Malaria along the southernmost fringe of its distribution in Africa: epidemiology and control *

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After more than thirty years of malaria control in northern Transvaal with residual insecticides, malaria prevalence has been reduced to a low level. However, low-grade transmission of Plasmodium falciparum *continues, with periodic focal outbreaks after abnormally high rainfall. From October 1973 to September 1976, the operational and epidemiological factors involved in this residual transmission were studied in over 17 000 people of an area of northern Transvaal. Incidence surveys based on the screening of fever cases revealed 42 autochthonous cases of malaria in 1974–75 and 10 cases in 1975–76. Parasite prevalence surveys were not sensitive enough to assess the malaria situation, and serological testing indicated different levels of infection according to the method used. One of the two principal vectors of malaria in Africa—*Anopheles funestus *Giles—was not detected in the project area, and the* A. gambiae *group (species A and B) was found in extremely low numbers, so that it could not have accounted for the low-grade transmission in the area. A recently discovered member of the* A. funestus *group somewhat resembling* A. aruni *Sobti, and a species hitherto undiscovered in the Transvaal, which is abundant in the area and is indistinguishable from* A. flavicosta *Edwards, may be involved. Both were found biting man—mostly outdoors during the four hours following dusk, when people frequently gather outside their houses and are thus vulnerable to mosquito bites.*

Experience in malaria control outside Africa cannot be extrapolated to African countries in view of their specific epidemiological, ethnic, social, and economic factors. Therefore the knowledge required for implementing realistic antimalaria programmes in Africa can be gained only through in-depth studies of the full range of prevailing epidemiological situations. The epidemiology of stable malaria and changes that can be produced with different intervention methods are being studied in East and West Africa. In order to complete existing knowledge on the epidemiology and control of malaria in Africa, it was found necessary to undertake a study in areas of unstable malaria. Unstable malaria is encountered in southern Africa below latitude 20°S, in an area encompassing Botswana, Mozambique, Namibia, Southern Rhodesia, South Africa, and Swaziland. The southernmost limits of malaria transmission extend to northern Transvaal and a portion of Zululand. In these areas where the disease has been reduced to low levels through land cultivation and antimalaria measures, malaria epidemics occur periodically against a background of non-immunity and result in waves of malaria morbidity and mortality. The surveillance system and the resulting antimalaria preventive and remedial measures have to be adapted to these specific situations. In order to identify practicable methods, field research was carried out in northern Transvaal from October 1973 to September 1976.

Prior to malaria control, northern Transvaal, which now includes rich agricultural areas, was largely undeveloped. An indication of the high levels of malaria existing then is given by Swellengrebel et al. (*9*), who found that 94% of children 2–5 years old, from the foothills of Tzaneen and Lebombo, were positive for the parasite. Mosquito surveys

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made in February–April 1931 showed significant numbers of *Anopheles gambiae* and *A. funestus* in houses, high proportions of these mosquitos (up to 11% of *A. gambiae* and 27% of *A. funestus*) having infected salivary glands.

House spraying with DDT was introduced in 1946. DDT was replaced by HCH in 1950 and reinstated in 1969. Good control of malaria was achieved by the early 1950s. In an unpublished report on the malaria situation in northern Transvaal, made by a WHO malaria assessment team late in 1959, a prevalence survey is stated to have indicated a crude parasite rate of 0.7%, all positive cases being infected with *Plasmodium falciparum*, associated in two instances with *P. malariae*. No *A. funestus* were detected in houses, but an average density of 0.11 *A. gambiae* per house was found by means of pyrethrum spray catches. The assessment team also reported that, out of a total population of 2 036 486 in northern Transvaal, 141 300 were at malaria risk. Between 1966 and 1975, the incidence of malaria was generally very low (with a yearly average of 964 cases), but a major flare-up occurred in 1972, when 3731 cases were detected.[a]

The 1972 peak of malaria cases was preceded by exceptionally heavy rainfall during the summer months (November 1971–March 1972). The close relationship between the number of malaria cases

