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Plasmodium ovale Malaria Acquired in Viet-Nam

N. N. GLEASON,[1] G. U. FISHER,[2] R. BLUMHARDT,[3] A. E. ROTH[4] & G. W. GAFFNEY[5]

Four cases of Plasmodium ovale *malaria are reported among US servicemen stationed in Viet-Nam between January 1966 and March 1969. Taken together with other cases cited by the authors, these provide strong evidence of the existence (sometimes disputed) of this* Plasmodium *in continental South-East Asia.*

None of the men had served in any other area of endemic malaria and their travel and medical histories suggest that all 4 infections were acquired by mosquito transmission. They constitute only 0.066 % of the 6036 malaria cases reported among servicemen returning from Viet-Nam during this period and represent only 0.11 % of the blood films from 3686 individuals examined at the US National Malaria Repository during the same period.

Serological testing for malaria antibodies with the indirect fluorescent technique corroborated the diagnosis of P. ovale *in 1 case. Speciation was not possible in the other 3 cases since titres to* P. vivax *and* P. ovale *antigens were identical. Only 1 of the patients reported previous experience with vivax malaria.*

Most of the parasites seen in thin blood films were developing trophozoites and immature schizonts; ring forms and gametocytes were rare; mature schizonts were not found. The morphology of the parasites was typical of P. ovale, *with more than 50 % of the infected cells showing fimbriations, an oval shape or both.*

Mosquito transmission of *Plasmodium ovale* malaria is common in West Africa according to Bruce-Chwatt (1963) and Garnham (1966) and has been documented on rare occasions in the Philippines (Garcia, 1941; Jeffrey & Young, 1954; Alves, Schinazi & Aniceto, 1968). It has also been reported from eastern New Guinea by Jackson (1944) and McMillian & Kelly (1967). A case of ovale malaria was also reported in 1942 by Yao & Wu, but the existence of this species on the South-East Asian subcontinent has recently been disputed by Lysenko & Beljaev (1969). The following case reports provide further evidence that *P. ovale* does exist in continental South-East Asia.

[1] In Charge, National Malaria Repository, Protozoology Laboratory, Helminthology and Protozoology Unit, Parasitology Section, Microbiology Branch, Laboratory Division, National Communicable Disease Center, Atlanta, Ga. 30333, USA.

[2] Resident in Medicine, Stanford University Hospital, Palo Alto, Calif. 94304, USA. Formerly, Epidemic Intelligence Service Officer, Malaria Surveillance, Parasitic Disease Branch, Epidemiology Program, National Communicable Disease Center, Atlanta, Ga. 30333, USA.

[3] Chief, Internal Medicine, 98th General Hospital, APO New York 09305, USA. Formerly, Assistant Chief, Department of Medicine, US Army Hospital, Fort Carson, Colo., USA.

[4] Bethany Hospital, Kansas City, Kansas 66102, USA. Formerly, Chief, Pathology Service, US Army Hospital, Fort Carson, Colo., USA.

[5] Medical Staff, Loch Raven Veterans Administration Hospital, Baltimore, Md., USA.

CASE REPORTS

Case 1

On 10 November 1967, while on duty in California, a 21-year-old white American soldier developed chills and fever. Six days later he was hospitalized at a US Army hospital; blood smears were obtained and malaria parasites, thought to be *P. vivax*, were detected. However, upon later review of the smears by the National Malaria Repository, the parasites were identified as typical *P. ovale* (Fig. 1A–1C). Serum was obtained from the patient 14 days after the onset of his illness and analysed for the presence of antibodies to malaria by an indirect fluorescent antibody (IFA) test: the serum dilution end-points were 1 : 256 against *P. ovale* and *P. vivax*, 1 : 64 against *P. malariae* and 1 : 16 against *P. falciparum*.

