SOME ASPECTS OF MALARIA PREV ALENCE, VECTOR INFECTIVITY AND D DT RESIST AN CE STUDIES IN GAMBELIA REGION, SOUTHERN WESTERN ETHIOPIA

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ABSTRACT: Prevalence of Plasmodium falciparum and Plasmodium vivax in thehuman population, infectivity and DDT resistance of Anopheles mosquitoes were studied on samples collected during the peak malaria season of 1990 from Gambella, South West Ethiopia. Mosquito vectors collected were assorted into species and their infectivity with malaria parasites was determined by the enzyme-linked immunosorbent assay (ELISA). In the human population out of a total of 821 individuals examined from nine villages, 4.1% (34) were found to be positive for malaria parasites. Of the 34 positive individuals 5.9% (2) were positive for Plasmodium vivax and 94.1 (32) for Plasmodium falciparum. Although relatively high positivity rates for malaria were observed in 1-4 and 5-14 age groups, the difference in the rates of positivity was not statistically significant for the whole population ($P = 0.5077$). However, a significant difference in parasite prevalence was detected between the nine localities ($P < 0.05$). Compared to that of 1989, the overall malaria prevalence rate in the human population significantly decreased in 1990 ($P < 0.05$). Insecticide susceptibility studies revealed the presence of DDT resistant Anopheles gambiae s.l. mosquitos in Itang. Furthermore, a strong evidence would suspect the vectorial status of A. pharoensis was obtained by detecting salivary gland sporozoite antigens of P. vivax in the head region of two mosquitos. Sporozoite rates of 0.76% (P. falciparum) for A. gambiae s.l. and 0.47% (P. vivax) for A. pharoensis were determined. [Ethiop. J. Health Dev. 1994;8(1):1

INTRODUCTION

Ethiopia has three geoclimatic zones that have characteristic malaria endemicities (1). The "kolla" or the hot zone below 1500m altitude has seasonal or perennial malaria transmission depending on local conditions with moderate to high endemicity .This roughly constitutes 46% of the territory. The "woina dega" or temperate zone (46% of the territory) is between 1500m and 2500m altitude and has malaria transmission characterized by sporadic outbreaks of unstabie malaria resulting from sudden climatic changes such as heavy rains or floods. The "dega" or cold zone (8% of the territory) which is found above 2500m altitude is free of malaria transmission.

On this basis, at present, about 75% of Ethiopia is considered to be either malarious or potentially malarious with about 64% of the population at risk of infection (2). Previous studies have shown that the malaria situation in Gambella is characteristically hyper-endemic with stable malaria transmission along the Baro River basin (3, 4). However, the appearance of drug resistant of P. falciparum (5), insecticide resistant of A. gambiae s.l. and the high influx of people from non-malarious regions of the country (reset tIers from the highlands of Tigrai, Wollo, Shoa, etc.), with the concomitant agricultural expansion programs, would be expected to influence the epidemiology of malaria transmission in the area.

Lack of adequate understanding of the epidemiology of malaria transmission has remained to be one of the major impediments to malaria control in Ethiopia. The present study attempts to provide an additional information on the vectors responsible for malaria transmission and on malaria parasite prevalence in the human population in Gambella. It is hoped that it would assist the planning of intervention strategies against malaria in the Gambella region.

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METHODS

STUDY AREA

Gambella, situated at go N, 35° E, is a tropical lowland in South West Ethiopia. Its altitude is in the range of 500 to 6OOm above sea level. The wet season extends from late April to mid November and the dry season is between late November and early April (3). The area is traversed mainly by the Baro, Alwero, Gillo and Akobo rivers flowing with highly varying volumes of water at different times of the year . Nine localities, namely, Gambella town, Abobo (Tegnei), Chobo, Perbongo, Ukuna,

Uballa, Pegnudo, Finkio (Baro Abol) and Itang with relatively stable populations were selected for the study. Out of these sites. Parasitological investigations were not conducted in Gambella town, Abobo (Tignei) and ltang. Since some resettlement villages were closed and others had very few inhabitants, during the study period, it was necessary to limit the study to the nine localities. The population of the region comprises the indigenous Anuak, Neur and Mesango nilotic nationalities and the Hametic-Semitic settlers that have immigrated fromahe highlands. The region is one of the areas in the country where the control of malaria is operational by the National Organization for the Control of Malaria and other Vector-borne Diseases (NOCMVD).

