J Occup Health 1997; 39: 105–112

Journal of Occupational Health

Prevention of a Malaria Outbreak among Non-Immune Japanese Workers Engaged in the Construction of a Thermal Power Plant in Sonebhadra, India

Hironobu KATSUYAMA[1], Shigeyuki KANO[2], Mamoru SUZUKI[2], Kiyofumi SAIJOH[3], Kimiaki SUMINO[4] and Goro TSUCHIYA[1]

[1]Department of Health, Mitsubishi Heavy Industries, Kobe Shipyard,
[2]Department of Parasitology, Gunma University School of Medicine,
[3]Department of Hygiene, Kanazawa University School of Medicine and
[4]Department of Public Health, Kobe University School of Medicine

Abstract: Prevention of a Malaria Outbreak among Non-Immune Japanese Workers Engaged in the Construction of a Thermal Power Plant in Sonebhadra, India: Hironobu KATSUYAMA, *et al.* Department of Health, Mitsubishi Heavy Industries—Continuous consumption of anti-malarial drugs is not always recognized as the first choice for prevention of malaria among workers residing in malarious areas for long periods. In Japan, personal protective measures have been primarily recommended. However, a high incidence of malaria was observed among workers engaged in construction of a thermal power plant in the Sonebhadra district, Uttar Pradesh, India, during the period from February to August, 1991. On-site inspection revealed the difficulties of preventing mosquito bites among personnel who had lived in industrialized countries and were not immune to malaria. Repeated education regarding the danger of malaria and rigorous precautions from mosquito bites dramatically reduced the incidence of malaria among such personnel. Not only proper usage of personal protection measures such as long sleeved shirts, mosquito repellents, mosquito nets etc., but also an effort to reduce the number of mosquitoes in living and working areas by such means as insecticides, spraying operations, etc. should be emphasized. On the other hand, chemoprophylaxis still seemed to be the most effective protective measure for workers compelled to remain outdoors until late at night. The utility and availability of protective divices may depend on the working status and circumstances, so that on-site inspection helps to ensure occupational health, and also affords an opportunity to instruct the workers on malaria prevention.
(*J Occup Health 1997; 39: 105–112*)

Received April 11, 1996; Accepted Sept 17, 1996
Correspondence to: H. Katsuyama, Department of Public Health, Kawasaki Medical School, 577 Matsushima, Kurashiki-city 701-01, Japan

Key words: Malaria, Non-immune Japanese workers, On-site inspection, Protective measures

Large-scale epidemics of malaria in the tropics are still being reported. The WHO[1,2] has reported that 42% of the world's population, or about 2,280 million persons, live in areas with varying degrees of malaria risk. Among them, 1,780 million live in areas where endemic malaria has been considerably reduced or even eliminated but transmission still occurs. As many as 490 million people inhabit areas where endemic malaria remains basically unchanged and most control programs are still at the planning or initial implementation stage. Although accurate information on the global incidence of malaria is difficult to obtain, the most recent estimates indicate that the worldwide incidence of malaria may be of the order of 300–500 million clinical cases per year.

Recent development projects and industrial enterprises in the tropics may produce ecological and social changes conductive to outbreaks of malaria. Their facilities are often planned for construction in undeveloped areas such as wild jungles where ecological changes can easily trigger malaria outbreaks. Their large labor forces may include newcomers to the area who are not immune to local strains of Plasmodia and, moreover, may introduce strains to which the local population is particularly susceptible[3]. Engineers from highly industrialized countries or cities, who usually lack any immunity to malaria, must cooperate with local workers who are occasionally asymptomatic carriers. Such situations amplify the danger of contracting malaria, particularly for non-immune personnel who must remain at the sites for long periods.

The Centers for Disease Control, USA recommends the use of anti-malarial drugs even when necessary for extended

periods, since prophylactic doses of chloroquine rarely cause serious adverse reactions, in addition to personal protective measures[4]. On the other hand, the WHO recommends administration of anti-malarial drugs only for short-term travelers but advises long-term residents to consult malaria specialists[5]. Mass chemoprophylaxis may not always be desirable as the first-choice countermeasure with respect to the resistant strains prevailing at the present time. Careful studies are necessary to prepare guidelines for prevention of malaria in accordance with the local conditions of each particular project.

