An Observation on the Possible Effect of O'Nyong-Nyong Fever on Malaria *

by J. DE ZULUETA,[a] J. P. WOODALL,[b] J. R. CULLEN,[a] M. C. WILLIAMS,[b] G. W. KAFUKO,[a] *and* J. D. GILLETT[b]

In the course of epidemiological investigations in the southern part of Masaka district, Uganda, during the year 1960, the finding of a considerable drop in malaria rates and malaria transmission was associated with the discovery in the area of o'nyong-nyong fever, a new epidemic virus disease of East Africa.

The malaria work had been planned to investigate seasonal fluctuations in anopheline densities and possible variations in the transmission of the disease during a one-year period in a representative area of central Uganda such as the southern part of Masaka district. These observations will be discussed elsewhere and we shall deal here only with those which seem to point to the possible effect of o'nyong-nyong fever on malaria in the area.

O'nyong-nyong fever, an epidemic virus disease clinically resembling dengue, was found for the first time in 1959 in north-western Uganda. From this area it spread to the south and east, across the Kenya border, and reached the northern shores of Lake Victoria. For more information on o'nyong-nyong fever the reader is referred to a recent publication by Haddow, Davies & Walker.[c] It may be sufficient to say here that the disease is characterized by fever, headache, an itching rash and, above all, very marked joint and back pains—hence the north Uganda name of o'nyong-nyong or " joint-breaker ". In Masaka district, however, the disease was referred to as " kikonyogo ", which literally means hit or beaten by " enkonyogo ", a kind of short, rough stick.

Investigations carried out by the East African Virus Research Institute and the Medical Services of Uganda and Kenya showed that the disease had spread in 1959 through a large area of northern Uganda and Kenya, and that there had probably been about 750 000 cases, with no deaths.[c] Until our observations in Masaka district in May 1960, there had been no indication that o'nyong-nyong fever had reached that part of Uganda. Regarding the transmission of the disease, the evidence obtained by the East African Virus Research Institute indicates that the two main vectors of malaria in Africa, *Anopheles gambiae* and *A. funestus*, can harbour the virus of this disease and that at least *A. funestus* can transmit it.

Our malaria investigations in southern Masaka, carried out monthly, were centred in the small town of Rakai (Saza or County Koki). Our study area covers approximately 350 square miles (about 900 km^2), stretching from the shores of Lake Victoria (altitude 3720 feet (1134 m)) to approximately 60 miles (about 95 km) inland. The country, flat or rolling, originally a savanna country, is all below the 4700 feet (1430 m) level. Rakai itself has an altitude of 4100 feet (1250 m). Rainfall data for this station, covering our period of observations, are given in Table 1. Malaria conditions in the area have been investigated by us since December 1959. The degree of endemicity varies, according to the localities, between hyperendemic conditions (spleen-rate in children 2-10 years over 50%) and mesoendemic conditions (spleen-rate in children 2-10 years from 11% to 50%). The two vectors in the area are *A. gambiae* and *A. funestus*. Collection of blood films for the study of the monthly infant parasite-rate was started in the area in April 1960, and the results obtained are summarized in Table 2. Monthly house captures by flitting were started in May 1960, and the results of *A. gambiae* and *A. funestus* catches are summarized in Table 3. Monthly dissections of the salivary glands of *A. gambiae* and *A. funestus* were also started in May 1960, and the results are given in Table 4. Originally a twelve-month period of observations had been envisaged for the house captures and dissections (from May 1960 through April 1961) but, in view of the peculiar findings of May 1960, to which we shall refer later, it was decided to continue the captures and dissections during May 1961.

Rainfall records (Table 1) show two peaks of rainfall during the year 1960, one in April and another less marked in September. Two periods of

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[a] Uganda Malaria Eradication Pilot Project, Kabale.

[b] East African Virus Research Institute, Entebbe, Uganda.

[c] Haddow, A. J., Davies, C. W. & Walker, A. J. (1960) *Trans. roy. Soc. trop. Med. Hyg.*, **54**, 517.

