titrated, while on other days only the undiluted serum was tested. Sufficient virus was present in serum taken June 3 to cause the death of all the inoculated mice. On June 4 the titre of virus in the serum of the lamb was 1 in 34,000, and on June 5 it was 1 in 69 (Table II). No virus was present in blood taken on other days.

A protection test employing as virus the serum taken from Lamb 3 on June 4 against normal and Rift Valley fever immune sera, showed that the infective agent present that day in the serum of the lamb was specifically neutralized by the Rift Valley fever immune serum (Table II). Additional protection tests, employing preinfection serum, and specimens taken at various stages in the convalescence of the lamb, against stock Rift Valley fever virus, showed that demonstrable protective antibody was present 7 days after the onset of fever, or 10 days after the animal was bitten by infected mosquitoes (Table III).

Histological studies were made of the liver of a mouse inoculated with serum taken from Lamb 3 on June 3. These revealed the presence of the characteristic lesions of Rift Valley fever, in further confirmation of the transmission.

TABLE II.—*Results of the Intraperitoneal Titration of the June 4 Serum of Lamb 3, and of a Protection Test which showed that the Pathogenic Agent in that Serum was Rift Valley Fever Virus.*

Serum.	June 4 serum, Lamb 3, dilution, log.	Deaths of mice (days after inoculation).						Summary of result.		Titre of virus, 1 in—
								Died.	Lived.	
Normal rhesus . . .	0 . . .	2,	2,	2,	3,	3,	3	6	0	..
” ” . . .	2 . . .	3,	3,	3,	3,	3,	5	6	0	..
Immune human . . .	0 . . .	S,	S,	S,	S,	S,	S	0	6	..
” ” . . .	2 . . .	S,	S,	S,	S,	S,	S	0	6	..
Diluent only, for titration of virus in serum of Lamb 3	0 . . .	2,	2,	2,	3,	3,	3	6	0	} 34,000
	1 . . .	3,	3,	3,	3,	3,	4	6	0	
	2 . . .	2,	3,	3,	3,	3,	3	6	0	
	3 . . .	3,	3,	3,	3,	3,	S	5	1	
	4 . . .	3,	3,	3,	3,	3,	S	5	1	
	5 . . .	3,	3,	S,	S,	S,	S	2	4	
	6 . . .	S,	S,	S,	S,	S,	S	0	6	

S indicates that the mouse survived.

It could not be determined whether Lamb 3 was infected by mosquitoes of Lot 1 or Lot 7. However, in the light of results which follow, it seems probable that it was infected by the mosquitoes of Lot 1, and that the extrinsic incubation period was 20 days.

TABLE III.—*Results of Two Intraperitoneal Mouse Protection Tests employing 1 per cent Rift Valley Fever Virus, Showing the Acquisition of Humoral Immunity by Lambs Infected through the Bites of Eretmapodites chrysogaster Group.*

Serum.	Days after infecting bites.	Deaths of mice (days after inoculation).						Summary of result.	
								Died.	Lived.
Lamb 3, preinfection	2,	2,	2,	2,	3,	4	6	0
June 7 . . .	6 . . .	3,	3,	3,	3,	3,	3	6	0
” 8 . . .	7 . . .	3,	3,	3,	3,	3,	3	6	0
” 9 . . .	8 . . .	3,	3,	3,	3,	4,	4	6	0
” 10 . . .	9 . . .	5,	5,	6,	6,	S,	S	4	2
” 11 . . .	10 . . .	4,	S,	S,	S,	S,	S	1	5
” 17 . . .	16 . . .	S,	S,	S,	S,	S,	S	0	6
Lamb 4, normal	2,	2,	2,	2,	3,	3	6	0
5, ”	2,	2,	2,	2,	3,	3	6	0
1, immune	S,	S,	S,	S,	S,	S	0	6
Lamb 4, normal	2,	2,	2,	2,	2,	2	6	0
5, ”	2,	2,	2,	2,	2,	5	6	0
6, preinfection	2,	2,	3,	3,	3,	3	6	0
6, July 5 . . .	11 . . .	S,	S,	S,	S,	S,	S	0	6
1, immune	S,	S,	S,	S,	S,	S	0	6

S indicates that the mouse survived.