The present report concerns the results of our research on prevention of a malaria outbreak among non-immune Japanese workers engaged in the construction of a thermal power plant in Sonebhadra, India, as a minor group among large numbers of domestic workers hired at the site. A rational malaria control manual was particularly required for the non-immune Japanese workers from the viewpoint of occupational health. This study should be applicable to health programs in similar types of plant projects.

Subjects and Methods

Location of work site

The site of the present project was as shown in Fig. 1A. The area was selected because of a promising potentiality for industrial development. A coal mine and rich water reservoir in the Rihand lake were considered to provide the essential background for future development of this area regardless of the present limitations in the basic infrastructure such as transport, communication, etc. Owing to this high potentiality for industrial development, a variety of international project teams have constructed a number of facilities around the Rihand lake. A thermal power plant is being constructed in one such project area in Anpara, as shown in Fig. 1B. This project has been in progress since 1990.

Workers and location of their residential facilities

Approximately 2,500 domestic laborers were engaged in the construction work. Eight to nine Japanese construction engineers and 2–3 office businessmen were working regularly at the site. Each individual remained at the site continuously for 6 months to 2 years or longer, with 2 to 4 week home leaves at 6-month intervals. Their accommodation was a prefabricated dormitory with attached recreation hall and dining room, located at the southwest corner of the project area. Local laborers lived in the villages along the Singrauli-Pipri road, and the distance from the south end of the village to the Japanese camp was approximately 1.5 km (Fig. 1C). A forest lies to the west of the station beyond the villages. North and south of the station are heights with small rivers and forests, and a swamp and the Rihand lake extend along the east end of the station. Such humid places may provide breeding grounds for mosquitoes.

Diagnosis of malaria

Malaria was diagnosed at the on-site clinic by microscopic examination of Giemsa stained blood smears from the suspected cases. The diagnosis was reconfirmed by laboratory examination after the patients had returned to Japan. Specific antibody titration with an indirect fluorescent antibody test in accordance with the method of Kano[6] was used for retrospective diagnosis. All workers were subjected to health checkups including serological examination after each return trip to Japan. Since the period of stay varied from worker to worker, the incidence was calculated by using person-month methods for three sub-periods after looking at the data, i.e. before February, 1991, from February, 1991 to August, 1991 and after August, 1991.

Results

Incidence of malaria

A total of 46 workers (all male, ages ranging from 24 to 51 years) were sent to the Anpara station during the period from May, 1990 to July, 1995. Before July, 1991, the number of workers sent to the site was limited. Nonetheless, among the total of 19 workers at the site during the period from February to August, 1991, eight were diagnosed as having malaria, including one suspicious case (case 6) (Figs. 2 and 3). Owing to this high incidence, on-site inspection was performed at the end of April, 1991. Although 3 cases were diagnosed after on-site inspection, their infection was presumed to have occurred around April. After construction of the boiler for the No. 4 unit was completed in June, 1991 (Fig. 2), the number of workers was increased in order to start construction of a new boiler for the No. 5 unit as well as to operate and maintain the previously completed boilers. However, only one of these workers contracted malaria.

Cases

Patients 1 to 5 complained of clinical symptoms such as fever, diarrhea and nausea, etc., attended the on-site clinic, and were diagnosed as being infected by *P. vivax* on the basis of microscopic examination. Patient 5 manifested fever, and microscopic examination revealed his infection to be malaria in August. In July and August, the number of mosquitoes was relatively small. He was more likely to have been exposed to mosquitoes during a short term stay in April, hence his infection was presumed to have occurred in April rather than July or August. Although case 6 was suspected to have contracted malaria because he experienced vomiting and diarrhea, microscopic examination did not confirm the diagnosis. He was under prophylactic treatment with chloroquine. Even repeated microscopic and serological examination failed to yield positive findings in this case. All patients were treated with chloroquine after the diagnosis regardless of the previous institution of chemoprophylaxis or otherwise. The total dose of chloroquine varied from 0.75 g to 13.5 g but symptoms usually disappeared before the dose reached 1 g. In some