TABLE 1
RAINFALL IN RAKAI, SOUTHERN MASAKA (IN INCHES)

Year	January	February	March	April	May	June	July	August	September	October	November	December
1960	2.18	2.64	3.99	6.07	3.61	0.45	0.15	1.34	3.08	2.55	1.42	0.74
1961	0.43	3.29	4.79	3.36	2.20	—	—	—	—	—	—	—

TABLE 2
RESULTS OF MONTHLY INFANT PARASITE-SURVEYS IN SOUTHERN MASAKA

	1960									1961			
	April	May	June	July	August	September	October	November	December	January	February	March	April
Number examined	86	322	194	90	135	73	113	96	108	176	145	86	131
Number positive	16	36	52	30	22	19	19	17	15	47	26	18	21
Infant parasite-rate (%)	18.6	11.2	26.8	33.3	16.3	26.0	16.8	17.7	13.9	26.7	17.9	20.9	16.0

TABLE 3
RESULTS OF MONTHLY HOUSE CAPTURES OF *ANOPHELES GAMBIAE* AND *A. FUNESTUS* IN 46 CATCHING STATIONS IN SOUTHERN MASAKA

Species	1960								1961				
	May	June	July	August	September	October	November	December	January	February	March	April	May
A. gambiae	2 336	601	115	33	47	31	12	11	24	23	52	150	141
A. funestus	1 540	2 044	2 262	3 636	1 629	612	458	875	835	719	941	903	1 107

dry weather towards the middle and the end of the year are also noticeable. This is the normal pattern of rainfall for Masaka district and indeed for most of central and western Uganda. *A. gambiae* density, as judged by our house captures (Table 3), had its peak in May, immediately after the April peak of rainfall. *A. funestus* reached its highest density in August, shortly after the June-July dry period. *A. funestus* densities tended to increase again in December, during the second dry period.

The association of high densities of *gambiae* with abundant rainfall and of high densities of *funestus* with periods of dry weather follows the general pattern encountered for these two species in East Africa. What is unexpected in our observations is the finding of high densities of *A. gambiae* and *A. funestus* during the month of May 1960, with favourable meteorological conditions for transmission associated with a very low sporozoite-rate (Table 4). Of 533 *A. gambiae* dissected in May,

TABLE 4

RESULTS OF SALIVARY GLAND DISSECTIONS IN *ANOPHELES GAMBIAE* AND *A. FUNESTUS* IN SOUTHERN MASAKA

Year	Month	*A. gambiae*			*A. funestus*		
		Number dissected	Number positive	Sporozoite-rate (%)	Number dissected	Number positive	Sporozoite-rate (%)
1960	May	533	3	0.6	295	0	0.0
	June	390	19	4.9	402	2	0.5
	July	80	5	6.3	369	5	1.3
	August	24	0	0.0	370	3	0.8
	September	44	0	0.0	581	4	0.7
	October	27	1	3.7	410	6	1.5
	November	49	0	0.0	537	0	0.0
	December	25	2	8.0	610	1	0.2
1961	January	24	1	4.2	399	1	0.3
	February	22	0	0.0	540	3	0.6
	March	53	0	0.0	501	3	0.6
	April	113	0	0.0	507	1	0.2
	May	118	3	2.5	503	5	1.0

only three showed sporozoites in their glands, and no infection was found in 295 *A. funestus* dissected during the same month. This gives a sporozoite-rate of 0.6% for the first species and one of 0.0% for the second. The over-all sporozoite-rate for *A. gambiae* from June 1960 through May 1961, based on 969 specimens, was 3.2%, and the over-all sporozoite-rate for *A. funestus* during the same period of time, based on 5729 specimens, was 0.6%.

A sporozoite-rate of 0.6% in *gambiae* is difficult to explain. It is, in fact, even more difficult if we consider that in Rakai and neighbouring inland catching stations no infection was detected in a total of 386 *A. gambiae* gland dissections. The three positive specimens were found among 147 dissected from capture stations close to Lake Victoria. It is less significant that no infections were found in any of the *A. funestus* dissected, since the over-all sporozoite-rate in this species is much lower than in *A. gambiae* in our series of observations. In fact, later on in the month of November, we found no sporozoite infections in a large sample (537 specimens) of *funestus* dissected. It may also be mentioned here that in May 1960, in addition to the gland dissections whose results are shown in Table 4, we made 50 stomach dissections of *A. gambiae* and 50 of *A. funestus* from the Rakai area, all of them negative for malaria parasites. Moreover, the gland dissections carried out in May 1961 yielded normal results.