The patient was born and raised in the USA and did not leave the country until 16 November 1966, when he travelled by air to duty in the Republic

of Viet-Nam; he served in various locations in that country and did not leave until April 1967, when he flew to Japan for 7 days of rest and recuperation. He then returned directly to Viet-Nam and was well until July 1967, when he was hospitalized with a fever of unknown origin; no diagnosis of malaria was made at that time. On 21 August 1967, he was hospitalized in Viet-Nam for treatment of a shrapnel wound and received one blood transfusion. On 28 September 1967, he was transferred by air to a hospital in Japan. On 8 October 1967, he returned by air to the USA, where he remained until the onset of his illness. While in Viet-Nam he had taken the standard malaria chemoprophylaxis (300 mg chloroquine base and 45 mg primaquine base in a combination tablet once weekly) but denied having continued this regimen after leaving Viet-Nam. Attempts to locate the donors of the blood he received were unsuccessful.

Case 2

On 13 January 1969, a 21-year-old white American serviceman developed chills and fever. He was admitted to a veterans' hospital in the USA; blood smears were obtained and malaria parasites detected, but the hospital laboratory could not identify the *Plasmodium* species. The smears were reviewed by the National Malaria Repository, and typical *P. ovale* organisms were identified (Fig. 2A–2C). Serum was obtained from this patient 10 weeks after the onset of illness and analysed for malaria antibodies by the IFA test; the serum dilution end-points were 1 : 64 against *P. ovale* and *P. vivax*, 1 : 16 against *P. malariae*, and 1 : 4 against *P. falciparum*.

The patient was born in the USA and did not leave the country until April 1968, when he travelled by air to Viet-Nam, with an interim stop in Japan. In August 1968 he travelled directly to Taiwan for 5 days' leave, after which he flew back to Viet-Nam. In October 1968 he was treated for vivax malaria at an evacuation hospital. He returned to field duty, and on 3 January 1969, he travelled by air to the USA with one stop in Japan. He had never received blood transfusions and denied using shared syringes.

Case 3

On 1 February 1969, a 21-year-old Negro American serviceman developed a febrile illness. He was hospitalized at a military installation in the USA.; blood smears were obtained and found positive for *P. ovale* parasites; the diagnosis was confirmed by the National Malaria Repository (Fig. 3A–3C). Serum obtained from this patient 17 days after the onset of illness was analysed for malaria antibodies by the IFA test; the serum dilution end-points were 1 : 256 against *P. ovale* and *P. vivax*, 1 : 64 against *P. malariae*, and 1 : 16 against *P. falciparum*.

The patient was born and raised in the USA. On 31 October 1966, 5 months after induction into the US Army, he flew directly to a duty station in Germany. In the next 10 months he travelled only in Germany, France, and Holland. On 3 August 1967, he flew back to the USA, where he remained until 16 September 1967, when he travelled by air to Viet-Nam; his aircraft stopped briefly for fuel in Hawaii and Okinawa. Except for 5 days of rest and recuperation leave in Bangkok, Thailand, in May 1968, he spent the next 15 months on duty in a number of locations in Viet-Nam. On 16 December 1968, he returned by air from Viet-Nam to the USA, with 1-hour stops in Okinawa and Hawaii. Thereafter, he remained in the USA until the onset of his illness. He had never received blood transfusions or used shared syringes and had no history of malaria or unexplained febrile episodes.