Transmission 2.—The 33 *E. chrysogaster* mosquitoes comprising Lot 7 received infective feeds from mice on May 18. One gorged on and 2 probed a normal mouse on the 6th day after the infective meal, and another probed a normal mouse on the 11th day, but no transmission occurred. Four of the mosquitoes gorged on a normal lamb on the 10th day, without transmitting virus; another 4 bit a normal lamb (No. 3) on the 14th day, probably with negative result. The final exposure to normal animals occurred on the 20th

day (June 7), when 4 of the insects bit a normal mouse (Table I). The mouse remained well during 48 hours, but was found dead on the morning of the 3rd day. It was inadvertently discarded before a protection test was made or the liver was obtained for histological examination. However, the death of the animal within the appropriate period of time and after an illness of less than 24 hours is indicative of Rift Valley fever. Moreover, all the mosquitoes that bit this mouse were shown to be infected. On the 21st day after the infective feeds the 13 mosquitoes remaining alive, including the 4 which bit a normal mouse the previous day, were tested individually for the presence of virus. Each of the 13 contained virus. Sixteen other tests for virus were made on individual mosquitoes of this lot between the 8th and the 20th day after the infective meals; 14 gave positive results. The tests on this lot, therefore, showed that 27 of 29 mosquitoes tested had retained the virus, 13 of them for as long as 21 days. The extrinsic period of incubation in this transmission was 20 days, and the intrinsic period was 3 days.

Transmissions 3 and 4.—The 44 *E. chrysogaster* group mosquitoes of Lot 11 received their infective feeds on Lamb 3 on June 4, at a time when the serum of the lamb contained 567,800 LD₅₀ of virus per ml. The lamb itself had been infected by *E. chrysogaster* group mosquitoes. Mosquitoes of Lot 11 bit normal mice on the 3rd, 5th, 7th, 9th, 11th, 13th and 15th days after their infective meals without transmitting virus. A normal mouse bitten by 2 of the mosquitoes on the 19th day (June 23) became infected (transmission 3). Individual mosquitoes probed normal mice on the 22nd and 25th days, but got no visible blood, and did not transmit virus. Five gorged on and 2 probed non-immune Lamb 4 on the 17th day, but transmission did not occur. One gorged on and 2 probed normal Lamb 6 (Table I) on the 20th day (June 24), and this lamb became infected (transmission 4). Two mosquitoes probed normal Lamb 4 on the 22nd day and one on the 25th day, but got no blood and did not infect that lamb. One mosquito gorged on normal Lamb 4 on the 29th day and again on the same lamb on the 31st day without result. This mosquito was tested on the 31st day and found to contain virus. Its failure to transmit is unexplained, but probably indicates that the vector potential of mosquitoes of the *E. chrysogaster* group is variable. Forty-two tests of individual mosquitoes of this lot showed that 33 of them retained virus.

In summary, it may be said that transmission from lamb to lamb and from lamb to mouse was effected with mosquito Lot 11, with extrinsic incubation periods of 20 and 19 days respectively, and that 78 per cent of the mosquitoes tested were proved by inoculation to have retained the virus, the longest period of retention being 31 days.

The normal mouse bitten by mosquitoes of this lot on June 23 (transmission 3) remained well during 4 days, but was sick on the morning of the 5th day. It was sacrificed, and a suspension of a portion of its liver was used as virus in a Rift Valley fever protection test. Of the 6 mice which received this suspension mixed with non-immune serum, 4 were dead on the 2nd and 2 on the 3rd day. Each of the 6 mice receiving the liver suspension mixed with Rift Valley fever immune serum remained well during 10 days. The result showed that the liver of the mouse contained an infective agent which was specifically neutralized by antibody against Rift Valley fever virus. The transmission was further confirmed by the fact that the liver of the mouse infected by the mosquitoes of

Group 11 exhibited specific lesions of Rift Valley fever. The incubation periods in this transmission were: extrinsic, 19 days; intrinsic, 5 days.

Lamb 6, bitten by mosquitoes of Lot 11 on June 24 (Transmission 4), remained afebrile and appeared well until the afternoon of June 26, when its temperature was slightly elevated and it seemed listless. The temperature was further elevated the next day (Fig. 1), and did not return to normal until June 29th. Tests in mice of serum taken from Lamb 6 showed that virus was present in its blood on June 26, 27, and 28, but not on other days. Quantitative tests were done on June 27 and 28, and titres of virus in the serum were 1 in 15,800 and 1 in 372 respectively. The liver of a mouse ill as result of inoculation with the serum of Lamb 6 taken on June 26 was used (as virus) in a Rift Valley fever protection test. Five of 6 mice receiving the suspension of this liver mixed with normal serum succumbed on the 2nd and 3rd days, while 5 of 6 receiving the liver suspension mixed with Rift Valley fever immune serum remained well; one mouse receiving the normal serum mixture survived, and one receiving the immune serum mixture died. This test showed that the infective agent in the serum of Lamb 6 was Rift Valley fever virus.