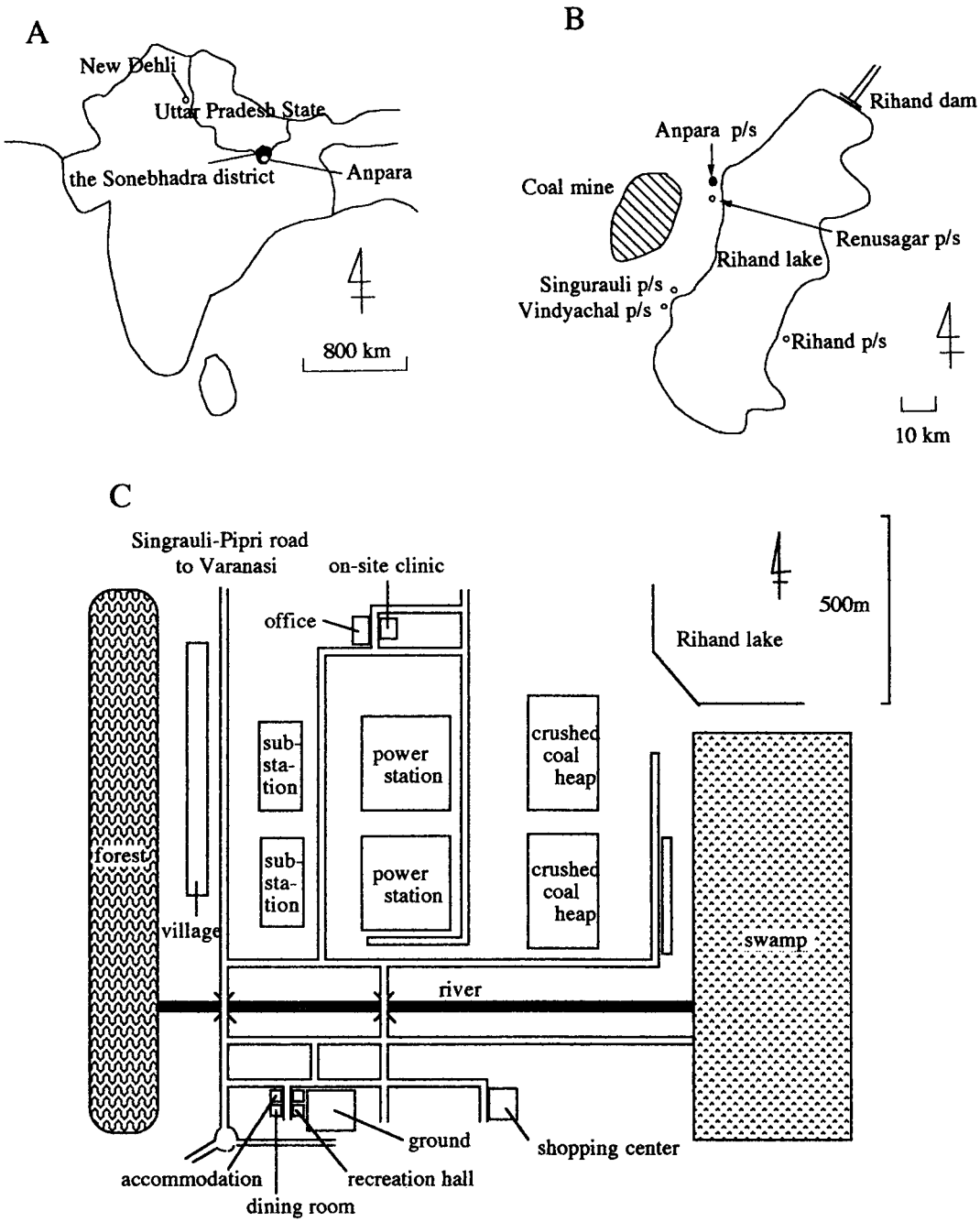

Fig. 1. Location of the Sonebhadra district (A) and Anpara (B), and layout of the Anpara power station (C).

cases, however, anti-inflammatory drugs or antibiotics were also administered.