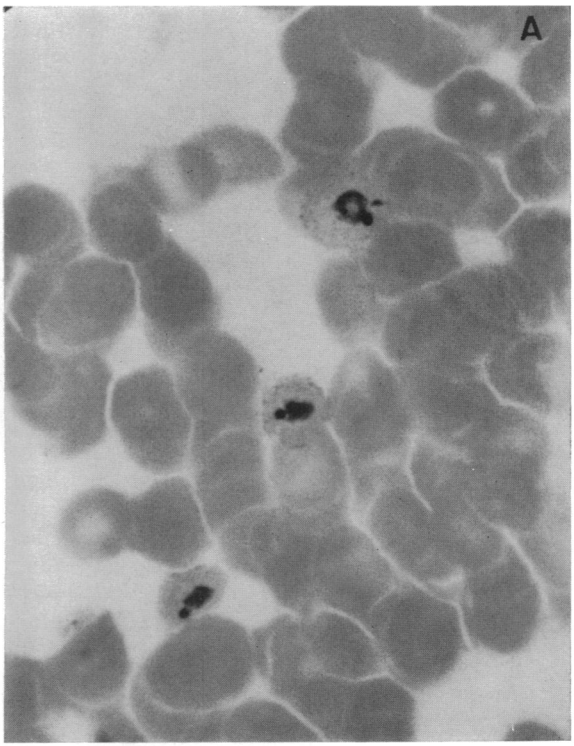
Case 4

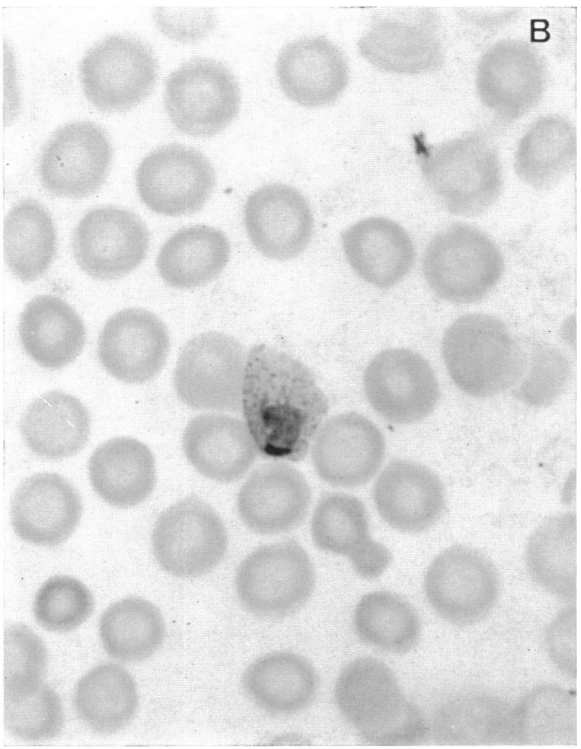
On 16 March 1969, a 22-year-old Negro serviceman developed chills and fever and was admitted to a civilian hospital in the USA. Blood smears were obtained and the hospital laboratory technicians identified malaria parasites (species unknown). These parasites were later identified as typical *P. ovale* by the National Malaria Repository (Fig. 4A–4C). Serum obtained from the patient 7 days after the onset of illness was analysed for malaria antibodies by the IFA test; the serum dilution end-points were 1 : 1024 against *P. ovale*, 1 : 256 against *P. malariae*, and 1 : 64 against *P. vivax* and *P. falciparum*.

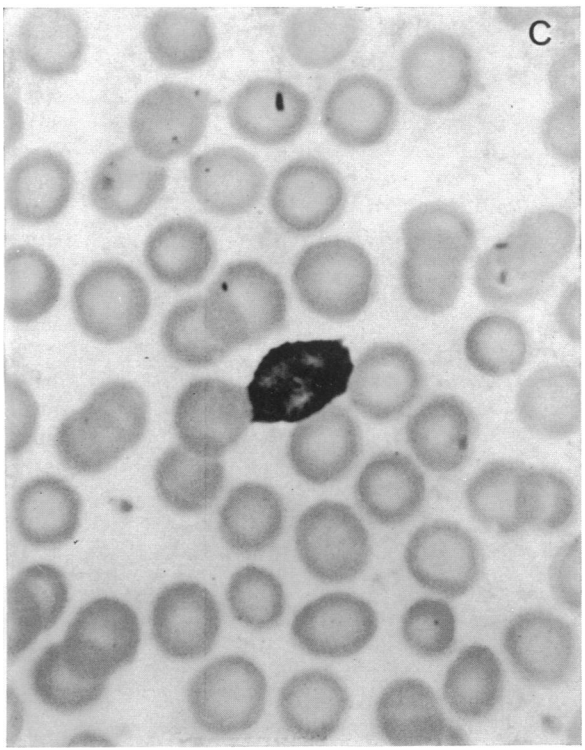
The patient was born in Texas and remained in the USA until late 1967, when he departed for duty in Viet-Nam. His aircraft stopped for a few hours at Clark Field, a military base located in a non-malarious area 50 miles (80 km) north of Manila, Philippines; his flight then continued directly to Viet-Nam. He served in a variety of locations in Viet-Nam until mid-February 1968, when he returned by air, *via* Tokyo, to the USA, where he remained until the onset of his illness. He had never received blood transfusions or used shared syringes and had no previous history of malaria.