STUDY POPULATION

Based on the list of inhabitants obtained from NOCMVD centers, 10% of the residents were randomly selected from each locality .A short questionnaire was filled by asking the study subjects. It included Demographic inforn1ation such as name, age, sex, place of residence, duration of stay and previous residence.

PARASITOLOGICAL STUDY

Thick and thin blood films were collected from each of the study subjects. The slides were air dried and stained for 30 minutes in 3% Giemsa solution within 18 hours of collection and were later examined at a field station in Gambella. All positives and a random selection of 10% of the negatives were re-examined by another senior microscopist in Addis Ababa at the. National Research Institute of Health (NRIH).

ENTOMOLOGICAL STUDY

Mosquito collections were made from indoor resting sites in the morning hours (6:00-9:00 A.M.), and by using human baits stationed indoors and outdoors in the evening hours (5:00-8:00 P.M.) through aspirators. Indoor collections were kept and treated separately from those obtained outdoors. All mosquitoes collected were identified to the species level based on morphological descriptions (6, 7). Sporozoite detection in mosquitoes: Parous mosquitos were stored in a desiccator in silica jel vials and transported to the NRIH laboratory for further analysis. Mosquitoes of the indoor-resting collections (IRC)and the human bait-capture collections were assayed using the sandwich enzyme linked inununosorbent assays (ELISA) for the detection of P. falciparum and P. vivax circumsporozoite (CS) proteins. The ELISA protocol followed was essentially that developed by several workers (8,9) and later-on standardized as akit by Wirtz (10). In brief, P. faldparum and P. vivax anti-sporozoite monoclonal bodies were adsorbed to Immunol 2 (Dynatech Laboratories, U.S.A)" microtiter plates. After overnight incubation at room temperature (20-23°c) a blocking buffer (10gm albumin bovine fraction V, 5gm Casein, 0.1gm Thimerosol in PBS) W18 ; added and incubated for another one hour at room temperature. Following aspiration of the blocking buffer, the head-thorax or abdomen mosquito triturates were added to the wells. The plates were then incubated for one hour at room temperature and washed twice with phosphate buffered .saline- Tween 20 (pBS- TW 20) and horse peroxidase labelled anti-sporozoite ,nonoclonal antibody added and incubated for two hours followed by washing three times with PBS-TW20. Finally, ABTS [2, 2'-azino-bis-(3- ethylbenzthiazoline -6-'sulphonic acid)], a peroxidase substrate (Sigma Co. St. Louis, MO,) was added to the wells. The absorbance 4talues. were then read using a Multi-Scan Spectrophotometric (Dynatech Laboratories, U.S.A.) ELISA plate reader at 405nm. Values greater than the mean plus three standard deviation of the seven negative controls of male mosquitoes were considered positive. Positive controls that consisted of recombinant R32 peptide of P. faldparum cs protein and a synthetic P. vivax CS peptide were used asdescribed by Wirtz et al. (10).

Insecticide suscebility studies: The specimens for this study were collected from Itang during the months of September and October from 1988 to 1990. Blood-fed indoor resting A. gambiae s.l. were exposed to 4% DDT

impregnated paper using the standard WHO test-kit (11). Twenty mosquitoes per test-kit were exposed for one, two and four hours. Another group of twenty were exposed to oil impregnated paper for an equal period of time and used as controls. Each of these tests was carried out in duplicates. The
mortality values obtained from the three exposure periods were recorded.

