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CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA ON THE PACIFIC COAST OF COLOMBIA*

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The first documented chloroquine-resistance in malaria was that for a strain of *Plasmodium falciparum* from the Magdalena Valley in the northeastern part of Colombia.[1, 2] Although drug-resistant malaria has been reported since from other countries in South America, none has been reported from the Pacific coast of that continent.[3]

In March 1967 three North American servicemen, with no previous history of malaria, were seen in the Canal Zone with falciparum malaria. These men had participated in studies for a sea-level canal route in northern Colombia. Their activities had been centered at the proposed western terminus of the route at Curiche, Colombia. All gave a history of having taken the military chemoprophylaxis regimen of 300 mg chloroquine base and 45 mg primaquine weekly.[4]

When treated with therapeutic regimens of chloroquine, the infections responded abnormally. This led to an investigation to determine if the malaria parasites in these servicemen, as well as in the natives of the area where the servicemen acquired the infections, were resistant to chloroquine. The results are set forth in this report.

MATERIALS AND METHODS

The servicemen were treated with chloroquine at the U.S. Army Hospital, Fort Clayton, Canal Zone, and in Gorgas Hospital, Ancon, Canal Zone. Thick-film blood examinations were performed every 12 hours during hospitalization and daily thereafter for varying periods of up to 14 days; where possible, slides were made on days 14, 21, and 28. Parasites were counted by the method of Earle and Pérez.[5] Urine samples were collected for determination of the presence of chloroquine (Haskins test).[6] The treatment regimens and the parasitologic response in these men are shown below.

The history revealed that the most likely place for these men to have contracted the infection was in the area of the Pacific base camp for the canal studies. This camp is located on Humbolt Bay on the Pacific coast of Colombia south of the Colombia-Panamá border near the native communities of Curiche, Coredó, and Guarin. All native settlements in the area are located on the seacoast. The area immediately inland from the base camp is uninhabited.

In May 1967, a preliminary malaria survey was conducted in the area in question. Three communities were surveyed: Curiche, Coredó, and Guarin, and the laborers at the canal-study base camp (Camp Curiche).

On 1 August 1967 thick and thin films were made from blood of the 186 residents of villages Guarin and Coredó. Beginning 3 August, the team of investigators went from house to house treating with chloroquine the 76 persons shown to have malaria. The following procedures were used.

Each person was weighed on a bathroom scale prior to treatment and was given the drug under the supervision of one of the investigators; care was taken to see that the medicine was swallowed. The treatment was based on 1.5 g of chloroquine base for adults, adjusted for weight (Table 1). The drug was given in single daily doses as recommended by the World Health Organization.[3] Based on actual body weights, the total 3-day dosages ranged from 16.9 mg per kg to 41.3 mg per kg.

Thick and thin blood films were made on Days 0, 2, 3, 7, 14, 21, and 28, Day 0 being the first day of treatment. The films were stained with Giemsa stain. Parasites found were recorded as the ratio of parasites to 600 white blood cells, taken to be the equivalent of 0.1 cm of blood.

Co-operation of the community was very good during the treatment period and, while reluctance to give a blood sample had developed before the

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TABLE 1

Chloroquine-dosage schedule used in treating malaria

Weight (*lbs*)	Single doses of chloroquine base (*mg*)			
	Day 0	Day 1	Day 2	Total amount
100 to 150	600	600	300	1,500
50 to 99	300	300	150	750
20 to 49	150	150	75	375
Under 20	75	75	75	225

month of observation was completed, an adequate sample was obtained; 67 of the 76 persons were followed for 28 days.

RESULTS

Clinical malaria occurred in the three North Americans, who claimed that they were following the chloroquine-primaquine chemoprophylaxis regimen weekly. Before the therapeutic trials were begun, the urine of E.W. and R.M. was positive for chloroquine by the Haskins test. The patients then were started on a regimen of chloroquine base of 1.5 g base or more.

These three patients differed in parasitologic response to chloroquine treatment (Table 2).

Forty-eight hours (Day 2) after the initial dose of chloroquine, parasitemia in patient E.W. had increased about five times to the dangerous level of 116,290 per cm (RIII grade of resistance[3]). His clinical condition was worsening. To the chloroquine regimen was added quinine (10 grains three times a day for 10 days), pyrimethamine (25 mg three times a day for 3 days), and sulfadiazine (500 mg four times a day for 6 days). There was a rapid clinical response with clearance of parasites 4 days later. The patient's blood films remained negative for the remainder of the 28-day observation period. He remained well for 6 months, when he left the country.