FIG. 1

PLASMODIUM OVALE FROM CASE 1







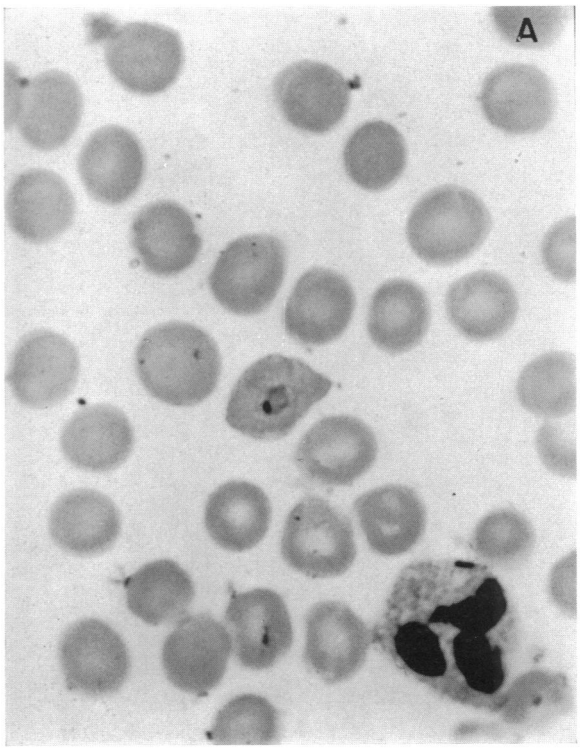
A: Compact trophozoites with large chromatin mass in fimbriated, coarsely stippled cells.

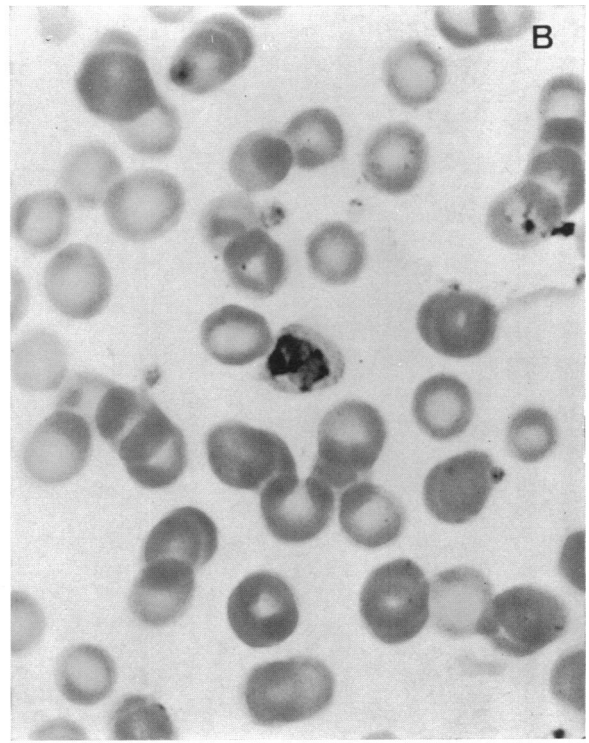
B: Compact trophozoite with large chromatin mass in oval, coarsely stippled cell.

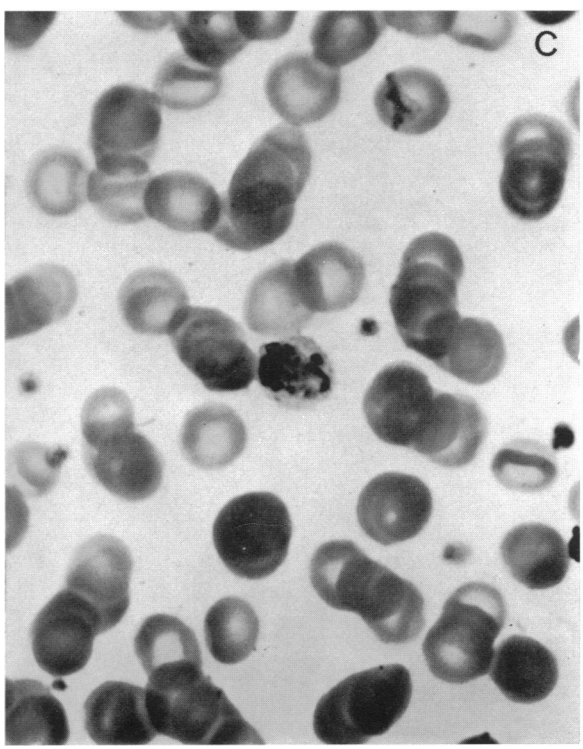
C: Old trophozoite in oval, fimbriated cell.