Further proof of the transmission was the observation that Lamb 6 developed protective antibody against Rift Valley fever virus as a result of the experiment (Table III). The incubation periods in this instance were: extrinsic, 20 days; intrinsic, 3 days.

Lots of E. chrysogaster with which no transmission was effected.—Thirteen *E. chrysogaster* group mosquitoes, Lot 3, received infective feeds from mice. Two of them (Table I) probed a normal mouse on the 10th day but got no blood; another bit a normal mouse on the 24th day, and one bit a lamb on the 13th day. No transmission occurred. Ten of the mosquitoes were tested individually for virus 13 to 25 days after their infective feeds. Six of these were positive, 4 on the 25th day out of 6 tested that day. In the light of other findings, the only opportunity for transmission with this lot was in the case of the mouse bitten on the 24th day, and we cannot be certain that the mosquito which bit that mouse contained virus.

Three other lots of *E. chrysogaster* group were included in the experiments. All the mosquitoes in 2 of the lots were dead by the 15th day, none having bitten normal animals after the 5th day; none of the third lot bit normal animals after the 18th day. No transmissions occurred. The 30 mosquitoes of the 3rd lot (Lot 12) received their infective feeds from Lamb 3, but after the peak of circulating virus in the animal was past, and when the titre of virus in its serum was 1 in 69, representing only 1152 effective (for mice) units of virus per ml. of serum. Each of the 30 insects in this lot was tested individually for virus. Only 6 of the 30 tests were positive, in sharp contrast with the results obtained with Lot 11, which fed on the same lamb at a time when the serum of the animal contained 567,800 LD₅₀ of virus per ml. of serum, and in which 33 of 42 mosquitoes tested were found to have retained the virus. Thus the causes of failures in transmission were probably the following: (1) failure of the insects to bite normal animals after an appropriate extrinsic incubation period, and (2) low levels of virus in the serum of the animals which were used to infect the mosquitoes.

Table 4 shows the consolidated data on all the lots of the *E. chrysogaster* group, and of other species of mosquitoes tested, and includes the results of tests for virus by inoculation as well as results of the successful and unsuccessful

TABLE IV.—*Summary of Tests for Virus by Inoculation, and of Attempts to Transmit Rift Valley Fever Virus with 4 Species of Mosquitoes.*

Species of mosquitoes.	<i>Aedes aegypti</i> .	<i>Eretmapodites chrysogaster</i> group.	<i>Taeniorhynchus fuscopennatus</i> .	<i>Taeniorhynchus uniformis</i> .
Number of lots	4	7	12	3
Number of mosquitoes	58	177	644	79
Tests for virus by inoculation into mice	{ Number* 49(49)	{ 121(122)	{ 263(348)	{ 15(15)
Latest test for virus, day	{ Positive 6	{ 78†	{ 40	{ 6
Latest positive test for virus, day	{ 37	{ 37	{ 38	{ 14
Survival after infective feed, days.	{ 26	{ 31	{ 29	{ 13
Mosquitoes alive 20th day	{ Minimum 1	{ 1	{ 1	{ 1
Last biting, day	{ Maximum 36	{ 36	{ 37	{ 13
Number of mosquitoes biting after 19th day	{ Mean 15.2‡	{ 14.4§	{ 7.4	{ 5.2
Successful transmissions	{ Number 25	{ 52	{ 34	{ 0
Incubation period, days	{ Per cent 43.1	{ 29.4	{ 5.3	{ 0
Lots failing to show virus	{ 34	{ 31	{ 34	{ 0
	{ All lots 63	{ 18	{ 77	{ 0
	{ Better lots 3	{ 18	{ 34	{ 0
	{ 0	{ 4	{ 0	{ 0
	{ Extrinsic	{ 19,20	{ . .	{ . .
	{ Intrinsic	{ 3-5	{ . .	{ . .
	{ 2/32	{ 0/-	{ 3/75	{ 2 lots not tested.

* Figures in parentheses are numbers of mosquitoes included in the tests.

