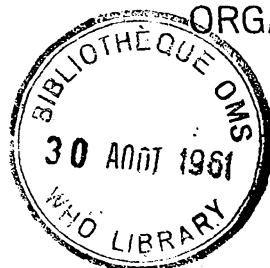


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WHO/Mal/305
2 August 1961

ORIGINAL: ENGLISH

AN OBSERVATION ON THE POSSIBLE EFFECT OF O'NYONG-NYONG FEVER
ON MALARIA¹

by

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In the course of epidemiological investigations in the southern part of Masaka district in 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 (ONN) 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 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 in a separate publication and we will deal here only with those which seem to point to the possible effect of ONN 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 ONN the reader is referred to a recent publication by Haddow, Davies & Walker (1960). It may be sufficient to say here that the disease is characterized by fever, headache, an itching rash and,

¹ Report of work carried out by the East African Virus Research Institute and the Uganda Malaria Eradication Pilot Project, supported jointly by the Ministry of Health, Uganda Protectorate, and the World Health Organization.

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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 been probably 750 000 cases with no deaths (Haddow, Davies & Walker, 1960). Until our observations in Masaka district in May 1960, there was no indication that ONN 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 Anopheles 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 has a surface of approximately 350 square miles and it stretches from the shores of Lake Victoria (altitude 3720 feet) to approximately 60 miles inland. The country, flat or rolling, originally a savanna country, is all below the 4700 feet level. Rakai itself has an altitude of 4100 feet. Rainfall data for this station, covering our period of observations, are given in Table I. 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 per cent.) and mesoendemic conditions (spleen rate in children 2-10 years between 11 and 50 per cent.). 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 were summarized in Table II. Monthly house captures by flitting were started in May 1960 and the results of A. gambiae and A. funestus catches are summarized in Table III. Monthly dissections of salivary glands of A. gambiae and A. funestus were also started in May 1960, and the results of them are given in Table IV. 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 will refer later, it was decided to continue the captures and dissections during May 1961.

Rainfall records (Table I) show two peaks of rainfall during the year 1960, one in April and another less marked in September. Two periods of dry weather towards the end and the middle 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 III) 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 tend to increase again in December during the second dry period.

The association of high gambiae densities with abundant rainfall and of funestus high densities 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 funestus during the month of May 1960, with favourable meteorological conditions for transmission associated with a very low sporozoite rate (Table IV). Out of 533 A. gambiae dissected in May, only three showed sporozoites in their glands, and no infection was found in 295 A. funestus dissected the same month. This gives a sporozoite rate for the first species of 0.6 per cent. and of 0.0 per cent. for the second. The over-all sporozoite rate for A. gambiae from June 1960 through May 1961, based on 969 specimens, has been 3.2 and the over-all sporozoite rate for A. funestus during the same period of time, based on 5729 specimens, has been 0.6.

A sporozoite rate in gambiae of 0.6 is difficult to explain. It is, in fact, 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. The finding of no infections in all the A. funestus dissected is less significant, since the over-all sporozoite rate in this species is much lower than in A. gambiae in our series of observations. We found, in fact, later on in the month of November, no sporozoite infections in a large sample of funestus dissected, 537 specimens.

We may add here that in May 1960, in addition to the gland dissections shown in Table IV, we made 50 stomach dissections of A. gambiae and 50 of A. funestus from the Rakai area, all of them negative for malaria parasites. Also that gland dissections in May 1961 yielded normal results (Table IV).

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 II). We have carried out three general spleen and parasite surveys in schoolchildren in our study area in Masaka district, and we have obtained the following results:

	<u>Number examined</u>	<u>Enlarged spleen</u>	<u>Spleen rate (%)</u>	<u>Positive bloods</u>	<u>Parasite rate (%)</u>
1st survey, December 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 parasite rates from December 1959 through December 1960. This decrease, however, is not 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:

<u>Date</u>	<u>Number examined</u>	<u>Enlarged spleen</u>	<u>Spleen rate (%)</u>	<u>Positive bloods</u>	<u>Parasite rate (%)</u>
3 December 1959	76	46	60.5	40	52.6
19 May 1960	149	98	65.8	16	10.7
30 November 1960	76	41	53.9	16	21.0

The drop in the parasite rate in Rakai schoolchildren from 52.6 per cent. (December 1959) to 10.7 per cent. (May 1960) is much more marked than anything seen in the rest of Masaka district, and in fact was compensated later by a slight increase

to 21.0 per cent. (November 1960), bringing the parasite rate there 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 ONN 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 (1961) and those for haemagglutination-inhibition by Clarke & Casals (1958), and by Williams, Woodall & Porterfield (in press).