Table 1. Prevalence of Plasmodium vivax and P. falciparum by Age (Gambella, October-November, 1990)

	Precent			
Age group (groups)	No. pos./No. Exam	P.vivax	P. falciparum	Total
1-4	9/159	1.3	4.4	5.7
5-14	13/266	0	4.9	4.9
15-49	10/329	0	3.0	3.0
≥	2/67	0	3.0	3.0
Total	34/821	0.24	3.9	4.1

Table 2. prevalence of P. vivax and P. falciparum by Locality (Gambella, October-November, 1990)

No			Percent positive		
	Locality	No. pos. No.Exam.	P. Vivax	P. falcp	Total
1	Uballa 27	1/50	0	2.0	2.0
2	Uballa 28	6/100	0	6.0	6.0
3	Chobo 9	3/118	0	2.5	2.5
4	Chobo	1/50	0	2.0	2.0
5	Perbongo 10	0/80	0	0	0
6	Prebongo 19	3/92	0	3.3	3.3
7	Ukuna 22	7/81	0	8.6	8.6
8	Ukuna 24	4/50	0	8.0	8.0
9	Baro Abol (Finkio)	9/200	0	3.5	4.5
	Total	34/821	1.0	3.9	4.1

Table 3. Infectivity of the Common Endophilic Anopheline Mosquitoes Assayed by Uusing Santi-circumsporozoite Monoclonal Antibodies Against P. falciparum and P. vivax. (Gambel1a, October-Novembor, 1990)

Collection area	A.z	A. ph	A.g	A.n	A,c.	A.z	A. ph	A.g	A.n	A,c.
				**						
Gambella town	-	-	2(140)	-	-	-	-	0(140)	-	-
Abobo (Tegnei)	-	-	0(2)	-	-	-	-	0(2)	-	-
Chobo	0(1)	0(3)	-	-	-	0(1)	0(3)	-	-	-
Perbongo	0(1)	0(2)	0(32)	0(9)	0(1)	0(1)	0(2)	0(23)	0(9)	0(1)
Ukuna	-	0(1)	0(2)	0(1)	-	-	0(1)	0(2)	0(1)	-
Pegnudo	-	-	0(6)	-	0(1)	-	-	0(6)	-	0(1)
Baro Abol (Finko)	0(4)	0(424)		*** 0(6)	0(4)	0(4)	2(424)	0(79)	0(6)	0(4)
Total	0(5)	0(428)	2(246)	0(16)	0(6)	0(5)	2(428)	0(264)	0(16)	0(6)
% of mosquitos infected	0	0	0.76	0	0	0	0.47	0	0	0

Table 4. **Infectivity of the Common Outdoor human-bait Collected Anopheline Mosqutoes Assayed using Anti-circumsporozoite Monoclonal Antibodies Against *P. Falciparum and P. vivax.* (Gambella, October-November, 1990)**

	ELIAS (.falciparum)								ELESA (P.vivax)							
Collection area	**z	A.ph.	A.g.	A.n	A.c	A.s	A.pa	A.f	A.z	A.ph	A.g.	A.n	A.c	A.s	A.pa	A.f
Gambella town	0(4)	0(15)	0(1)	-	-	-	-	-	0(4)	0(15)	0(1)	-	-	-	-	-
Abobo (Tignei)	-	0(6)	-	0(2)	-	-	-	-	-	0(6)	-	0(2)	-	-	-	-
Chobo	-	0(11)	-	-	0(33)	0(4)	-	-	-	0(11)	-	-	0(33)	0(4)	-	-
Pegnudo	0(7)	0(12)	0(1)	0(2)	0(2)	-	-	-	0(41)	0(132)	0(3)	0(2)	0(2)	-	-	-
Ukuna	0(35)	0(18)	0(1)	-	0(10)	-	0(15)	0(4)	0(35)	0(18)	0(1)	-	0(10)	-	0(15)	0(4)
Uballa	0(3)	0(40)	-	-	-	0(26)	-	0(3)	0(3)	0(40)	-	-	-	0(26)	-	0(3)
Pegnudo	0(70)	0(21)	0(1)	-	-	-	-	-	0(70)	0(21)	0(1)	-	-	-	-	-
Baro Abol (finkio)	-	0(87)	0(1)	-	0(7)	0(1)	-	-	-	0(87)	0(1)	-	0(7)	0(1)	-	-
Itang	0(34)	0(132)	0(2)	0(3)	-	-	-	-	0(34)	0(132)	0(2)	0(3)	-	-	-	-
Total	0(187)	0(462)	0(9)	0(7)	0(52)	0(31)	0(15)	0(7)	0(187)	0(462)	0(9)	0(7)	0(52)	0(31)	0(15)	0(7)
% of mosquitos infected	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