In addition to patient 5, two more workers were diagnosed with malaria in July and August (Fig. 3). The diagnosis of these cases was established by significantly high titers of antibody to *P. falciparum* observed in serological tests performed after their return to Japan. Patient 7 had neither apparent clinical symptoms nor episodes of intense exposure to mosquitoes while he had stayed at the site since January, 1991. His infection also might have occurred around April. Although patient 8 developed fever and diarrhea in June as well as in August, he did not attend the clinic because he was confident of protection by chemoprophylaxis. This patient started staying at the site in May, hence contraction seemed to occur just after on-site inspection when the re-instruction was not yet effective.

None of the patients diagnosed as infected with *P. vivax* by microscopic examination, i.e., patients 1 to 6, were

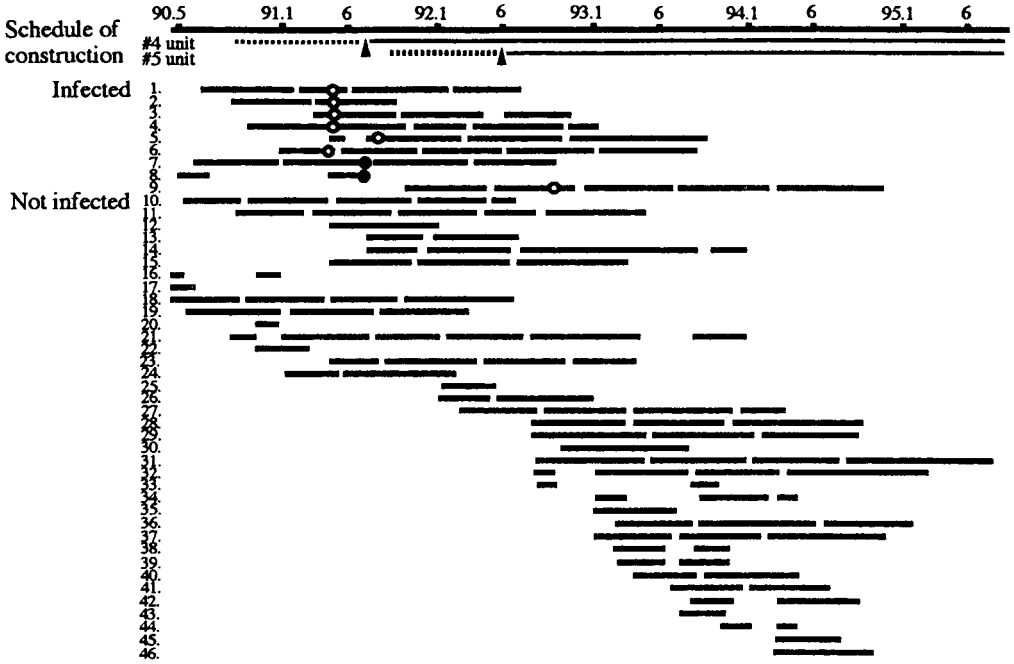

Fig. 2. Timetable of workers staying at the Anpara power station. The boiler was under construction (--), completed (▲), and under operation (—). Diagnosed by microscopic examination (○) and serological tests (●) as having contracted malaria.