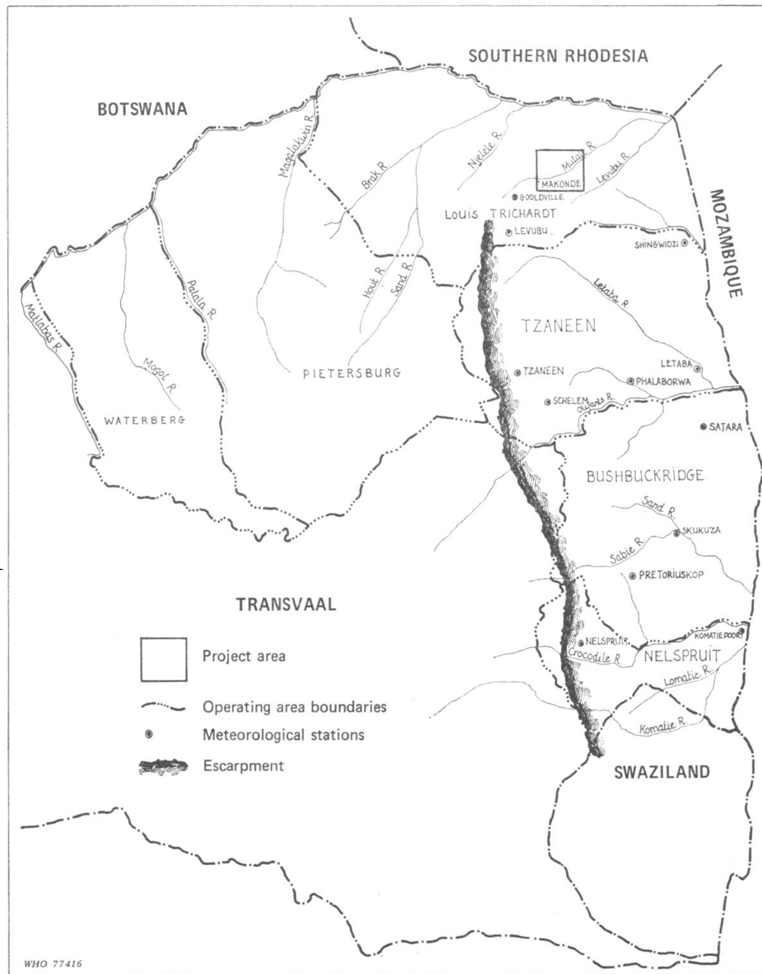

Fig. 1. Map of northern Transvaal, showing the project area.

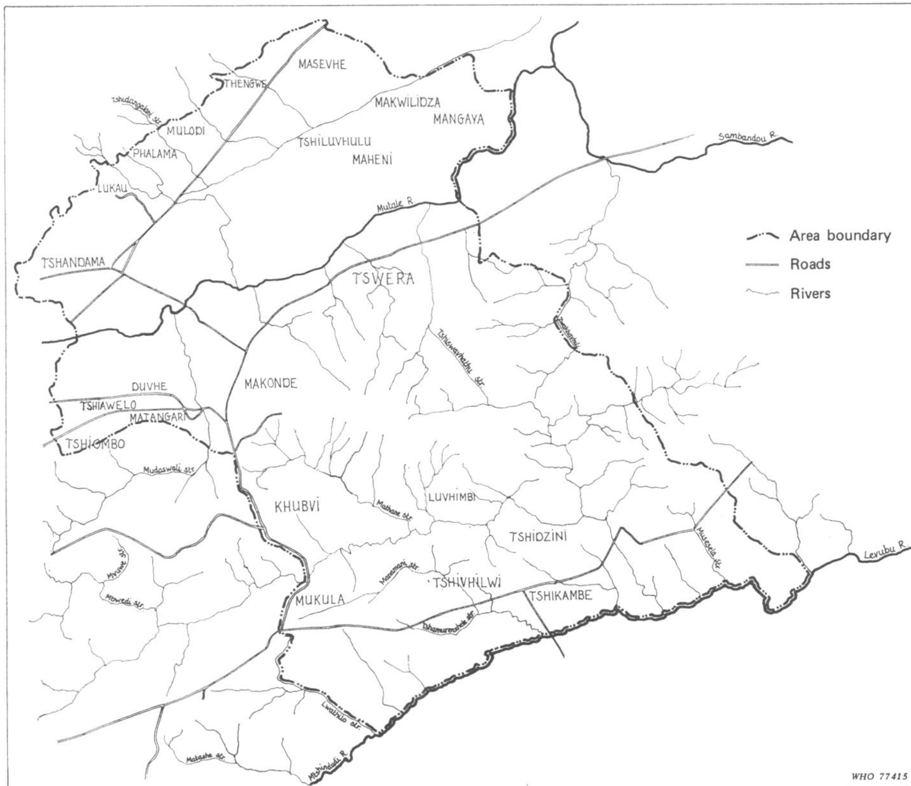

Fig. 2. Makonde project area.

and summer rainfall [a] was also indicated by the reverse situation, in 1970, when there were exceptionally few malaria cases (147) and unusually low rainfall (117 mm) during the period January–March compared with a mean of 348 mm for the corresponding period in the years 1969–73.