** A.z. (Anopheles ziemanni), A.Ph. (A. pharoensis). A.g. (A. gambiae s.l). A.n. (A.nili) A.c. A. Acoustani, a.s. (A. squamosus).
A.Pa (A.paludis) or A.f. (A. funestus)

N.B. Figures in parentheses are the number of mosquitos

Table 5. **Result of the DDT Susceptibility Tests (1990)**

CONE	EXP	NO. EXPOSED		NO. DEAD	% MORTALITY
			1988		
DDT 4%	1 HR	99		21	21.2
Control		79		0	0
DDT 4%	2 hrs	40		18	45
CONTROL		40		0	0
DDT 4%	4hrs	60		30	50
CONTROL		40		0	0
			1989		
DDT 4%	1hr	97		0	0
CONTROL		90		0	0
DDT 4%	2hrs	77		0	0
CONTROL		75		0	0
DDT 4%	4hrs	120		0	0
CONTROL		60			
			1990		
DDT 4%	1hrs	140		14	10
CONTROL		60		0	0
DDT 4%	2hrs	100		11	11
CONTROL		60		0	0
DDT 45%	4hrs	160		24	15
CONTROL		60		0	0

- = concentraion of insecticide (DDT)
- = exposure preiod

RESULTS

The prevalence of P. vivax and P. falciparum by age is shown in Table 1. Out of a total of 821 individuals examined, from nine villages, 4.1% (34) were found to be positive for malaria parasites. Of the 34 positive
individuals 5.9% (2) were positive for P. vivax and.94.5% (32) were positive for P. faldparum. P. vivax was detected only in the 1-4 year age- group; P. faldparum was diagnosed from all age groups, with the highest (4.9-%) prevalence rate in the 5-14 year age-group, amd the lowest (3.0%) prevalence in the age-group above 15 years. However, the difference was not statistically significant (P = 0.5077).

The prevalence rates of P. vivax and P. falciparum in the nine villages are presented in Table 2. P. vivax was found only in Finkio village while P. falciparum was detected in the eight localities with the highest prevalence (8.6%) in Ukuna 22.

A comparison of malaria prevalence between 1989 and 1990 is shown in Figure 1. The data suggested that as compared with the year 1989, the overall malaria prevalence decreased by 3.3 fold in 1990. The reduction in prevalence was greatest in the age-group 30-34 (10.1 fold) followed by the age-group 40-44 (7.7 fold). The lowest was in the 45-49 (1.03 fold) md 35-39 (1.6 fold) age-groups. Among children and in those aged 50 years and above, the reduction in prevalence ranged from 2.7 to 3.9 fold while the decrease the 15-29 age- group was 4.9 fold.

The epidemic mosquitoes assayed for the detection of CS proteins included: 6 A ziemanni, 430 A. pharoensis, 264 A. gambiae s.l., 16A. nili and 6A. coustani. Of these, only 0.76% (2) A. gambiae s.l. and 0.47% (2) A. pharoensis were found positive for P. falicparum and P. vivax, respectively (Table 3).

The infected A. gambiae s.l. were collected from the town of Gambella while the infected A. pharoensis was from Finkio. Of the two infected A. gambiae s.l. the infection in one mosquito was located in the head-thorax region and that of the second mosquito was abdominal. The infections in A. pharoensis were both located at
the head-thorax region.