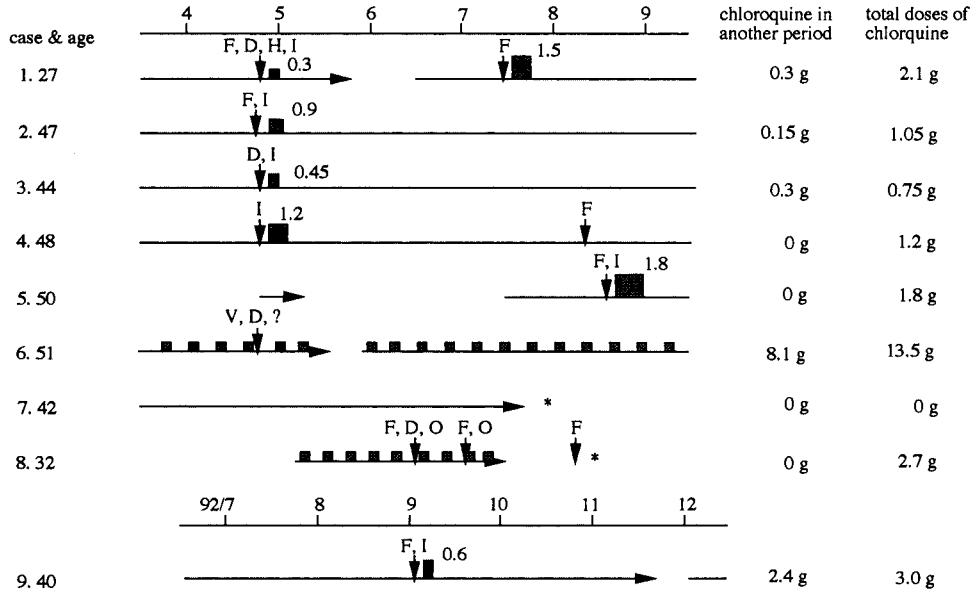

Fig. 3. Cases of malarial contraction and their progress. F, fever; H, hematuria; D, diarrhea; V, vomiting; O, treatment with Ofloxacin; I and ?, respectively diagnosed or suspected as P. vivax infection by microscopic examination; *, positive to P. falciparum antibody; and ▬, administration of chloroquine and doses.

positive in anti-malarial antibody examinations performed in Japan.

On-site inspection was performed from 30 April, 1991 to 5 May, 1991 in order to clarify the reasons why so many workers had contracted malaria during the same period and to improve protective measures.

On-site inspection

1. Purpose of on-site inspection: Following the advice of specialists in Japan, personal protective measures including mosquito coils, mosquito nets, insecticide sprays, etc., were recommended as the first choice for countermeasures to malaria. Spraying operation around the housing areas was also recommended. Chemoprophylaxis was advised only in cases where workers were exposed to higher malaria risks. The first purpose of on-site inspection was to ascertain whether the workers had abided by the recommendations, and whether the recommendations themselves were appropriate and applicable to the site, etc.

2. Daily life of workers at site: Both outdoor and office workers were obliged to work for 11–12 hr a day from April, 1991 to August, 1991 and from January, 1992 to March, 1992 in order to complete the boiler, and from December, 1992 to February, 1993 to start firing the boiler (Fig. 2). Excluding such busy periods, they usually worked approximately 10 hr a day. Moreover, they were occasionally compelled to leave the site in order to negotiate with other companies, to purchase equipment or materials, etc., and to stay outdoor for long periods and until late at night. All workers were also compelled to go out for meals in the dining room outside the dormitory (Fig. 1C). They usually spent their free time at night with their colleagues in the recreation hall which was also located outside the dormitory. They therefore often remained outdoors even after sunset.

3. Protective measures at the work site: Only one worker was under chemoprophylaxis when on-site inspection was performed, but he had contracted malaria (case 6). The protective measures in the dormitory and the office seemed to be well organized and were effective in preventing the entry of mosquitoes, but only one mosquito coil was provided in the dining room (80 m^2) and the recreation hall (30 m^2), though many people were frequently entering and leaving. Since the workers were off duty and relaxed, they were often careless about preventing mosquito bites or repelling mosquitoes (Table 1).

Personnel working outside the power station usually carried personal protective devices. However, they did not always carry these devices when they left the power station, especially when they expected to finish their tasks in a short time, or to work indoors at the assigned locations, etc. Contrary to their expectation, they were sometimes compelled to remained outdoors for long periods or until late at night and subjected to unexpected exposure to mosquitoes. Even when the workers brought personal protective devices, they were not always appropriate or sufficient for such occasions.

4. Malaria risk and information available at the work site: The transmission pattern has been generally documented as seasonal but not perennial in the northern and central parts of India including the Sonebhadra district[7]. That is, only a small number of mosquitoes exist during the hot season in May and June. After the rainy season from late June to early September, mosquitoes proliferate and remain numerous until November. December and January are cool and the number of mosquitoes decreases, but the mosquito season resumes again in February and continues until just before the hot season. Thus, concomitantly with increased numbers of mosquitoes, malaria transmission increases bimodally before the hot season and after the rainy season.

Nevertheless, neither a nation-wide nor a regional epidemiological surveillance system for malaria exists in India. Aside from national trends in the incidence and prevalence of malaria in the whole of India, even the WHO regional office for South-East Asia cannot always ascertain precise information for each limited area. Although the prognosis of non-immune patients who had first contracted malaria depends on the prevalent species of malarial parasite and resistance to anti-malarial drugs, etc., information regarding the characteristics of temporary endemic malaria was not available even in hospitals and clinics in the endemic regions. The local physicians, including those of the on-site clinic, seemed to regard malaria with the same attitude as a common cold and not as a serious infection.

