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en français)

IN VIVO SENSITIVITY OF PLASMODIUM FALCIPARUM TO CHLOROQUINE, AMODIAQUINE AND SULFADOXINE/PYRIMETHAMINE, ZANZIBAR, UNITED REPUBLIC OF TANZANIA

by

A. Matteelli, H. Haji, S. Issa, K. Mohamed and J. Muchi
Zanzibar Malaria Control Programme, Ministry of Health
Box 236, Zanzibar, United Republic of Tanzania

CONTENTS

1. INTRODUCTION

Zanzibar consists of two islands, Unguja and Pemba, situated in the Indian Ocean 30 km off the coast of mainland Tanzania. The total area of the two islands is 2332 km^2, with an estimated total population of 620 300 divided more or less equally between the two islands (Fig. 1).

Malaria is holoendemic in most of the areas and is the major public health problem in the country. Plasmodium falciparum represents about 90% of the malaria infections, the remainder being composed mainly of P. malariae followed by P. ovale. Chloroquine resistance is present in P. falciparum and constitutes one of the major constraints in the control of the disease. The importance of monitoring drug sensitivity has increased since P. falciparum resistance to chloroquine and amodiaquine was first detected in Zanzibar Town in 1982 (Schwartz et al., 1983; Campbell et al., 1983).

This paper reports the findings of a survey to assess P. falciparum sensitivity to chloroquine, amodiaquine and sulfadoxine/pyrimethamine (Fansidar) carried out in four different localities of Unguja and Pemba from September 1988 to February 1989.

2. MATERIALS AND METHODS

2.1 WHO standard 7-day field test

The following localities were chosen (refer to Fig. 1):

(a) Zanzibar Town (Kilima Hewa), Urban District, West-Urban Region, Unguja.

(b) Chaani, North A District, North Region, Unguja.

(c) Makunduchi, South District, South Region, Unguja

(d) Chake Chake, Chake District, South Region, Pemba.

Children were recruited from primary schools, with an age range of 6-14 years.

Over the period September 1988 to February 1989, 2447 blood samples were examined for malaria parasites (thick and thin films). Children were recruited for the trial if, on the initial examination, they presented with: P. falciparum infection only; parasite density greater than 800 asexual forms/mm^3 blood; negative Dill-Glazko urine test for 4-aminoquiolines.

The recruited children were divided into strata with low, medium and high parasite densities (low, 800-1500 parasites/mm^3 blood; medium, 1501-3000 parasites/mm^3 blood; high, greater than 3000 parasites/mm^3 blood). Roughly one third of the children in each stratum was allocated to one of the following three regimens:

(a) Chloroquine: 25 mg/kg body weight over 3 days (10; 10; 5 mg/kg/day)

(b) Amodiaquine: 25 mg/kg body weight over 3 days (10; 10; 5 mg/kg/day)

(c) Fansidar: 1.25 mg pyrimethamine and 25 mg sulfadoxine/kg body weight single dose.

Quality controlled drugs were used, supplied by the WHO/Malaria Action Programme, Geneva, in the form of chloroquine tablets of 150 and 50 mg; amodiaquine tablets of 200 mg; Fansidar tablets of 25 mg pyrimethamine/500 mg sulfadoxine.

Treatment was administered under careful supervision, the tablets being swallowed with water and biscuits. The children were observed for at least one hour after drug administration in order to exclude cases of vomiting.

On day 2 the absorption of 4-aminoquinolines was checked by the Dill-Glazko urine test. Absorption of Fansidar was not tested because urine tests for sulfonamides are reputed to be unreliable (M. Lemge, pers. com.).

Thick and thin blood films were collected every day from day 0 to day 7, stained with Giemsa 5% for 30 minutes and examined under 100X oil immersion objectives. At least 100 fields were searched before a blood film was considered to be negative; in positive blood films asexual parasites were counted against 500 leukocytes, and the parasite density per mm^3 was calculated assuming a leukocyte count of 8000 per mm^3 of blood.

The slides were read by microscopists at the Zanzibar Malaria Control Programme (ZMCP) where there is regular cross-checking both within the laboratory and by colleagues at the Parasitology Department, Muhimbili Medical Center, Dar es Salaam.