None of the outdoor human-bait collected mosquitoes were found positive for the two malaria parasites assayed (T1ble 4). Similarly, indoor human-bait collected A. pharoensis mosquitoes from Finkio were also found negative.

The result of DDT susceptibility tests on A. gambiae s.l. for the years 1988 to 1990 is presented in Table S. The mortality values for one, two and four hours exposure periods showed values below the 80% susceptibility threshold, indicating the presence of DDT resistant A. gambiae s.l. in the study area with the highest level of resistance recorded for 1989.

DISCUSSION

The higher rate of parasite positivity in children aged between 1-4 years could be the result of relatively low acquisitiol of immunity. The parasite positivity rate in the older children (5-14 age-group) was also relatively high, because of the partial immunity these children of the reset the population have developed over about 5 years of stay in the region. Differences in prevalence between the nine localities could be attributed to fact rs such as the presence of convenient breeding sites for mosquitoes. For instance, the higher prevalence in Ukuna can be explained by the proximity of the Seri River, and of marshy areas in Uballa 28 and Finkio.

The overall reduction in the prevalence of malaria in 1990 when compared to that of 1989 could be explained by the insecticidal pressure due to" Actellic " spraying (Personal communication, Gambella region NOCMVD office) that drastically reduced the indoor-resting anopheline population in 1990. This measure
was undertaken following the 1989 severe malaria epidemic which could not be contained as a result of DDT resistance observed in the area (13). Except in the age-group above 35 years old, the majority of whom were
indigenous and showed a lower reduction in prevalence rate, the pattern of prevalence reduction in all age-groups was relatively high. The lower reduction in prevalence in the indigenous population where more than 95% did not take antimalarial drugs for treatment or prophylaxis, is most probably the result of stable malaria in the region. This observation is similar to other in populations of holo or hyper- endemic areas of the world (12). The higher reduction in prevalence of infection in the age- groups that constituted that constituted the reset tier population could be due to increasing immune status as they stayed longer in the region and the intensive drug usage (about 98%) for treatment and prophylactic purposes. Previous investigations suggested that the species responsible for malaria transmission in Gambella were A.gambiae s.l, A..funestus and A.nili (3, 14, 15). The role of A. pharoensis was not clear (4). Our findings were partly in conformity with that reported earlier (16). This study has provided evidence that reinforces the report of Fissehaye et al. (17) incriminating A.pharoensis as a malaria vector species specifically carrying p. vivax.

Over the past 19 years the ecological changes that took place in Gambella as a result of increased activities such as agricultural development projects and the resettlement programs appear to have created a conducive
environment for the breeding of A.pharoensis . A prefeued breeding environment in the form of abundant rice farm and marshy areas produced by the over flooding Baro river may account for the large population of A.pharoensis .This has been proved by the collection of a large number of this species in Finkio. The connection between the presence of P. vivax patients in Finkio only and the infection of the two A.pharoensis in this village with P. vivax may be explained by th;., fact. For A.gambiae s.l. which is known as an efficient vector of P.falciparum. such breeding places are not conducive (2).

On the basis of this investigation it also appeared that only the indoor-resting mosquitoes constituted important vectors while the outdoor avid biters did not. This could be explained by the fact that the outdoor human bait collected vector mosquitoes are by and large newly emerged tenerals that have not previously fed on human blood to pick parasites.

Since 1959, DDT spraying at a dosage of 2g./m2 was carried out twice yearly first as a pilot control programme and then as an active control operation (18). Although we do not have the DDT susceptibility test data for other areas of the Gambella region, the present finding indicated the presence of DDt resistance of
A.gambiae s.l. in Itang. This may be explained by the fact that, after 1986 DDt-spraying was widely used in vast areas of the region due to the increasing activities of resettlement and agricultural development schemes which may have resulted in the occurrence of resistante strains within a chort period. For a better understanding of the specific epidemiology of malaria and its consequences in the local population of Gambella, similar follow- up studies should be carried out.

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