The numbers of *A. gambiae* group (677 specimens) and of *A. funestus* group (31 specimens) collected in approximately 10 000 houses during the periods July 1971–June 1972 and July 1972–June 1973 by means of pyrethrum spray catches,[a] clearly show that relatively many *A. gambiae* (547 specimens) were present during the period February–May 1972, i.e., during the malaria resurgence in 1972. On the other hand, the extremely low numbers of *A. funestus* (12 in 1971–72 and 19 in 1972–73) appear to have been unrelated to the malaria flare-up of 1972.

[a] Detailed tables have been deposited in the WHO Library and photocopies are available at cost of reproduction from Chief, Office of Health Literature Services, World Health Organization, 1211 Geneva 27, Switzerland.

The factors involved in the low-level transmission persisting in northern Transvaal after more than 30 years of control activities and in the periodic resurgence of the disease were assessed by methods sensitive enough to monitor this unstable situation. This article describes the results of the studies.

PROJECT AREA AND OPERATIONAL INFRASTRUCTURE

The area selected for the study (Fig. 1 and 2) lies in the Sibasa District in the extreme north of the Transvaal, its centre being situated at approximately 22°45′S and 30°35′E. It extends over 250 km² in two broad river plains—the Mutale in the north and the Mutshindudi in the south. Altitudes range from 500 to 1000 m above sea level. Data from the Gooldville Meteorological Station, situated 12 km south-west of the area, show a mean annual rainfall of 1069.6 mm, the wettest months being generally between October and April. The hottest season is

between October and March, when mean maximum temperatures range from 26.7° to 29.1°C and the coldest season is between May and August, when mean minimum temperatures range from 12.0° to 14.4°C.

The study population comprised 17 300 Venda people living in 22 villages. Houses have circular mudbrick walls and conical grass-thatched roofs. Approximately one-quarter of the houses are re-plastered (or mud-washed) in December in preparation for the Christmas holidays, when many of the men return home from working outside the area. The land is used primarily for grazing cattle, which are penned outdoors at night, but there is small-scale domestic cultivation of maize, millet, and sorghum throughout the area.

Parasitological surveys were periodically carried out and the incidence of new malaria cases was measured longitudinally by examining 100 microscope fields of thick blood smears collected from persons with fever (passive detection system) attending the four clinics and hospital serving the area. In August 1975, an active detection system, based on monthly house-to-house visits, was developed. The procedure for screening fever cases and examining their blood for the presence of malaria parasites was the same.

Blood films were taken for parasitological examination and, at the same time, blood samples were taken for serological testing by means of the immunofluorescence antibody (IFA) test described previously (*2*). For this purpose, *P. fieldi* antigen was obtained from splenectomized *Cercopithecus aethiops* monkeys at the Virus Research Institute, Rietfontein, Johannesburg. Wellcome antihuman immunoglobulin (sheep), an IgG product that also cross-reacts with IgM and IgA, was used. In addition, *P. falciparum* antigen, obtained from the Gambia, was used for comparative studies with *P. fieldi* antigen.

The entomological studies basically consisted in monthly observations in 6 sample villages. Methods included the use of exit-window-traps and pyrethrum spray catches in houses; the collection of anophelines resting outdoors in artificial pit-shelters; and indoor and outdoor night-biting catches from human beings.

Except for 136 houses in the remote village of Luvhimbi, situated in the central mountainous region, houses in the project area were sprayed annually, between August and November, with 75% DDT (wettable dispensable powder) at 2 g per m^2, by means of Hudson X-pert sprayers with 8002E CHSS nozzles. In 1974, about 90% of the houses were sprayed. In 1975, at least 94% were sprayed and the quality of application was satisfactory.

PARASITOLOGICAL AND SEROLOGICAL SURVEYS AND CASE-DETECTION ACTIVITIES

The methods described above were used both in the project area and outside the project area in a malaria focus and in an area where malaria had not been detected for 15–20 years.