5. Chemoprophylactic regimen: Workers did not always comply with the chemoprophylactic regimen. Although side effects, such as gastrointestinal disturbances, dizziness and headache, etc., were minor, they were sometimes regarded as intolerable by workers who were not infected. Moreover, they were apprehensive concerning serious adverse reactions such as retinopathy, despite their rarity, and did not recognize the importance of continuous preventive administration of drugs. Thus, regular prophylactic medication was neglected by many workers. The workers complied with anti-malarial drug regimens only when they were diagnosed as infected, but abandoned the regimen as soon as possible after the symptoms disappeared.

Re-instruction and improvement of protective measures

The information collected was fragmentary. Even though the workers occasionally suffered from mosquito bites, no episodes of intense exposure to mosquitoes occurred. The reason why malarial infection occurred mainly in April but not in February, March or autumn remained obscure. However, several problems were found in application of protective measures especially in the prevention of mosquito bites, so that protective measures which were useful for preventing mosquito bites were instructed (Table 1). Moreover, the chemoprophylaxis regimen was amended (Table 2). Frequent use of an insect spray was advised in order to reduce mosquitoes not only in the dormitory but

Table 1. Problems in protective measures against mosquito bites found at on-site inspection and indication after on-site inspection

	on-site inspection	indication after on-site inspection
Personal protection at outside		
• clothes		
in daytime	•not always wear a long sleeved shirt	•in high seasons, wear a long sleeved shirt and trousers.
at night	•not always wear a long sleeved shirt and trousers	•wear a long sleeved shirt and trousers at any time
•application of repellents to parts of the body that are not covered by clothes	•sometimes forget to take along repellents when going out or the workers do not always recognize the necessity of repellents when they expect to go out for only a short period	•use every 2–3 hr even when the workers have to go out for only a short time, take along repellents
Accommodation		
•the entrance door	•keep opening	•use mosquito gauze
•mosquito net for the bed	•usually used	•continue using
•insecticides and mosquito coils		
in the dining room and recreation hall	•insufficient and many people are going in and out	•an increase in the number of mosquito coils and frequent use of insecticides are required
in the personal room	•usually used when the workers are in the room	•in high seasons, even when the workers are absent, keep using them in private rooms
in the toilet	•not always used	
•spray operation	•not to be performed	•once a week and more frequently when warned of increasing malaria risk
Office building		
•the entrance door	•not always closed and many people going in and out	•keep closing to prevent the entry of mosquitoes.
•insecticides and mosquito coils		
in the office	•used but not sufficient	•an increase in the number of mosquito coils and frequent use of insecticides are required
in the conference room	•insufficient and many people going in and out	
in the drawing room		
in the toilet	•not always used	
•spray operation	•not to be performed	•same as at the accommodation

Table 2. Indication of chemoprophylaxis against malaria after on-site inspection

1. Workers who have to stay outside frequently at night have to take chloroquine in the high season of malaria
2. Other workers had better take chloroquine when information concerning increased malaria risks is obtained
3. Suggested regimen for drug prophylaxis:
   chloroquine for adults - 300 mg base - once a week
4. Check for side effects (after each return to Japan)
   total amount of chloroquine
   periodic ophthalmologic examination
   symptoms, etc.

also throughout the living area including the dining room and the recreation hall. The workers were strictly required to carry personal protective devices when going out, especially at night. Chemoprophylaxis was, however, recommended only for workers who had to stay outside frequently at night.

We also requested the physicians of nearby hospitals to provide any available information concerning increased malaria risks, e.g., that the numbers of mosquitoes or patients were increasing in the surrounding areas. Once such information was obtained, more stringent measures, including chemoprophylaxis, would be recommended to all the workers.