† 24 of 30 in one lot were negative. Excluding these, the number retaining virus was 72 out of 91. The one poor lot was infected on a lamb after the peak of circulating virus was passed.

‡ 7 mosquitoes were sacrificed on the 29th day, thus reducing the mean.

§ 19 mosquitoes were sacrificed 20 and 24 days after the infective feeds, thus reducing the mean.

|| Numerator shows the number of lots, the denominator the number of mosquitoes tested in these lots.

attempts to transmit by bite. It will be noted that the *E. chrysogaster* group mosquitoes retain the virus much better than other species, and that they alone were successful in transmitting it.

Experiments with other species.

Transmission experiments with 4 lots of *Aedes aegypti*, 12 lots of *Taeniorhynchus fuscopennatus*, and 3 lots of *Taeniorhynchus uniformis* which had taken blood from infected mice, were unsuccessful. Three of the 4 lots of *A. aegypti* tested may be dismissed from consideration, as they evidently got little or no virus with their infective feeds. Five of the 8 mosquitoes tested in the remaining lot (Lot 5) contained virus, but normal mice bitten by insects of this lot on the 5th, 7th and 12th days after their infective feeds, and a normal lamb bitten on the 10th, 14th, 18th, 21st and 24th days (Table I), did not become infected. The mosquitoes usually fed avidly on either the mouse or the lamb offered. Their failure to transmit to mice could have been due to the fact that insufficient time had elapsed between the infecting feed and the latest attempt to transmit. This was probably not the case with the lamb, however, as this animal was bitten as late as the 24th day after the mosquitoes fed, and the tests for virus in the insects of this lot showed that the lamb was bitten on every occasion by 1 or more mosquitoes which were proved to contain virus. From this evidence we conclude that *Aedes aegypti*, if it can transmit Rift Valley fever at all, is a very poor vector.

Only 2 of 12 lots of *T. fuscopennatus* which fed on infected mice could be regarded as suitable for transmission experiments. The other 10 lots either succumbed *en masse* before transmission could have been expected to occur, or

the tests by inoculation failed to show more than a very small percentage of the mosquitoes to have retained the virus. Even the remaining two lots could not be considered good, as only 16 of 71 tests for virus in Lot 15 and 10 of 98 in Lot 17 were positive. Although mosquitoes of these 2 lots bit normal mice on 6 occasions up to the 13th day after their infective feed, they failed to transmit the virus, possibly because of an inadequate period of incubation. The 6 bitings of a normal lamb by Lot 15 (Table I) were all by the same mosquito, which contained no virus when it was found dead on the 38th day. A test for virus in a mosquito of Lot 17 found dead on the 29th day was positive, so that there was at least one infected insect in the lot each time the mosquitoes bit the lamb up to and including the 27th day. Whether or not the lamb was ever bitten by a mosquito containing virus cannot be stated. The mosquitoes that bit the lamb on the 29th and 31st days gave negative results in tests for virus.

No tests for the presence of virus were made in 2 of the 3 lots of *T. uniformis* which had fed on infected mice, and no mosquitoes of these 2 lots bit normal animals during the maximum period of 12 days that any of them lived. None of the mosquitoes of the other lot were exposed to a lamb, and none could be induced to take blood again from a mouse. All were dead by the 14th day. Fifteen mosquitoes of this lot were tested individually for virus as they died, and it was found to be present in 6 of these (Table IV). The longest period of demonstrated retention of the virus was 13 days.

Susceptibility tests on animals used in unsuccessful transmission.

Experiments.—The lambs employed in unsuccessful attempts at transmission were tested for susceptibility at the end of the experiments, either by inoculation with fully virulent pantropic virus or by serum protection tests. None was immune. All the mice bitten by mosquitoes, other than the 2 previously mentioned as having been infected, were given intraperitoneal test inoculations of virus at the end of the experiment. All were shown to be fully susceptible. It is therefore known that the 4 transmissions which we have described were the only ones that occurred.

Multiplication of virus in mosquitoes.

No tests were done with the express purpose of determining whether Rift Valley fever virus multiplies in any of the species of mosquitoes included in our experiments. Nevertheless, the tabulation of the results of inoculation tests with mosquitoes of the most satisfactory lots of each species, by intervals between infective feed and test for virus, gave information on this point which may be significant (Table V). It was seen that a high percentage of mosquitoes of the *E. chrysogaster* group harboured virus throughout the experiments. This does not prove that the virus multiplies in the group; but if it does not multiply, it must be exceedingly well maintained. That it actually does multiply in these mosquitoes is suggested by their ability to transmit the virus, but only after an extrinsic incubation period of 19 or 20 days.