Project area

Not a single malaria-positive smear was detected in over 6000 thick blood films collected in the course of prevalence surveys during the period October 1973–September 1976.

Passive case detection was done through Thengwe and Tshiombo clinics in the project area and Tshaulu and Sterkstroom clinics just outside it; these clinics were part of the Donald Fraser Hospital network, and the hospital itself played an important part in passive case detection. An active case detection system was also developed, and has operated since August 1975. Forty-two autochthonous cases of malaria were detected in the area in 1974–75 and ten in 1975–76. All were falciparum infections from scattered foci,[a] coming from 13 localities in 1974–75 and 4 localities in 1975–76.

IFA results from the project area, with *P. fieldi* [b] antigen are given in Table 1. The combined results for all villages showed positivity to titres of 1 : 20 and above in 22% of those in the age group 0–4 years and 46–47% of those in higher age groups. In surveys in which the filter paper method was replaced by the capillary tube technique, the corresponding values were 29% and 36%.

Outside the project area

An area of focal malaria. A sudden, localized outbreak of malaria developed during February 1975 at Mandlakazi—a small village outside the project area, about 20 km north-east of Tzaneen: 13 infections had been found by passive case detection during the previous 5 months, and 83 falciparum infections were detected among 768 blood smears

[a] See footnote *a* on page 97.

[b] When *P. falciparum* antigen obtained from the Gambia was used in parallel with *P. fieldi*, 85.6% of combined samples from within and outside the project area were negative to both antigens; 3.7% were positive to both antigens; and 10% were negative to one antigen but positive to the other.

Table 1. Levels of positivity recorded in IFA tests using *P. fieldi* antigen: Makonde study area, 1975

Sampling technique	Age group (years)	No. examined	Titre			
			⩾1 : 20		⩾1 : 40	
			No. positive	% positive	No. positive	% positive
Filter paper						
	0-4	403	90	22	30	7
	5-9	924	427	46	175	19
	⩾10	1425	664	47	267	19
Capillary tube						
	0-4	773	221	29	48	6
	5-9	1017	367	36	105	10
	⩾ 10	202	72	36	29	14

Table 2. Fluorescent antibody tests with the filter paper technique and *P. fieldi* antigen Mandlakazi, March 1975

Site	Age (years)	No. examined	Titre ⩾1 : 20		Titre ⩾1 : 40	
			No.	%	No.	%
Adjacent to river	0–4	49	15	30.6	9	18.4
	5–9	68	21	30.9	16	23.5
	10–19	19	13	68.4	12	63.2
	20–40	88	72	81.8	65	73.9
1 km from river	0–4	18	1	5.6	0	0.0
	5–9	24	0	0.0	0	0.0
	10 +	31	5	16.1	1	3.2
School (pupils from both sites)	5–9	109	57	52.2	29	26.6
	10–19	96	50	52.1	30	31.3

taken during the second half of February. Among children aged 0–4 years who lived close to the local river, 30.6% were found to have serological titres equal to or above 1 : 20 (considered as positive), compared with 5.6% for children living 1 km away from the river. Further details are given in Table 2.

Malaria-free area. An area in which malaria had not been detected for 15–20 years was selected for control purposes. Blood samples were taken from 750 people at Mamabolo, situated 70 km east of Tzaneen: all were parasitiologically negative. IFA tests were performed with *P. falciparum* antigen, and the findings showed negative titres for children in the age group 0–4 years and positive titres rising from 3% in the age group 5–9 years to 24% in people aged 40 years or over. Details are given in Table 3.

Table 3. *P. falciparum* titres in Mamabolo, selected for use as a malaria-negative control area

Age groups (years)	Mean age group	No. examined	Titres ⩾ 1 : 16 observed	PTI	MTI	MPTI
0–4	(2.2)	151	0	0	0	0
5–9	(7.2)	105	3	2.9	0.02	1.0
10–19	(15.4)	245	20	8.1	0.10	1.3
20–39	(26.8)	151	13	8.6	0.17	2.1
40 +	(51.8)	98	24	24.4	0.58	2.4
Total		750	60	8.0	0.15	1.8

ENTOMOLOGICAL OBSERVATIONS

Of the 37 wild-caught larvae of the *A. gambiae* group collected from the project area, 12 were identified cytotaxonomically as species A and 25 as species B.