2.2 WHO standard 28-day extended field test

An extended test was performed in Makunduchi because it is a dry coral area and the vector, Anopheles gambiae s.l., occurs only at exceptionally low densities; it is therefore likely that the intensity of malaria transmission is relatively low. In addition to the slides taken daily for the first seven days, slides for the extended test were taken on days 14, 21, and 28 and processed as described above.

3. RESULTS

3.1 WHO standard 7-day field test

Among the 2447 children examined, 627 (25.6%) were found positive for P. falciparum infection only (Table 1). Only 2 cases of P. malariae were detected in Unguja, while 10 cases of mixed P. falciparum/P. malariae and/or P. ovale infections, and 3 cases of P. malariae alone were detected in Pemba.

Of the 232 children recruited in the trial, 206 (88.8%) completed the tests, i.e. they took the drugs, did not vomit, had a blood film taken on each day of the follow up, and, for children treated with 4-aminoquinolines, had a positive Dill-Glazko urine test on the last day of treatment. The initial asexual parasite counts ranged from 800 to 110 200/mm^3 (mean 1786). Of the 206 children who completed the tests, 78 received chloroquine, 57 received amodiaquine and 71 Fansidar.

The positivity rates and geometric mean parasite densities from day 0 to day 7 for each of the three drugs are presented in Figs 2 and 3.

The results of the chloroquine tests are shown in Table 2. Resistance was found in 30 children (38.5%): 15 (19.2%) at RI, 12 (15.4%) at RII, and 3 (3.9%) at RIII levels of resistance.

A comparison of the daily parasite density and the daily parasite rate observed in previous chloroquine sensitivity tests conducted in Zanzibar Town in 1982 (Schwartz et al., 1983) and in the current study, is shown in Figs 4 and 5.

For the analysis of chloroquine resistance results by area, the S and RI responses were pooled to form one group, and the RII and RIII another group (the latter being

regarded as resistance of clinical importance) giving a 4 x 2 contingency table. The X^2 value for this table was 9.61 (P=0.022), suggesting a significant difference between the areas. However, these results should be treated with caution as expectations below 5 were involved in the calculation.

The greatest contribution to the total X^2 value came from Makunduchi, and pair-wise comparisons between areas by Fisher's exact test suggested only two significant contrasts: between Makunduchi and the urban district of Kilima Hewa (P=0.047) and between Makunduchi and Chake (P=0.0077). The comparison between Makunduchi and Chaani was of borderline significance (P=0.068); all other comparisons were non-significant (P>0.3). Although the numbers involved are rather small, the results suggest that the frequency of chloroquine resistance (RII and RIII) is similar in Kilima Hewa, Chaani and Chake but greater in Makunduchi.

In the amodiaquine treatment group, 1 case (1.8%) of RII and 2 cases (3.5%) of RIII resistance were detected (Table 3). In the Fansidar treatment group all children were cleared of parasitaemia before day 7, except 1 child (1.4%) who presented a RI response (Table 4). Two of the 3 cases of resistance to amodiaquine and the only case of resistance to Fansidar, were also found in Makunduchi.

The mean parasite clearance times (calculated only in the S/RI response groups) were 3.25 days for chloroquine, 2.96 days for amodiaquine, and 2.33 days for Fansidar.

3.2 WHO standard 28-day extended field test

The results of the extended test in Makunduchi are shown in Fig. 6. All the children (15 in each drug regimen) who completed the 7-day field test were successfully followed up in the extended test.

By day 28 all 15 children who were given chloroquine had become positive. This means that the 8 whose parasitaemia had cleared by day 7 suffered either a recrudescence or a fresh infection during the subsequent 3 weeks.

Human biting catches by the ZMCP and light trap catches by C. Maxwell (pers. com.), have indicated that people in Makunduchi receive less than one bite a week from *Anopheles* mosquitos. While this does not rule out the possibility of malaria transmission in the area, it does mean that the likelihood of receiving a fresh infection in any one month is very low. The 8 children in whom parasites reappeared between day 7 and day 28 did not sleep outside Makunduchi during this time and must therefore be considered to be resistant at the RI level. This suggests that strains of *P. falciparum* fully susceptible to chloroquine are now rare in Makunduchi.

Among the children receiving the amodiaquine regimen, 4 (26.7%) were fully sensitive to the drug (S response group). The remainder were RI (9 children, 59.9%), or RII/RIII cases (1 child each, 6.7%).