R.M. received 2.1 g of choloroquine over a 5-day period and had a good initial clinical and parasitologic response. He took the standard military prophylactic tablet of 300 mg of chloroquine base and 45 mg of primaquine on Days 14 and 21. Parasites were found in his thick smears on Day 35 after the patient had complained of fever for 2 days (RI grade of resistance[3]). His urine was positive for chloroquine. He had another course of 1.2 g of chloroquine with good clinical and parasitologic response before leaving for the United States, when he was lost to our follow-up.

W.H. received an initial treatment of 2.4 g of chloroquine in 6 days; his clinical and parasitologic response was excellent. He returned to duty and took the standard military chemoprophylactic tablet of 300 mg of chloroquine base and 45 mg of primaquine on Days 14, 21, and 28. He became febrile on Day 28 and when seen on Day 30 again had malaria parasites in the thick blood smear (RI grade of resistance[3]). His urine was positive for chloroquine. On Days 30, 31, and 32 he received a standard regimen of 1.5 g of chloroquine base, which eliminated the parasitemia in 5 days. He then left for the United States and was lost to follow-up.

The history, clinical course, and early relapse of these men were sufficient to cause us to investigate the response of indigenous cases of malaria to chloroquine in the area of the Curiche base camp.

The preliminary malaria survey of the native population in May 1967 had revealed malaria prevalence rates of over 50% in the nearby native villages of Coredó and Guarin (Table 3). A survey made on 1 August 1967 in Coredó and Guarin resulted in the diagnosis of malaria on 76 of the 186 blood smears, a ratio of 40.9%. These infections were distributed as follows: *P. falciparum* rings, gametocytes, or both, 58; *P. vivax*, 14; mixed *P. falciparum* and *P. vivax*, four. The highest infection rates were in the younger age groups (Table 4).

The persons shown to have malaria were treated with chloroquine according to the schedule in Table 1. Of the 57 persons with *P. falciparum* parasites, in 24 (42%) the parasites either did not disappear or relapsed within 28 days (Table 5). Two, and probably three, never lost the parasites, indicating the RII grade of resistance.[3] An additional four persons (28%) lost the parasites by Day 3, but parasites were present again on Day 7. Seven additional patients had parasites on Day 14, five more on Day 21, and five more on Day 28. Those in the last four groups are indicative of the RI grade of resistance.[3]

Urine samples collected on Day 7 from four of those in whom treatment failed gave positive Haskins tests for chloroquine.

Of considerable interest is the age distribution of those infected at the beginning of treatment

TABLE 2

Parasitologic response of falciparum malaria in three nonimmune persons treated with chloroquine

Days after treatment	E.W.		R.M.		W.H.	
	Trophozoites per mm	Chloroquine base (mg)	Trophozoites per mm	Chloroquine base (mg)	Trophozoites per mm	Chloroquine base (mg)
0	26,550*	900	17,610*	900	14,380	900
1	128,390	300	67,250	300	350	300
2	116,200*	300†	180*	300	10*	300
3	16,740		10	300	0	300
4	350		0	300	0	300
5	20		0		0	300
6	0		0			
7	0					
8	0					
9	0					
10	0					
11	0					
12	0					
13	0					
14	0		‡			‡
21	0		‡			‡
28	0					‡
30					1,090*	900
31					5,650	300
32					6,310	300
33					260	
34					20	
35			6,040*	600	0	
36			150	300	0	
37			0	300	0	
38			0		0	
39			0		0	

* Urine positive for chloroquine.

† The following additional treatment was begun: quinine, 10 gr t.i.d. × 10, pyrimethamine 25 mg t.i.d. × 3, and sulfadiazine 500 mg q.i.d. × 6.

‡ The standard military prophylactic tablet of chloroquine base 300 mg plus primaquine 45 mg had been taken.

(Table 4). No person over age 40 had a positive blood film when first seen; 14 persons ages 19 to 40 had malaria; 62 persons 18 years of age or younger had malaria.

All *P. falciparum* failures and relapses occurred in the age group 18 years and younger; in fact, 22 of the 24 occurred in persons 10 years of age or younger. The seven persons who did not lose parasitemia or whose blood was positive on Day 7 were 10 years of age or younger; five were younger than 6 years. No relapses occurred in any person who weighed over 100 pounds who had received 1.5 g of chloroquine base.