FIG. 2

PLASMODIUM OVALE FROM CASE 2







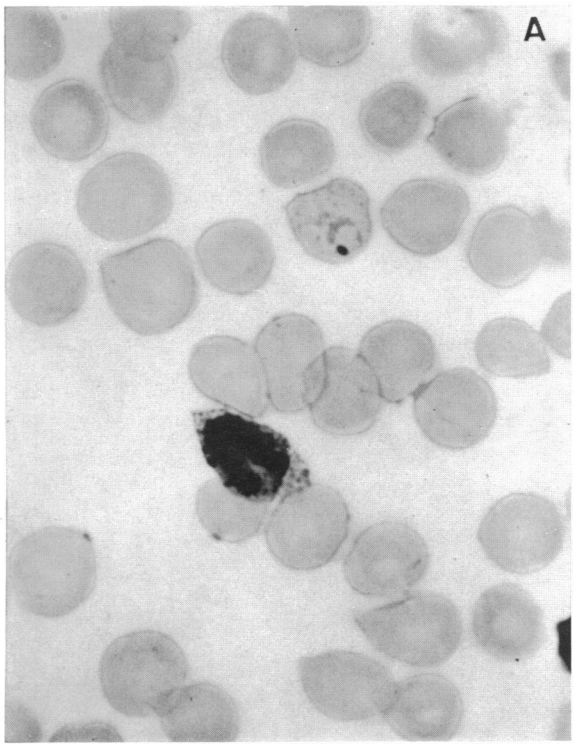
A: Young trophozoite with early pigment in oval, stippled cell.

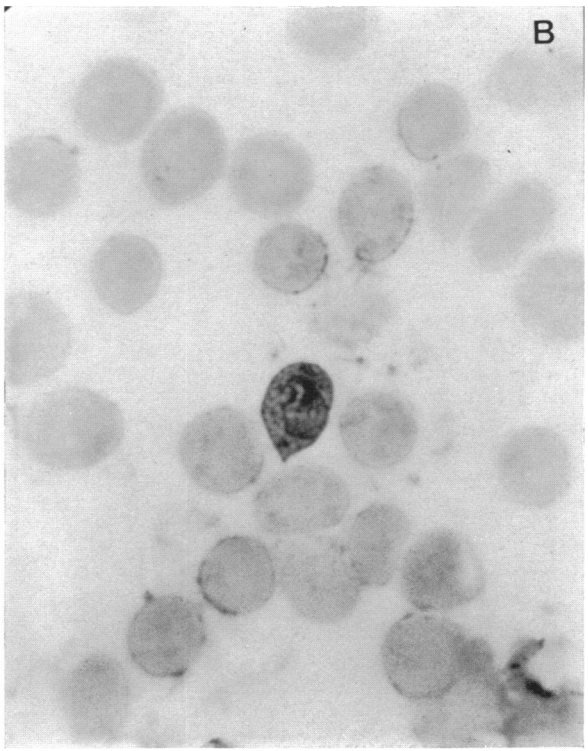
B: Older trophozoite with large chromatin mass, heavy pigment and fimbriated cell.