Among the children who were given Fansidar, 12 (80%) remained negative till day 28; 3 children (20%) were resistant at the RI level.

4. DISCUSSION

The results of this survey confirm the presence of strains of *P. falciparum* resistant to chloroquine. The total percentage of resistant cases (38.5%), as determined with the 7-day test, is slightly higher than in previous surveys conducted in the islands in 1982 (34%, Schwarz et al., 1983) and 1984 (31.6%, unpublished data ICP/PDP/001/Nairobi). However, comparison between the 1982 and 1988 daily parasite rates and parasite densities of *P. falciparum* infections treated with chloroquine (Figs 4 and 5) in Zanzibar Town showed no apparent deterioration of chloroquine sensitivity. The average parasite density observed on each day of the test was in fact higher in the 1982 survey than in the current one. This may be due to the fact that the present survey was conducted during the season of relatively low malaria transmission, whereas the 1982 survey had been carried out during the high transmission season.

In 1984, a 7-day test was conducted by a WHO Intercountry Team in several localities of Unguja and Pemba; it showed 68.4% of S/RI, 22.8% of RI, 7.6% of RII, and 1.3% of RIII responses to chloroquine. There is much more similarity between the responses of P. falciparum to chloroquine in the 1982 and 1988 surveys than with those observed in 1984. The percentage of chloroquine-resistant cases, in particular RII and RIII cases, is lower in the 1984 study than in the 1982 and 1988 surveys. It can be speculated that the drop in percentage of chloroquine-resistant infections observed in 1984 may have been due to the withdrawal of the chloroquine chemoprophylaxis programme in the country. Subsequently resistance started to increase, because chloroquine was widely available both from dispensaries and commercially, and it has now reached approximately the level of 1982.

In clinical terms the results suggest that chloroquine may fail to produce a satisfactory response in a high proportion of patients. In Makunduchi, especially, where a high percentage of RIII infections was detected, the benefit of using chloroquine as the first-line drug is questionable.

Amodiaquine, a drug not widely available in the islands, cleared parasitaemia in 94.7% of the children in the 7-day test. This implies that some parasites were resistant to chloroquine but were nonetheless sensitive to amodiaquine. However, the contrast between the two drugs was mainly in the proportion of RI infections, while the percentage of RIII infections was the same with both. A study conducted by Campbell et al. (1983) in Zanzibar Town in 1982 showed RIII resistance to chloroquine but not to amodiaquine. On the basis of those results it was suggested that amodiaquine might be preferable as a first-line drug to chloroquine. The detection of RIII resistance to amodiaquine in the present study does not support this suggestion. Consistent with the previous study, the extended test with amodiaquine in Makunduchi showed the occurrence of a high rate of late recrudescences, with an overall cure rate of only 26.7%. This limits the usefulness of amodiaquine as a second-line drug, to be used in case of chloroquine failure: such a drug should ideally be of benefit in all cases and fully effective in most. These criteria are not met by amodiaquine.

Finally, Fansidar, which was tested in the islands for the first time in the present study, was highly effective and cleared the parasites in almost all (98.6%) of the infections tested. This proportion is within the normal range of response to this drug in conditions of complete drug sensitivity (WHO, 1984); the 2 cases of RI response do not constitute any evidence for the existence of Fansidar resistance in Zanzibar.

5. CONCLUSIONS

The survey confirmed the presence of chloroquine resistance in Zanzibar; as many as 38.5% of the infections tested proved to be resistant in the 7-day test, mainly at RI, but also at RII and RIII levels of resistance.

The results of the small number of 28-day tests performed suggest that, at least in some areas, strains of P. falciparum fully sensitive to chloroquine are rare.

Despite the fact that chloroquine may fail to produce a satisfactory response in a high proportion of the treated cases, there is no adequate alternative to chloroquine as a first-line antimalarial drug.

Resistance to amodiaquine does not seem to be as common as that to chloroquine. In practice however, there is little or no advantage in using amodiaquine as the first-line drug because the frequency of RIII cases, in which there is a danger of acute mortality before a second-line drug can be administered, is apparently the same with both amodiaquine and chloroquine. Nor does amodiaquine seem to have any practical advantage as a second-line drug, again because of the existence of RIII strains, and also because of the high frequency of late recrudescences.