The most reliable means of identification of a wild-caught member of the *A. funestus* group is to examine its larval and adult progeny: these can be obtained readily under good insectary conditions. Of 23 members of the *A. funestus* group collected from pit-shelters and examined in this way, 14 from five sample villages were identified as *A. aruni* and 9 from a sample village (Tshidzini) as *A. leesoni* Evans. No other members of the *A. funestus* group were identified in the project area. The designation " *A. aruni* " for the material from the project area is provisional, since there are small but constant differences between the project " *A. aruni* " and *A. aruni* Sobti (*8*).

The most abundant group of anophelines caught in the project area were apparently members of the *A. marshallii* group, most of which (91% in houses, 73% in pit-shelters, and 26% biting man) were of a pale unidentified species, hitherto undetected in South Africa and very closely resembling *A. flavicosta* Edwards. Specimens of this species have been deposited in the British Museum and are being studied further. *A. marshallii* (Theobald) occurred in typical adult form, but also exhibited a confusing range of polymorphism with pale-wing forms predominating during the hottest months (November–March) and dark-wing forms during the coldest months (June and July). Other species of anopheline caught in the project area were: *A. pretoriensis* (Theobald); *A. rufipes* (Gough); *A. maculipalpis* Giles; *A. squamosus* Theobald; *A. theileri* Edwards; *A. cydippis* De Meillon; *A. coustani* (Laveran); *A. rhodesiensis* Theobald; *A. nili* (Theobald); and *A. natalensis* (Hill & Haydon), multicinctus form.

Pyrethrum spray catches made between August 1974 and April 1976 showed average densities of 0.01 for the *A. gambiae* group, 0.02 for the *A. funestus* group, and 0.21 for the *A. marshallii* group in untreated houses, with corresponding values of 0.004, 0.005, and 0.03 in DDT-treated houses. Exit-window-trap catches made between February 1975 and April 1976 showed no members of the *A. gambiae* or *A. funestus* groups and average densities per trap of 0.1 for the *A. marshallii* group for untreated houses, with corresponding values of 0.001, 0.001, and 0.06 for DDT-treated houses. Precipitin-test examination of blood-fed anophelines (one from the *A. funestus* group, 11 from the *A. marshallii* group, and one from the *A. rufipes* group from pyrethrum spray-catches; and 4 from the *A. marshallii* group from exit-window-traps) showed all to have fed on cattle and none on man.

Numerous borrow-pits (2–10 m in circumference and 1–3 m deep) exist in most villages, being formed by the removal of earth for brick-making or plastering walls. Pigeon holes dug in thin walls to form pit shelters were found to be excellent resting places for mosquitos. Two collections from pit-shelters were made each month in each of the six sample villages. Of the 8721 anophelines collected between August 1974 and April 1976, 0.65% were of the *A. gambiae* group, 12.0% of the *A. funestus* group, and 68.4% of the *A. marshallii* group, the greatest numbers of the two last-mentioned groups occurring between December and May. *A. pretoriensis* and *A. rufipes*

were relatively abundant from February to March 1976. Precipitin tests carried out on 3452 anophelines collected in pit-shelters indicated that only 0.2% had fed on human blood, whereas 99.8% had fed on cattle.

The densities of anophelines biting man indoors and outdoors were determined by means of catches by two men sitting indoors and two men sitting outdoors. A short (4-hours) night-biting catch commencing shortly before sunset was carried out in 4–6 of the sample villages each month and, in addition, an all-night biting catch was made in one or two villages each month.

During 62 short night-biting catches (corresponding to 496 man-hours) made between March 1975 and April 1976 in DDT-treated houses and outdoors, 622 anophelines were caught. Of these, 0.8% were caught inside DDT-treated houses and 99.2% outdoors. No members of the *A. gambiae* group were caught indoors and two were caught outdoors in the 496 man-hours of collection. Only one anopheline of the *A. funestus* group was caught indoors and 18 outdoors. All the *A. marshallii* (0.74 per man-hour) were caught outdoors.

During 6 short night-biting catches made in untreated houses and outdoors between December 1975 and April 1976, no anophelines were caught indoors, compared with 132 caught outdoors. Of these, 0.75% belonged to the *A. gambiae* group, 2.25% to the *A. funestus* group, and 55.3% to the *A. marshallii* group, and 15.2% were *A. squamosus*, 6.8% *A. maculipalpis*, 11.4% *A. rufipes*, 6.1% *A. pretoriensis*, and 2.25% *A. coustani*.