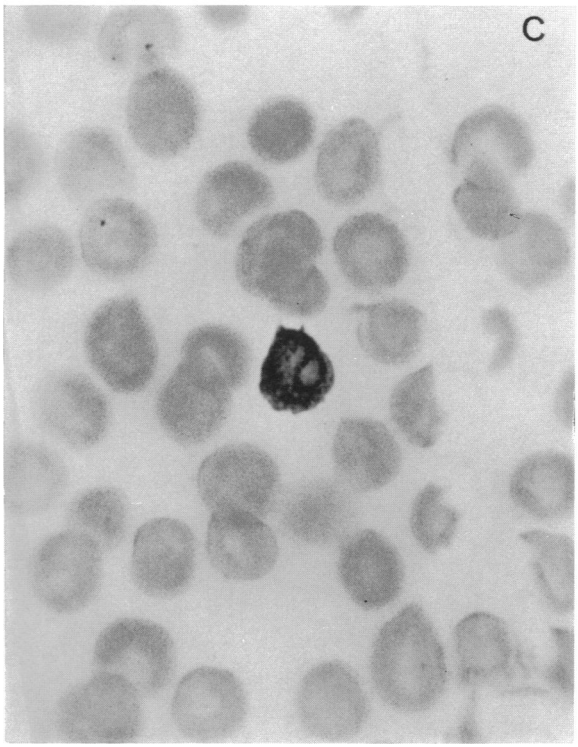
C: Immature schizont, heavy pigment, fimbriated, slightly enlarged cell.

FIG. 3

PLASMODIUM OVALE FROM CASE 3







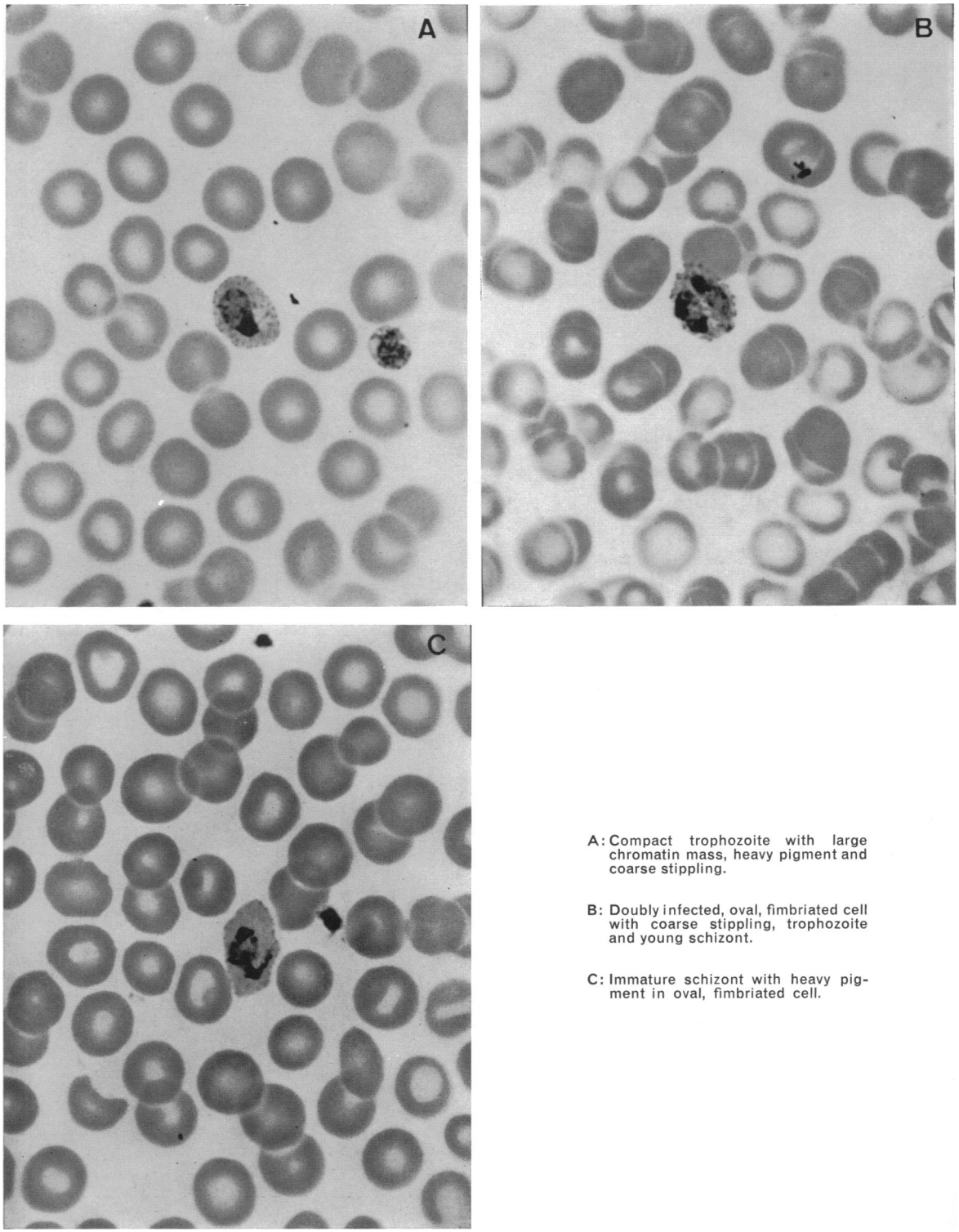
A: Ring stage with early pigment and developing schizont in oval, fimbriated cells.