Among the mosquitoes of the 2 species of *Taeniorhynchus*, on the other hand, the percentage containing virus was highest in the first 10 days after the infective feeds and then declined. This seems to indicate that, although a small proportion of *Taeniorhynchus* may maintain the virus for several weeks, in most mosquitoes of the species tested the virus slowly dies off in the insect without multiplying.

TABLE V.—*Summary of Results of Tests for Virus in Better Lots of Each Species of Mosquitoes by Intervals of 10 Days after Infective Feeds.*

Interval between infective feed and test (days).	<i>A. aegypti</i> , Lot 5.		<i>E. chrysogaster</i> group, Lots 1, 3, 7 and 11.		<i>T. fuscopennatus</i> , Lots 15 and 17.		<i>T. uniformis</i> , Lot 19.	
	Tested.	Positive.	Tested.	Positive.	Tested.	Positive.	Tested.	Positive.
1-10	2	1	22	16	117	22-1†	11	5
11-20	1	1	36	31	39	2	4	1
21-30	5	3	29	21-1*	11	1	0	..
Over 30	0	..	1	1	3	0	0	..

* 21 individual mosquitoes and 1 pool of 2 were positive, so that 22 or 23 contained virus.

† 22 individual mosquitoes and 1 pool of 2 were positive, so that 23 or 24 contained virus.

The *Taeniorhynchus* mosquitoes used in these experiments took their initial (infective) feeds well. Only mosquitoes which showed no visible blood were exposed to infected animals. The immobilized mouse could then be left in the Barraud cage unattended, and the mosquitoes which had taken blood could be easily identified when inspected a few hours later. A high percentage of those exposed to infected animals obtained blood within a few hours. However, it was only with difficulty and the exercise of considerable patience that many of them could be induced to take blood again for a transmitting feed. Moreover, the mosquitoes of this genus did not thrive well in captivity (Table IV), and it was difficult to keep them alive long enough to carry out the necessary procedures. Whether these mosquitoes are short lived in nature, or are ill suited to the environment of the laboratory, is unknown, but it seems possible that their aversion to repeated blood meals is a characteristic of the genus.

The *A. aegypti* tested were too few in number to permit conclusions with regard to multiplication of the virus in this species.

Entomological note on the E. chrysogaster group in the Kitinda area.

The *Eretmapodites chrysogaster* Graham group mosquitoes used in the transmission experiments were wild females caught in lake-side forest at Kitinda, about 2 miles west of Entebbe. The *E. chrysogaster* group as defined by Edwards (1941) contains 5 species; no wholly reliable characters are known by which the females of the group may be separated one from another. For the determination of the actual species used it was, therefore, essential to examine males derived from the same locality. Of 40 wild males collected there between December 11, 1947 and January 17, 1948, 37 were *E. chrysogaster* Graham and 3 were *E. intermedius* Edwards. It was considered possible, however, that wild females caught biting in the same locality might be of different species, so females were collected for egg-laying in order that males might be bred from them for examination. They were repeatedly offered blood meals in the laboratory. Those which fed were kept at about 24° C. for 48 hours in Barraud cages covered with damp cloths, and were allowed access to 5 per cent glucose solution. They were then isolated individually in glass tubes (15 × 85 mm.) lined with moist filter-paper and plugged with cotton. The tubes were stored at 30° C. until eggs were laid or the females died. Batches of eggs laid by individual females were hatched separately; the larvae were mainly fed with appropriate instar larvae of *Aedes (S.) aegypti* from a stock culture. *Eretmapodites* males hatching from these cultures were then examined to determine the identity of the original female which laid the eggs. In all, 608 females were collected between November

10, 1947 and January 19, 1948, and of these, 139 laid eggs. However, a considerable proportion of these egg batches failed to hatch, and only 48 were successfully reared through to yield adult males for examination; i.e., of the original 608 females, only 48, or 7.9 per cent, were identified by the male characters of their progeny. However, all these 48 were found to be *E. chrysogaster*. Although the proportion definitely identified is small, the fact that all were *E. chrysogaster*, taken together with the great preponderance of this species among the wild males, indicates that the *Eretmapodites chrysogaster* group population at Kitinda is composed almost entirely of *Eretmapodites chrysogaster* Graham itself, with only a small component of *Eretmapodites intermedius* Edwards.