During 16 all-night biting catches made in DDT-treated houses and outdoors between February 1975 and April 1976, 150 anophelines were caught biting man, 0.7% inside DDT-treated houses, and 99.3% outdoors. Most of them were caught shortly after dusk, the numbers then declining throughout the night; 85% of the anophelines biting outdoors were caught within the 4 hours and 71% within the 2 hours after dusk.

It was not possible to carry out susceptibility tests on *A. gambiae* from the project area because very few anophelines of that group were to be found there. Through the establishment of discriminating dosages (*13*), low degree DDT-resistance was shown to be present in colony material of *A. gambiae* species A. The colony material originated from wild-caught larvae collected in Nkuzaan village in the Hoogmoed area of Sibasa District in March 1974 (Table 4).

Table 4. Percentage mortalities in parental and selected populations of the Nkuzaan Colony

Exposure to 4% DDT (hours)	Parent population	1st selected generation	2nd selected generation	3rd selected generation
1	78 (375) [a]	52 (50)	50 (130)	24 (37)
2	92 (196)	94 (438)	82 (262)	50 (28)
3	94 (176)	—	94 (1036)	—
4	—	—	—	95 (1853)

[a] Numbers tested are shown in parentheses.

DISCUSSION

From the findings obtained between October 1973 and September 1976 it is clear that longer-term observations, along the lines established in the project, are necessary to elucidate the epidemiology of the persisting low level of residual transmission, and also to provide the data required for the epidemiological interpretation of any malaria resurgence and for the organization of an adequate monitoring system leading to preventive action.

In the study area, where transmission has been drastically reduced, prevalence surveys were not sensitive enough to quantify the degree of persisting residual malaria transmission; instead, surveillance activities (based on both active and passive case detection) led to the detection of 42 autochthonous cases in the 1974/75 season and 10 in the 1975/76 season among the 17 300 inhabitants of the project area. The immunofluorescence antibody test with *P. fieldi* antigen proved a promising but as yet unrefined epidemiological evaluation tool, since the results varied according to the method of collection used (filter paper or capillary tube). In addition, a titre of 1 : 20 (or even 1 : 40), considered as positive, may have been due to a false interpretation or to causes not directly related to malaria. The decision to use *P. fieldi* for the preparation of an antigen for the test was taken on account of the local availability of *Cercopithecus aethiops* and the lack of *Aotus trivirgatus* monkeys. Since then it has been shown that *P. falciparum* antigen, obtained from *in vitro* culture, is suitable for the test (*10*) and that antigen could be prepared from local human infections. Consequently, there is a need for further testing of serological methods in the field, and for the results to be subjected to careful interpretation (*7*) and

compared with those obtained by more conventional techniques. In this way, a better assessment of the IFA test could be obtained, in both epidemiological (*1*) and statistical terms, and a level established above which a serological titre could be considered as an indication of malaria infection (*5*).

The results of the Mandlakazi tests (Table 2) indicate how localized malaria foci may be and how the percentage titre index can be influenced by taking and grouping specimens from a wider area. Where similar conditions existed, a smaller number of specimens taken over a wide area could be used to determine the presence of transmission foci. Thereafter, in the defined foci, the extent and epidemiology of transmission could be determined by taking a larger number of specimens.

Among the traditional vectors of malaria in Africa *A. funestus* Giles was not detected in the study area and members of the *A. gambiae* group were present in extremely small numbers. Whether or not, under the prevailing conditions, the observed low densities of *A. gambiae* species A and B could be solely responsible for maintaining the low level of residual malaria transmission could not be determined on the basis of existing data. In view of its ubiquitous presence and higher level of man-biting rates in the study area, *A. aruni* may be considered to play a role in the residual level of malaria, through outdoor transmission during the early hours of the night. In addition, the hitherto undiscovered species that closely resembles *A. flavicosta* and is predominant in the project area is probably involved, since *A. flavicosta* has been found sporozoite-positive in West Africa, where it was thought to play a limited part in malaria transmission (*4*, *6*). Fourteen other species of anophelines have also been found in the project area and the possible role as malaria vectors of the more abundant of these cannot be entirely ignored. Elsewhere, sporozoite infections have been recorded in a number of these species, for example, 2 (1.7%) of 114 *A. pretoriensis* caught in the Letaba foothills in 1931 were found to be infected (*9*).