The 17 cases of *P. vivax* parasitemia were

TABLE 3

Malaria survey of Camp Curiche area, May 1967

Locality	No. slides	*P. falciparum*	*P. vivax*	Mixed	Total	Percent positive
Curiche	150	18	0	0	18	12.0
Coredó	107	34	23	7	64	59.8
Guarin	38	10	9	1	20	52.6
Camp Curiche (laborers)	24	2			2	8.3
Total and average	319	64	32	8	104	32.6

TABLE 4

Prevalence of malaria, by age groups, in populations of Guarin and Coredó, August 1967

Age (years)	Number of persons	Positive for malaria	
		(No.)	(%)
<1	13	4	30.7
1–4	34	16	47.1
5–9	40	25	62.5
10–18	31	17	54.8
19–39	48	14	29.1
40–59	12	0	—
60+	8	0	—
Total and average	186	76	40.9

TABLE 5

The parasite response of P. falciparum *infections that were not cured by chloroquine, distributed by age groups*

Age (years)	<18	18+
Parasite response	Number	Number
Not cleared	3	0
Cleared but relapsed in		
7 days	4	0
14 days	7	0
21 days	5	0
28 days	5	0
Total failures	24	0
Total treated	46	11
Percent failures	52	0

cleared within 3 days after the beginning of treatment. There were three relapses, one within 14 days and two within 28 days. These were in children 3, 12, and 5 years of age, respectively.

DISCUSSION

The data cited from the three cases of falciparum malaria occurring in nonimmune North Americans and from the children in Coredó and Guarin are evidence of a chloroquine-resistant strain of *P. falciparum* malaria as recently defined.[3, 8] The following points support this conclusion. The parasites produced clinical disease while the nonimmune adults were taking weekly prophylactic dosages of chloroquine-primaquine. Positive urine tests for chloroquine supported the claim that the drug was taken. The parasites greatly increased in numbers in one nonimmune adult who was taking the standard therapeutic dose of 1.5 g of chloroquine base. In two other adults the parasites disappeared after the therapeutic regimen, but relapse occurred in one case while the patient was taking the weekly prophylactic regimen.

In the native children, a large proportion either did not lose the parasites, or had relapses within 28 days after the taking of the standard dosages of chloroquine.

The difference in response of falciparum malaria parasites to chloroquine in the native adults as opposed to some of the children may be explained by several hypotheses. Presumably more years in an endemic area and more experience with malaria conferred a higher level of immunity in the adults. This, coupled with the schizonticidal effect of chloroquine, may have been sufficient to "cure" the cases of malaria seen in adults where the combination of a weight-equivalent amount of chloroquine and a presumably lower level of immunity failed to cure some children.

The 1.5 g standard dose of chloroquine base for adults gives a dose of 25 mg per kg for a 60-kg adult and a maximum of 33 mg per kg for any 45.5-kg (100 pounds) person. Five of the seven children whose infections were not cleared, or in whom relapse occurred by Day 7, had received more than 25 mg of chloroquine base per kg of body weight, as had 10 others who had relapses by Day 28. One of the latter had received 41.3 mg per kg. Therefore the failures do not appear to be related to inadequate dosages.

No pattern of parasite response is discernible; relapses at Days 7, 14, and 21 appear to be independent of the dosage of chloroquine received. By contrast, the response was more varied in the three nonimmune North Americans. The parasites in one of these (E.W.) showed a high level (RIII) of resistance to chloroquine, as manifested by the parasites' increasing to a dangerous level while the drug was being taken.

Most resistant strains of *P. falciparum* malaria have been detected by the failure of the infections in nonimmune adults to respond adequately to standard doses of the drug. The present study is one of the first on the response to chloroquine of the malaria in native popu-

lations, undoubtedly semi-immune, compared with the response of apparently the same strains of malaria in nonimmune persons. The results indicate that the response of the resistant infections in the younger age groups of the native population is somewhat similar to that in the adult nonimmunes. Had only persons 19 years of age or older been tested, no evidence of chloroquine resistance would have been demonstrated in the native population. The response of the native adults in a highly endemic area with undoubtedly the same strains of malaria indicate that they are not adequate models for testing for drug-resistant parasites.

SUMMARY AND CONCLUSIONS

Data from three nonimmune North Americans who acquired malaria in a highly endemic area of Colombia, and data from a field trial with infected natives in the same area, indicated the presence of chloroquine-resistant *Plasmodium falciparum* in the area. This is the first report of chloroquine-resistant malaria from the Pacific coast of the Western Hemisphere.

Evidence for chloroquine-resistance in the natives was found only in the children. It is concluded that tests for suspected chloroquine-resistance in *P. falciparum* in native populations of endemic areas are best done in the younger age-groups.

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