B: Trophozoite with large chromatin mass, coarse stippling and heavy pigment in slightly enlarged, fimbriated cell.

C: Trophozoite with large chromatin mass and heavy pigment in oval, fimbriated cell.

FIG. 4

PLASMODIUM OVALE FROM CASE 4



A: Compact trophozoite with large chromatin mass, heavy pigment and coarse stippling.

B: Doubly infected, oval, fimbriated cell with coarse stippling, trophozoite and young schizont.

C: Immature schizont with heavy pigment in oval, fimbriated cell.

MORPHOLOGY OF THE PARASITES

Most of the parasites seen in blood smears from the 4 cases were developing trophozoites and immature schizonts; ring forms and gametocytes were rare; mature schizonts were not found. In each case, more than 50% of the parasitized red cells were fimbriated and/or oval in shape and less enlarged than cells seen in *P. vivax*. The parasites were more compact than typical *P. vivax*, with larger chromatin masses and earlier pigment formation. The outer edges of many of the infected cells were indistinct or invisible, with only the coarse, violet-tinged Schüffner's stippling apparent. The Schüffner's dots appeared less numerous than those generally seen in *P. vivax* and showed a marked tendency to align themselves around the margins of and over the parasites. These characteristics are typical of the descriptions of *P. ovale* as published by Wilcox, Jeffery & Young (1954), Field & Shute (1956), and Garnham (1966).

DISCUSSION

In all 4 cases the morphology of the parasite in thin smear preparations was that of typical *P. ovale*. The geographical source and mode of transmission of the infections can be determined from the medical and travel histories. Viet-Nam was the only malarious area visited by Cases 1, 2, and 3. (The city of Bangkok, Thailand, visited by Case 3, is malaria-free according to a personal communication from D. M. Holden,[1] as are the other countries visited by the 3 men. The status of malaria in these countries has been reported as " eradicated " (*Wkly epidem. Rec.*, 1968).) Cases 2 and 3 must have acquired their infections by mosquito transmission in Viet-Nam, since neither had received blood transfusions or used shared syringes. Although it is conceivable that Case 1 was infected as a result of blood transfusion, his malaria is much more likely to have been mosquito-transmitted, since over 95% of the blood used by the US Armed Forces in Viet-Nam is collected in the USA, where *P. ovale* transmission does not occur, according to R. C. Singer.[2] Furthermore, the time between the date of his transfusion and the onset of his illness was about 10 weeks, which would be an unusually long incubation period for a blood-induced infection, particularly since the patient denied concomitant ingestion of antimalarials.

Case 4, who must have acquired his infection by mosquito transmission, visited two malarious areas, Viet-Nam and the Philippines. Focal transmission of ovale malaria does occur in the Philippines, but it is extremely unlikely that he was infected during his visit there, since malaria has been eradicated from the particular region where his aircraft landed, according to Holden; furthermore, his stay there was extremely brief (about 2 hours).

In addition to these 4 cases, N. E. Wilks[3] has told us, in a personal communication, of a *P. ovale* case diagnosed in an American soldier in Viet-Nam. Cadigan & Desowitz (1969) have also reported 2 indigenous ovale cases recognized in the local population of Thailand. Also, at the time this paper was presented at the joint meetings of The American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists in Washington, D.C. (November 1969), Dr Meir Yoeli of the New York University School of Medicine reported that he had recently diagnosed a case of ovale malaria in a person who had returned from serving in the Highlands of Viet-Nam. Photomicrographs shown during the discussion were typical of ovale parasites. There can be little doubt that mosquito transmission of *P. ovale* malaria does occur in South-East Asia.