For these two reasons Fansidar is far preferable as a second-line drug and can be considered the drug of choice for P. falciparum infections which fail to be cleared by

chloroquine. Fansidar resistance has not yet been detected in Zanzibar, and, at the moment, the likelihood of treatment failures with both chloroquine and Fansidar seems low.

In conclusion, chloroquine resistance is present in P. falciparum in Zanzibar, but there is no alternative first-line drug with significant advantage. Since cases of treatment failure are to be expected, attention must be concentrated on the identification, referral, and/or management of such cases by health personnel working at both peripheral and central levels of the health system.

6. SUMMARY

A survey to assess P. falciparum sensitivity to antimalarial drugs was conducted in four localities of Zanzibar, United Republic of Tanzania, from September 1988 to February 1989. The WHO standard field test (7 days) and extended test (28 days) were conducted on symptom-free children using chloroquine (78 children), amodiaquine (57 children) and sulfadoxine/pyrimethamine (Fansidar) (71 children). The presence of chloroquine resistance was confirmed, with 38.5% of the infections tested being found resistant at the RI (19%), RII (15%), and RIII (4%) levels of resistance. In the extended test none of the 15 infections tested were fully susceptible.

With amodiaquine and sulfadoxine/pyrimethamine 94% and 98% of the infections were found to be sensitive to the respective drugs in the 7-day test.

RESUME

SENSIBILITE IN VIVO DE PLASMODIUM FALCIPARUM A LA CHLOROQUINE, L'AMODIAQUINE ET LA SULFADOXINE/PYRIMETHAMINE, ZANZIBAR, REPUBLIQUE-UNIE DE TANZANIE

Une enquête destinée à évaluer la sensibilité de Plasmodium falciparum aux médicaments antipaludiques a été effectuée dans quatre localités de Zanzibar, République-Unie de Tanzanie, de septembre 1988 à février 1989. Les tests de terrain standards de l'OMS (7 jours) et le test étendu (28 jours) ont été effectués sur des enfants asymptomatiques prenant de la chloroquine (78 enfants), de l'amodiaquine (57 enfants) et de la sulfadoxine/pyriméthamine (Fansidar) (71 enfants). L'existence d'une résistance à la chloroquine a été confirmée, avec 38,5 % des cas d'infection examinés ayant été trouvés comme résistants aux niveaux de résistance RI (19 %), RII (15 %) et RIII (4 %). Dans le test étendu, aucun des 15 cas d'infection examinés n'était totalement sensible.

Avec l'amodiaquine et la sulfadoxine/pyriméthamine, 94 % et 98 %, respectivement, des infections ont été trouvées comme étant sensibles à ces médicaments, dans le test de 7 jours.

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Schwartz, I. K., Campbell, C. C., Payne, D. & Khatib, O. J. (1983) In-vivo and in-vitro assessment of chloroquine-resistant Plasmodium falciparum malaria in Zanzibar. Lancet, i: 1003-1005.

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TABLE 1. IN VIVO SENSITIVITY TESTS OF P. FALCIPARUM TO CHLOROQUINE, AMODIAQUINE, FANSIDAR - ZANZIBAR, TANZANIA (SEPTEMBER 1988-FEBRUARY 1989)

	No of children			
Locality	Screened	Positive	(%)	Tested
Kilima Hewa	314	146	(46.5)	40
Chaani	447	237	(53.0)	50
Makunduchi	955	127	(13.3)	45
Chake Chake	731	117	(16.0)	71
TOTAL	2 447	627	(25.6)	206

TABLE 2. P. FALCIPARUM IN VIVO RESPONSE TO CHLOROQUINE - ZANZIBAR, TANZANIA (SEPTEMBER 1988-FEBRUARY 1989)

	No. (%) of patients in response groups					Mean parasite clearance time (days)
Locality	S/RI	RI	RII	RIII	Total	
Kilima Hewa	13 (65)	4 (20)	2 (10)	1 (5)	20 (100)	2.77 s.e. 0.38
Chaani	10 (55)	5 (28)	3 (17)	0	18 (100)	2.90 s.e. 0.20
Makunduchi	7 (47)	1 (7)	5 (33)	2 (13)	15 (100)	4.14 s.e. 0.67
Chake Chake	18 (72)	5 (20)	2 (8)	0	25 (100)	3.44 s.e. 0.49
TOTAL	48 (62)	15 (19)	12 (15)	3 (4)	78 (100)	3.25 s.e. 0.23