The most promising lines of further investigation to identify vectors of malaria would appear to be efficient entomological surveillance at the site of detection of malaria cases (including dissection of anophelines captured and identification of the wild-caught material by means of cytotaxonomic and electrophoretic procedures). In addition, an attempt should be made to infect suspected vectors experimentally with *P. falciparum*.

A feature of anophelism, in the study area, was the low degree of contact with man, as indicated by pyrethrum spray catches, exit-window-traps, and night-biting catches indoors and outdoors. Furthermore, while anophelines were abundant in the study area, as shown by collections from pit-shelters, they fed almost exclusively on cattle penned outdoors.

The prevalence of an almost entirely zoophilic anopheline population in the study area may possibly be attributed to the selection pressure imposed on the anopheline population by 30 years of house-spraying with DDT/HCH. Similarly, the development of a low level of DDT resistance in *A. gambiae* species A in northern Transvaal (Table 4) may be attributed to the long history of residual spraying. Nevertheless it is known that, in areas of northern Transvaal where house-spraying was discontinued some years ago, the traditional vectors have returned indoors in significant numbers. In terms of practical malaria control, therefore, the occurrence of low densities of members of the *A. gambiae* and *A. funestus* groups in pyrethrum spray catches and in exit-window-traps of DDT-treated houses endorses the effectiveness of the present residual spraying programme—i.e., DDT 75% (wettable dispensable powder) applied at 2 g per m^2 between August and November, with the option of a second round of spraying in the period February–April to combat any flare-up in presumptive vectors should it arise.

ACKNOWLEDGEMENTS

We thank Dr James Gilliland, Coordinating Director of the Department of Health; the staff of the Annecke Institute, including those working in the field; and the rural Venda population and their leaders, for their valuable assistance, facilities, and willing cooperation.

Special acknowledgement is made of the constant support given to this study by Dr T. Lepes, Director, Division of Malaria and Other Parasitic Diseases, WHO, Geneva; Dr J. H. Pull, Epidemiologist, WHO, Geneva; and Dr J. Lelijveld, Leader of the project until his departure in February 1975.

Thanks are due also to Dr J. H. E. Meuwissen, Dr R. S. Bray, and Dr C. C. Draper, for help with serological testing; to Dr G. Davidson for resistance tests, and to Dr M. W. Service and Dr G. B. White for assistance with taxonomic problems relating to anophelines.

RÉSUMÉ

LE PALUDISME DANS SA ZONE MARGINALE DE DISTRIBUTION LA PLUS AUSTRALE EN AFRIQUE: ÉPIDÉMIOLOGIE ET LUTTE

L'endémicité du paludisme au nord du Transvaal a été réduite à des niveaux de prévalence très bas — de l'ordre de 0,7% — à la suite de plus de trente ans d'opérations de lutte antipaludique faisant usage d'insecticides à effet rémanent. Faisant suite à une pluviosité exceptionnelle, des foyers épidémiques de paludisme surviennent sur un fond de transmission à *P. falciparum* peu élevée. D'octobre 1973 à septembre 1976 on a essayé de découvrir et d'étudier les facteurs opérationnels et épidémiologiques, causes de cette transmission résiduelle. Cette étude a été faite dans un petit projet couvrant une population de 17 000 personnes, à l'extrême nord du Transvaal. Les enquêtes d'incidence, basées sur le dépistage des fiévreux, ont permis de détecter 42 cas locaux de paludisme en 1974–75 et 10 en 1975–76. Les enquêtes de prévalence n'ont pas eu la sensibilité nécessaire pour mesurer la situation palustre et les tests sérologiques ont indiqué divers niveaux d'infection selon la méthode employée. *A. funestus* Giles, un des principaux vecteurs du paludisme en Afrique, n'a pas été collecté dans la zone d'étude; le groupe *A. gambiae* représenté par un très petit nombre d'espèces A et B ne peut être tenu comme responsable de cette petite transmission résiduelle. Sur une base épidémiologique, il paraît évident qu'un membre du groupe *A. funestus* ressemblant à *A. aruni* Sobti et qu'une espèce non identifiée jusqu'ici au nord du Transvaal, abondante dans la zone d'étude et très semblable à *A. flavicosta* Edwards, peuvent être considérés comme suspects. Ces deux anophèles ont été découverts piquant l'homme, presque toujours à l'extérieur et pendant les premières quatre heures après le crépuscule. C'est pendant cette période de la journée que les personnes se réunissent volontiers en dehors de leurs maisons, servant ainsi d'appâts aux moustiques.

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