The unexpectedly low sporozoite-rate during May 1960 is coupled with a low infant parasite-rate during the same month, the lowest, in fact, in our period of observations (Table 2). We carried out three general spleen and parasite surveys in schoolchildren in our study area in Masaka district, and obtained the following results:

	No. exam.	*Enlarged spleen*	*Spleen-rate (%)*	*Positive bloods*	*Parasite-rate (%)*
1st survey, Dec. 1959	691	302	43.7	234	33.9
2nd survey, June 1960	845	337	39.9	181	21.4
3rd survey, Nov.-Dec. 1960	691	215	31.1	104	15.0

Our data point to a slow decrease in both the spleen- and the parasite-rate from December 1959 through December 1960. This decrease is not,

however, comparable with the sudden drop in malaria rates found in the schoolchildren in Rakai in May 1960. The examination of the schoolchildren in this locality at three different times gave the following results:

	No. exam.	*Enlarged spleen*	*Spleen-rate (%)*	*Positive bloods*	*Parasite-rate (%)*
3 Dec. 1959	76	46	60.5	40	52.6
19 May 1960	149	98	65.8	16	10.7
30 Nov. 1960	76	41	53.9	16	21.0

The drop in the parasite-rate in Rakai schoolchildren from 52.6% (December 1959) to 10.7% (May 1960) is much more marked than anything seen in the rest of Masaka district; it was, in fact, compensated later by a slight increase—to 21.0% in November 1960—bringing the parasite-rate in Rakai in line with the findings in other parts of the district.

Turning now to the virological findings in the area, cases of fever clinically resembling o'nyong-nyong fever came to our notice in May 1960. Two surveys were made, in mid-June and mid-July, with the object of isolating the virus and obtaining serum samples for antibody studies. The techniques used for isolation have been described by Williams & Woodall [d] and those for haemagglutination-inhibition by Clarke & Casals [e] and by Williams, Woodall & Porterfield.[f]

The June survey yielded 14 isolations of o'nyong-nyong fever virus from acute cases in the malaria study area, and confirmed (by the detection of antibodies) that 35 other people who gave a suggestive history had been infected with this virus; 43 of the 49 gave a history of onset in the first two weeks of June. Mosquito catches made in June by spraying the huts of acute cases yielded two isolations of o'nyong-nyong fever virus from a total of 165 *A. funestus* and one more from a total of 21 *A. gambiae* from Kakuto, a locality 12 miles (about 20 km) south of Rakai, in the malaria study area, but none from 326 *A. funestus* and 11 *A. gambiae* from Rakai itself.

The July survey yielded only three new isolations from human cases and none from mosquitos (260 *A. funestus*), and the impression was gained that the epidemic had subsided. Paired convalescent serum samples were taken from such as could be traced of the people who had been seen as acute cases in June, and a serum survey was made of 13 inhabitants of four huts in the Rakai area which had been used in the malaria work for routine monthly captures of anopheline mosquitos. Although 9 of the 13 claimed to have suffered from o'nyong-nyong fever only 6 proved to have antibodies. This would indicate, however, that a good number of the *gambiae* and *funestus* dissected for the presence or absence of sporozoites had been in contact with o'nyong-nyong fever cases. Further information on the prevalence of the disease in the malaria study area was obtained in June 1961, when antibodies were found in 34 schoolchildren among 77 examined in the Rakai area. Regarding the onset of the o'nyong-nyong fever epidemic, the local people stated that the disease appeared in March 1960 around Rakai and spread from there to the rest of the malaria study area.

The virological findings thus indicate a high prevalence of o'nyong-nyong fever in the malaria study area with a large number of human cases in June 1960, suggesting a peak of mosquito infections in May (since there is probably an incubation period lasting one week or more in man and a latent period between infection and transmission in the mosquito). By July the epidemic which had probably started around March was clearly waning.