TABLE 3. P. FALCIPARUM IN VIVO RESPONSE TO AMODIAQUINE - ZANZIBAR, TANZANIA (SEPTEMBER 1988-FEBRUARY 1989)

	No. (%) of patients in response groups					Mean parasite clearance time (days)
Locality	S/RI	RI	RII	RIII	Total	
Kilima Hewa	-	-	-	-		
Chaani	16 (94)	-	-	1 (6)	17 (100)	2.69 s.e. 0.18
Makunduchi	13 (86)	-	1 (7)	1 (7)	15 (100)	3.54 s.e. 0.37
Chake Chake	25 (100)	-	-	-	25 (100)	2.84 s.e. 0.36
TOTAL	54 (94)	-	1 (2)	2 (4)	57 (100)	2.96 s.e. 0.20

TABLE 4. *P. FALCIPARUM IN VIVO* RESPONSE TO FANSIDAR - ZANZIBAR, TANZANIA (SEPTEMBER 1988-FEBRUARY 1989)

	No. (%) of patients in response groups					Mean parasite clearance time (days)
Locality	S/RI	RI	RII	RIII	Total	
Kilima Hewa	20 (100)	-	-	-	20 (100)	2.05 s.e. 0.16
Chaani	15 (100)	-	-	-	15 (100)	2.13 s.e. 0.09
Makunduchi	14 (93)	1 (7)	-	-	15 (100)	2.86 s.e. 0.25
Chake Chake	21 (100)	-	-	-	21 (100)	2.38 s.e. 0.24
TOTAL	70 (98)	1 (2)	-	-	71 (100)	2.33 s.e. 0.10

FIG. 1.
SKETCH MAP SHOWING THE FOUR LOCALITIES TESTED FOR P. FALCIPARUM CHEMOSENSITIVITY IN ZANZIBAR (ISLANDS OF PEMBA AND UNGUJA), UNITED REPUBLIC OF TANZANIA