Case discovered after inspection

The contraction of malaria by patient 8 occurred just after on-site inspection even though he was under chemoprophylaxis, as described above. This worker visited the site when the re-instruction was not yet effective and might have underestimated the importance of protection against mosquito bites because he was under chemoprophylaxis. Such an episode emphasized the importance of protection against mosquito bites irrespective of having chemoprophylaxis or not. Although no workers were subjected to chemoprophylaxis even after on-site inspection, only one patient (patient 9), not under chemoprophylactic treatment, was diagnosed as having malaria by microscopic examination when he became febrile in September, 1992. Since he failed to visit the clinic when he noticed fever in February, 1992, it is not obvious whether the fever in September was caused by primary infection or by recurrence. His symptoms disappeared after taking 0.6 g of chloroquine.

Chloroquine treatment and adverse reaction

A total of 18 workers had been using chloroquine, the average dose being 2.55 g/person (range 0.3 g–13.5 g), and no retinopathy was detected in periodic ophthalmologic examinations.

Incidence rate of malaria

No incidence was observed before April, 1991, when five cases were diagnosed. The diagnosis of patient 5 was established in August but this patient's infection was presumed to have occurred in April, as mentioned above. Since serological examination established the diagnoses of cases 7 and 8 in August and July, respectively, it is difficult to specify when their infection occurred. Considering the latent period of malaria and the high season of mosquitoes as well as the periods of the workers' stay, the incidence was calculated for the period February, 1991 to August, 1991 rather than April, 1991. The incidence rate during this period was high, i.e., 0.11, as shown in Table 3. After August, 1991, the rate decreased to 0.0024. The Relative risk of malarial infection from February, 1991 to August, 1991 versus that in other periods was 51.6 (CI: 6.5–425.4).

Table 3. Incidence rates in each period calculated by the person-month method

	observation period		
	91.2–91.8	before 91.2	after 91.8
Total person-month	73.5	60.5	413.5
Cases	8	0	1
Incidence rate	0.11	0	0.0024
		0.0021	
Relative risk	51.6 (CI: 6.5–425.4)		

Discussion

The latent period of malaria ranges from a few days to several years. It is not unusual for malaria patients to manifest symptoms only after they have left the endemic area and returned to their home country. Most physicians in industrialized countries lack expertise in the treatment of malarial patients, hence delay in diagnosis and treatment is often inevitable. Thus, repeated examination for malaria is desirable when non-immune persons must reside for long periods in malarious areas.

Although serological tests performed after the workers had returned to Japan also verified two cases of malaria, many of the cases in the present study were diagnosed by microscopic examination after the symptoms became apparent. Serological diagnosis was usually not attempted at the site and the diagnosis was usually based upon microscopic examination of blood. Since the malaria antibody becomes positive two weeks or more after the primary infection, serological tests appear to be of only limited value in the diagnosis of acute malaria[8]. Serological tests are more useful in epidemiological studies, in determining whether a person has had malaria in the past, and in examining the effect of anti-relapse treatment, etc. All the patients in which microscopic examination revealed *P. vivax* in the blood were negative in serological tests performed after their return to Japan. However, *P. falciparum* infection was diagnosed by the serological test but not by the microscopic examination. Although the persistence of the antibody may vary with delay in treatment, the degree of parasitemia, etc.[6], that of patients non-immune to malaria after a single infection is still controversial. Moreover, the persistence of the antibody for *P. vivax* may differ from that for *P. falciparum*. Prompt treatment may prevent antibody development in patients who have contracted the benign form of malaria caused by *P. vivax*.

Although it is difficult to specify when the transmission occurred, an extremely high incidence was observed only in the period from February to August, 1991, suggesting insufficiency in the protective measures themselves and/or their application. On-site inspection revealed problems in

complying with instructions. It appears very difficult for people who have lived in industrialized countries to remember the danger of malaria and to maintain vigilance against mosquito bites. Even if all workers on duty remembered to carry personal protective devices both day and night, they were not always so cautious when off duty and relaxed. It is absolutely essential that workers become familiar with general precautions against malaria before entering a malarious area. However, instructions appropriate for the specific circumstances at the work site are also important to prevent unexpected exposure to mosquitoes. Furthermore, occasional instruction should be repeated for workers who have became familiar with the circumstances of the site but have possibly forgotten the danger of malaria. The workers preferred to take antimalarial drugs as chemoprophylaxis, especially when they expected to stay at the site for only a short period, as in patient 8. However, malarial contraction occurred even under chemoprophylaxis if the workers neglect the importance of protection against mosquito bites. The importance of prevention from mosquito bites should be emphasized.