Discussion

As can be seen, all our entomological and malariometric data point to a very great reduction in malaria transmission during the month of May 1960, when from the favourable meteorological conditions and high vector densities high transmission was to be expected; and in fact the vector species, *A. funestus* and *A. gambiae*, were transmitting o'nyong-nyong fever virus very efficiently in the area during that time. This suggests that the virus may have inhibited the development of the malaria parasites either in the vector or in the human host.

We should like to emphasize that our observations simply point to the possible effect of o'nyong-nyong fever on malaria and that only indirect evidence is brought forward in this note. Experimental work undertaken by us in August and September 1960 to ascertain in the laboratory any possible relationship between the two entities could not be completed, owing to the lack of gametocyte carriers when these

[d] Williams, M. C. & Woodall, J. P. (1961) *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 135.

[e] Clarke, D. H. & Casals, J. (1958) *Amer. J. trop. Med. Hyg.*, **7**, 561.

[f] Williams, M. C., Woodall, J. P. & Porterfield, J. S. (in press).

were required in the experiments. The proof that a virus disease such as o'nyong-nyong fever has an effect on a protozoal disease like malaria will be of considerable importance and we recommend that further experimental work be undertaken at institutions where volunteers or patients undergoing malaria therapy are available and transmission experiments can be satisfactorily planned and carried out.[g]

We may mention here that at least two instances of the interaction of viruses and malaria parasites are known. The level of viraemia in western equine encephalitis seems to be lower in canaries inoculated with this virus and *Plasmodium relictum*[h] than in canaries without malaria. In another series of experiments the development of malaria parasites was retarded in ducklings inoculated with *P. lophurae* and a suitable dose of what is described by the author as " spleen necrosis virus ".[i] If some interaction does exist between viruses and avian malaria parasites, it is not impossible that the same could happen in the case of o'nyong-nyong fever and human malaria, particularly since the virus of the former disease is known to persist in the two main vectors of malaria in Africa, *A. gambiae* and *A. funestus*.

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[g] In addition to the possibility that the virus, if present in a sufficiently high proportion of mosquitos, may have inhibited the development of the malaria parasites, there is an alternative possibility that it may have shortened the average life of the malaria vector and thus interfered with transmission. This aspect may be worth investigating.—ED.

[h] Barnett, H. C. (1956) *Amer. J. trop. Med. Hyg.*, **5**, 99.

[i] Trager, W. (1959) *Proc. Soc. exp. Biol. (N. Y.)*, **101**, 579.

A Method of Determining Insecticide Persistence in Tsetse Fly Control Operations

by R. J. KERNAGHAN, *Senior Entomologist, and* M. R. L. JOHNSTON, *Entomologist, Ministry of Health, Northern Region of Nigeria*

Recent developments in the control of tsetse flies by insecticidal methods in the Northern Region of Nigeria have brought about a need for a standard method of determining the persistence of an insecticidal deposit. The method of control is normally a single application of insecticide to the vegetation of the habitat during the dry season, at which time the tsetse are concentrated in the thickets. The single-application technique is dependent on the effective persistence of the insecticide for the duration of the probable maximum pupal period (usually reckoned as 7-8 weeks). As Kirkby & Blasdale[a] point out, the times of maximum concentration are limited and uncertain (being influenced by climatic conditions), and the only means of extending the spraying season is to begin before the concentration is complete, depending on the persistence of the toxic effect of the insecticide throughout the period of concentration. The length of time by which persistence extends beyond the maximum pupal period is therefore important in determining the date when spraying operations should begin for optimum effectiveness.

Chemical assay of the residual deposit from normal spraying of an insecticide on vegetation is often impractical because of the irregular distribution of the deposit, difficulties of sampling, calculation of the actual sprayed area, etc.; in any case, it would give only a figure of the total insecticidal deposit present, irrespective of the quantity actually available to the insect. A bio-assay method is preferable because, although the sampling difficulties remain, it gives a measure of the activity of the deposit.

Owing to the conditions in which it may have to be used, any such method must be simple in design and

[a] Kirkby, W. W. & Blasdale, P. (1960) *Bull. ent. Res.*, **51**, 253.