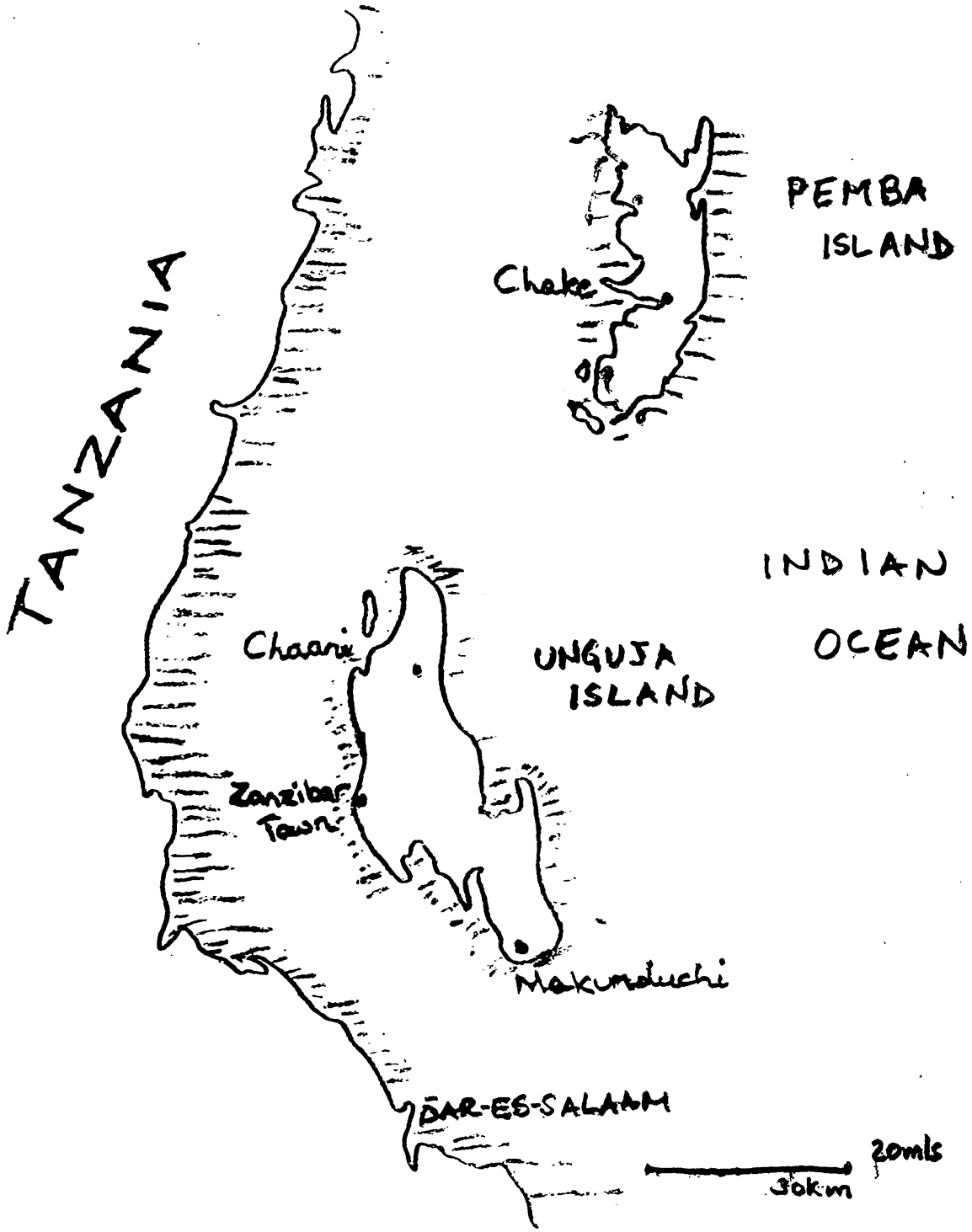


FIG. 2.
P. falciparum in vivo chemosensitivity
Zanzibar - United Republic of Tanzania
Daily parasite rate

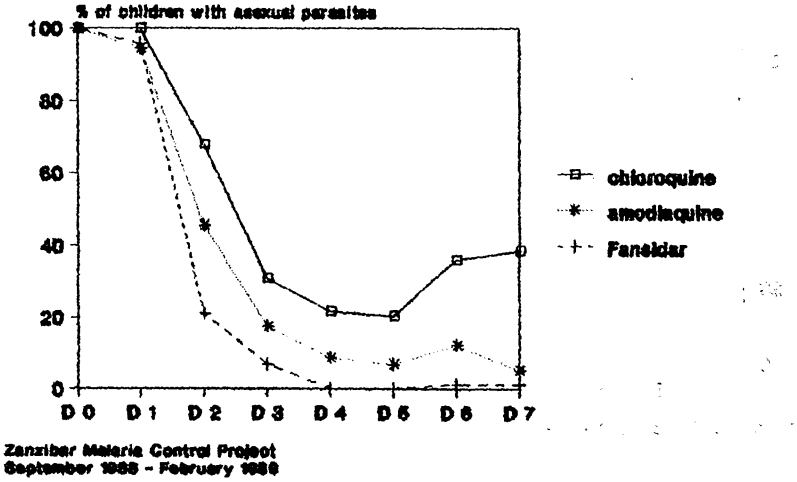


FIG. 3.
P. falciparum in vivo chemosensitivity
Zanzibar - United Republic of Tanzania
Daily parasite density

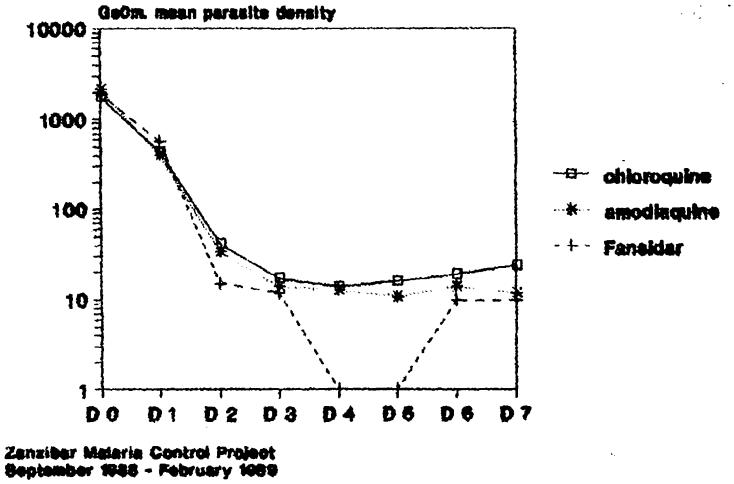


FIG. 4.
P. falciparum in vivo chemosensitivity
to chloroquine
Zanzibar Town - Zanzibar
Daily parasite rate