We are indebted to Mrs. E. C. C. van Someren for the determination of all but 18 of the terminalia examined; the remaining 18 have been identified by comparison with the material determined by her, and were all *E. chrysogaster*.

DISCUSSION.

The circumstances of the outbreaks of Rift Valley fever affecting humans and domestic animals on farms in Kenya (Daubney and Hudson, 1931, 1933), and those of the outbreak in the Semliki Forest in Bwamba County, Uganda (Smithburn, Haddow and Gillett, 1948), were sufficiently different to indicate that the vector insects probably were not the same. The country concerned in Kenya is open table land at an altitude of about 5000 feet, whereas the affected locality in Uganda is uninhabited virgin forest at an altitude of about 2500 feet. Daubney and Hudson (1933) found that the protection of susceptible animals from mosquito bites during the hours of darkness alone sufficed for almost complete prevention of the infection, and this was strong evidence in favour of a night-biting vector. On the other hand, all the mosquitoes from which virus was isolated in the Semliki Forest outbreak were taken on human baits during the hours of daylight. Furthermore, the mosquitoes here incriminated as the probable vectors in that outbreak, the *Eretmapodites chrysogaster* group, are, in Uganda, day-biting insects which are not commonly taken in night catches. Finally, it seems most improbable that this sylvan mosquito could propagate in the open country where the Kenya epidemics occurred.

The aforementioned facts, plus the apparent demonstration of virus in wild-caught *Taeniorhynchus fuscopennatus* in Kenya (Daubney and Hudson, 1933), caused us to become interested in the genus *Taeniorhynchus*, most, if not all, of the members of which are night-biting mosquitoes. In the original paper of Daubney and Hudson (1931) this mosquito was called *Taeniorhynchus brevipalpis*, while in a subsequent communication (1933) it was designated *Mansonia fuscopennata*. Throughout the present paper we have used the terminology of Edwards (1941). The 2 species studied here, *T. fuscopennatus* and *T. uniformis*, have not yet been ruled out as possible vectors owing to the difficulty encountered in keeping them alive and in inducing them to take blood at any time after the first (infecting) feed. Nevertheless, the tests for virus in mosquitoes of these 2 species showed a low percentage infected, and an apparent decline in the percentage containing virus with increasing passage of time after the infecting feed. Neither *T. fuscopennatus* nor *T. uniformis* has, to our knowledge, ever been incriminated as the vector of any disease. However, another member of

this genus, *Taeniorhynchus (Mansonioides) africanus* Theo., has been shown to be capable of transmitting yellow fever (Philip, 1930), and this or some other as yet untested species of the genus may be capable of transmitting Rift Valley fever.

The occurrence of Rift Valley fever in the Semliki Forest, doubtless involving as host(s) some species of wild animal, the isolation of the causative virus from sylvan mosquitoes, and the subsequent experimental transmission of the disease by insects of one of the species from which the virus was isolated, not only places Rift Valley fever among the diseases which can be transmitted by insects, but associates it as well with the increasing group of diseases having cycles of infection which do not include man or domestic animals.

While the experiments here reported do not incriminate a single species of mosquito as a vector of Rift Valley fever virus, they do show that at least one of the *E. chrysogaster* group can transmit it. The mosquitoes of this group are so closely related and so alike in their habits that it seems possible that the whole group may possess vector potentialities.

SUMMARY.

Rift Valley fever virus was transmitted experimentally from mouse to lamb, from mouse to mouse, from lamb to lamb, and from lamb to mouse by the bites of mosquitoes of the *Eretmapodites chrysogaster* group. The success of these experiments indicates that these mosquitoes, which were included in the *Eretmapodites* spp. from which the virus had previously been isolated, were probably responsible for the transmission of the virus in the Semliki Forest in 1944. The period of incubation in the *E. chrysogaster* group was 19 or 20 days at 30° C.

Attempts to transmit the virus with *Aedes aegypti* and with 2 species of the genus *Taeniorhynchus* were unsuccessful. It seems probable that the former cannot transmit this virus, but the experiments with *Taeniorhynchus* were inconclusive owing to technical difficulties. The virus may survive for a number of days in these mosquitoes, and it is possible that one or another species of this genus can serve as a vector.

The results seem to place Rift Valley fever definitely in the group of insect-borne virus diseases.

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