The June survey yielded 14 isolations of ONN 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 undergone infection with ONN; 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 ONN virus from a total of 165 A. funestus and two more from a total of 24 A. gambiae from Kakuto, a locality 12 miles 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 were used in the malaria work for routine monthly captures of anopheline mosquitos. Although nine of the 13 claimed to have suffered from ONN fever only six 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 ONN cases. Further information on the incidence of the disease in the malaria study area was obtained in June 1961 when antibodies were found in 34 schoolchildren among 77 examined in Rakai area. Regarding the onset of the ONN 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 incidence of ONN fever in the malaria study area with a high number of human cases in June 1960, suggesting a peak of mosquito infections in May (since there is probably an incubation period of a 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 malarimetric 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 ONN 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 would like to emphasize that our observations simply point to the possible effect of ONN on malaria and that only indirect evidence is brought forward in this paper. 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, due to lack of gametocyte carriers when they were required in the experiments. The proof that a virus disease like ONN 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 human volunteers or malaria-therapy cases are available and transmission experiments can be satisfactorily planned and carried out.¹

We may mention here that at least two cases are known on interaction of viruses and malaria parasites. The level of viremia in Western Equine Encephalitis seems to be lower in canaries inoculated with this virus and Plasmodium relictum (Barnett, 1956) than in canaries without malaria. In another series of experiments the development of

¹ In addition to the possibility that the virus if present in a sufficiently high proportion of mosquitoes may have inhibited the development of the malaria parasites there is an alternative probability that it may have shortened the average life of the malaria vector and thus interfered with transmission. This aspect may be worth investigating. [-Editor's remark]

malaria parasites was retarded in ducklings inoculated with Plasmodium lophurae and a suitable dose of what is described by the author as "spleen necrosis virus", (Trager, 1959). If some interaction may exist between viruses and avian malaria parasites, it is not impossible that the same could happen in the case of ONN and human malaria, particularly since the virus of ONN is known to persist in the two main vectors of malaria in Africa, A. gambiae and A. funestus.

SUMMARY

Field observations pointing to a possible effect of o'nyong-nyong fever on malaria are presented in this paper. O'nyong-nyong fever was found accidentally during epidemiological investigations of malaria in the southern part of Masaka district in Uganda. Unexpectedly low malaria transmission and parasite rates were observed in May 1960 when transmission of o'nyong-nyong fever was at its peak in the area. The possibility of this new virus disease having an effect on malaria is discussed and laboratory investigations to elucidate this point are recommended.

ACKNOWLEDGEMENTS

The authors wish to thank all those who have facilitated the carrying out of the work reported in this paper. They would like to thank in particular Dr P. Esmonde, M.C., Principal Medical Officer, Buganda, for much interest and assistance and Dr A. K. Kibaya, M.B.E., District Medical Officer, Masaka, for his great co-operation and personal help.

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TABLE I. RAINFALL RAKAI, SOUTHERN MASAKA
(in inches)

Year	Month											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
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 II. RESULTS OF MONTHLY INFANT PARASITE SURVEYS IN
SOUTHERN MASAKA

	1961												
	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	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 III. RESULTS OF MONTHLY HOUSE CAPTURES OF A. GAMBIAE AND A. FUNESTUS IN
46 CATCHING STATIONS IN SOUTHERN MASAKA

	May	June	July	1960			1961			April	May		
				Aug.	Sept.	Oct.	Nov.	Dec.	Jan.			Feb.	March
<u>A. gambiae</u>	2 336	601	115	33	47	31	12	11	24	23	52	150	141
<u>A. funestus</u>	1 540	2 044	2 262	3 636	1 629	612	458	875	835	719	941	903	1 107