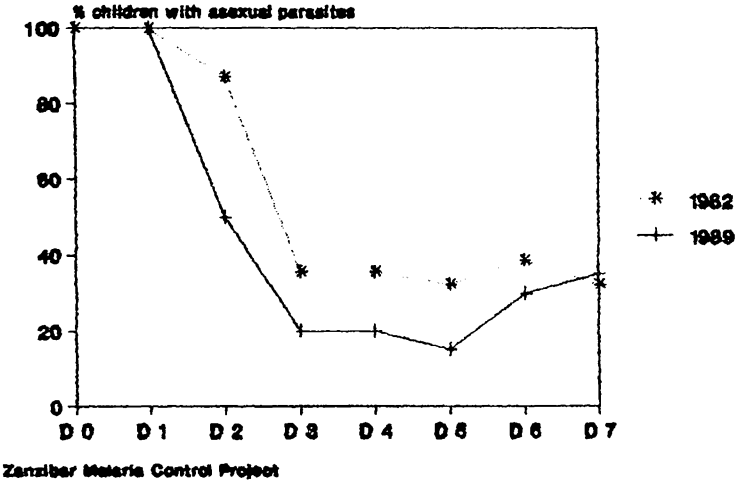


FIG. 5.
P. falciparum in vivo chemosensitivity
to chloroquine
Zanzibar Town - Zanzibar
Daily parasite density

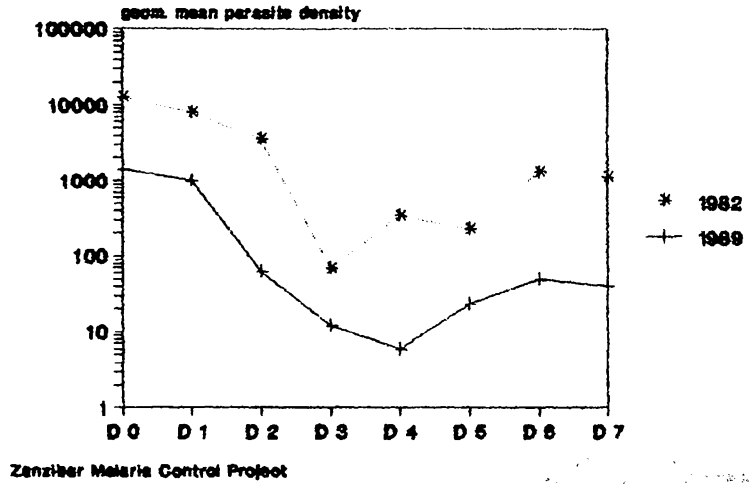
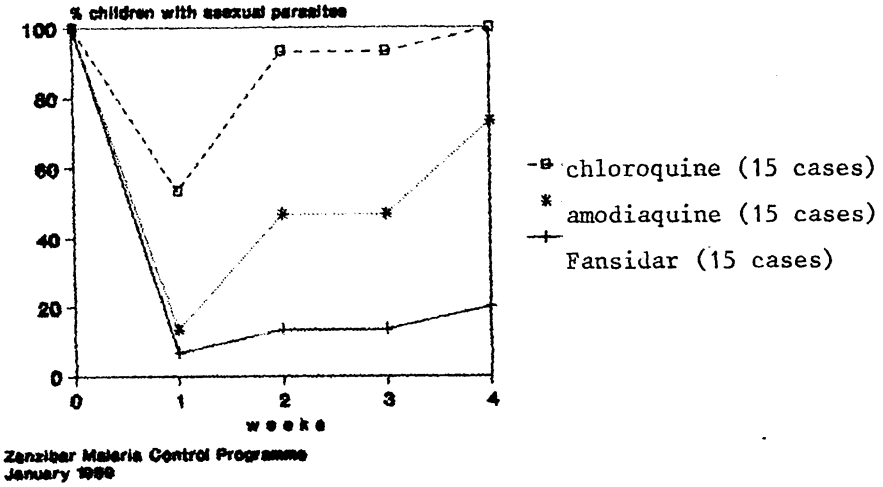


FIG. 6.
P. falciparum in vivo chemosensitivity
Makunduchi - Zanzibar, Tanzania
28-day extended field test



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