It is reasonable to consult physicians in the endemic area not only in cases where overt symptoms appear but also for the purpose of regular health examination, since they have more experience concerning malaria infection than physicians in industrialized countries. However, at the same time, it is also important to remind these physicians that non-immune people from industrialized countries easily ignore the danger of malaria. India has been classified as an RI area, i.e. clearance of asexual parasitemia can be achieved as for sensitive strains, but is followed by recrudescence within 28–42 days[8], but all the pathogens in the present study were fortunately sensitive to chloroquine without recrudescence. It is essential to detect *Plasmodium* by microscopic examination to confirm infection with malaria and to identify its species. However, microscopic examination alone is insufficient to ascertain whether or not the organism is resistant to drugs. Drug tolerance should be investigated immediately after the diagnosis is established. Information concerning the *Plasmodium* species involved and drug resistance, etc., may contribute to the management and prognosis of the patient. In addition, a more sensitive and reliable examination, such as *in situ* antibody examination[9,10], is desirable for workers displaying symptoms. When it is difficult to perform such further examinations at the on-site clinic, the company or, at least, the international project teams concerned should make an effort to refer the patients to nearby hospitals. The organization of miniature surveillance systems covering the work site, on-site clinic and nearby hospitals are also useful. International project teams should cooperate in establishing such systems to permit collection of accurate information and its prompt communication to each branch, even when the system is a very limited one.

Since only one patient developed malarial symptoms and was positive in microscopic examination after August, 1991, all the precautions adopted appear to have been effective in reducing malarial transmission. Repeated education helps workers to keep in mind the importance of such measures. This worker had not been under prophylactic medication. It is not unreasonable to suppose that chemoprophylaxis might have prevented overt symptoms in this case even though malarial infection was unavoidable. People, however, are generally unwilling to take medicine without having manifested symptoms, even when chemoprophylaxis is indisputably the most effective protective measure. It is also important to urge workers to faithfully take anti-malarial drugs during seasons of frequent transmission, although strict protective measures rather than chemoprophylaxis still seem to be preferential. The utility and availability of protective devices may depend on the working status and circumstances, so that on-site inspection helps to ensure occupational health, and also affords an opportunity to instruct workers on malaria prevention, including the safe use of prophylactic medication.

References

1) World Health Organization. World malaria situation in 1991, Part I. Weekly Epidemiol Record. Geneva: WHO, 1993; 34: 245–252.
2) World Health Organization. World malaria situation in 1991, Part II. Weekly Epidemiol Record. Geneva: WHO, 1993; 35: 253-258.
3) Gilles HM, Warrell DA. Bruce-Chwatt's essential malariology. Edward Arnold, 1993.
4) Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Service. Recommendations for the prevention of malaria among travelers. Morbid Mortal Weekly Report. Atlanta: U.S. Department of Health and Human Services, 1990; 39 (RR-3): 1–10.
5) World Health Organization. The informed prescriber: critical choices in drugs and therapeutic alternatives. Drug Bulletins Review. Geneva: WHO, 1990; 5. 5.
6) Kano S, Waki S, Igarashi I, Nakazawa S, Masuda G, Suzuki M. Retrospective malaria diagnosis by indirect fluorescent antibody titration on Japanese patients. Jpn J Parasitol 1990; 39: 475–481.
7) World Health Organization. Epidemiological considerations for planning malaria control in South-East Asia region. Geneva: WHO, 1987; South-East Asia Series No. 17.
8) World Health Organization. The clinical management of acute malaria. Geneva: WHO, 1990; South-East Asia Series No. 9.
9) Avraham H, Golenser J, Spira DT, Sulitzeanu D. *Plasmodium falciparum*: assay of antigens and antibodies by means of a solid phase radioimmunoassay with radioiodinated staphylococcal protein A. Transac Royal Soc Trop Med Hygiene 1981; 75: 421–425.
10) Mackey LJ, McGregor IA, Paounova N, Lambert PH. Diagnosis of *Plasmodium falciparum* infection in man: detection of parasite antigens by ELISA Bull WHO 1982; 60: 69–75.