These 4 cases are the only *P. ovale* infections diagnosed at the National Communicable Disease Center in servicemen returning from Viet-Nam during the 39 months from January 1966 through March 1969. They constitute only 0.066% of the 6036 malaria cases reported among servicemen returning from Viet-Nam during this period and represent only 0.11% of the 3686 blood smears obtained from such individuals and found positive at our laboratory during the same period. Since *P. ovale* is frequently mistaken for another *Plasmodium* species (Cases 1, 2, and 4), additional *P. ovale* cases quite possibly occurred among those infected ex-Viet-Nam servicemen whose smears were not available for review. None the less, it is apparent that *P. ovale* is exceedingly rare among such servicemen.

The prevalence of *P. ovale* among the Viet-Namese themselves cannot be estimated from our experience with US servicemen who have served in that country because of significant differences between the two populations in genetic background, acquired immu-

[1] Formerly Regional Malaria Officer, Malaria Eradication Program, Manila, Philippines.

[2] Chief, Communicable Diseases Branch, Department of the Army, Washington, D.C.

[3] Parasitologist, Armed Forces Institute of Pathology, Washington, D.C.

nity, sex and age distribution, and use of chemoprophylaxis. Furthermore, our experience is drawn from clinical cases, whereas the true prevalence of the parasite can be determined only by sampling both ill and well individuals. Since ovale malaria is characterized by a mild, short-lived illness and by both early and late asymptomatic parasitaemias, as reported by James, Nicol & Shute (1949) and Jeffery, Young & Wilcox (1954), it is conceivable that its frequency in a series of clinical cases such as ours is a significant underestimate of its true prevalence in the general population. Finally, ovale malaria may occur only in certain circumscribed areas of Viet-Nam where American troops rarely venture. In this instance, the prevalence of *P. ovale* among returning troops with malaria would be low, but its prevalence among the local population relatively high. We do not have sufficient information about the travels of our 4 patients during their duty in Viet-Nam to comment on this possibility.

Large-scale blood-smear surveys of populations in South-East Asia, performed by skilled technicians using thin-smear techniques, would be helpful in determining the true prevalence of *P. ovale* and would lead to a better understanding of the epidemiology of this parasite in these areas. If such surveys showed *P. ovale* to be as rare as the paucity of case reports would suggest, it would be difficult to explain how the parasite survives. There are two possible explanations.

(1) *P. ovale* might actually be a simian *Plasmodium* with man functioning as a rare and accidental intermediate host. There is some support for this hypothesis, since *P. schwetzi*, a parasite of chimpanzees, has been transmitted by mosquitos from monkey to man, and in man the parasite appears to be similar to *P. ovale*, according to a study by Coatney (1963).

(2) Blood smear surveys may not be sensitive enough to detect most ovale infections. The tendency of *P. ovale* to produce low-grade, intermittent parasitaemia and the ease with which it is masked by the presence of other *Plasmodium* species support this possibility.

Serological testing for malaria antibodies with the indirect fluorescent technique of Sulzer, Wilson & Hall (1969) corroborated the diagnosis of *P. ovale* in Case 4. In the other 3 cases, speciation was not possible, since the titres to *P. vivax* and *P. ovale* antigens were identical. In previous studies of US personnel returning from Viet-Nam with vivax malaria, as reported by Gleason et al.[1] using the IFA technique and *P. vivax* and *P. falciparum* antigens, the titre to *P. vivax* has been shown to exceed that to *P. falciparum* by at least a 4-fold difference in 79% of the cases and to equal that of *P. falciparum* in only 12%. Our experience thus suggests that there is more antigenetic similarity between *P. vivax* and *P. ovale* than between *P. falciparum* and *P. vivax*.

[1] Gleason, N. N., Wilson, M., Sulzer, A. J. & Runcik, K., *Serological speciation of* Plasmodium vivax *and* P. falciparum *infections by the malaria IFA test.* Paper presented at the meetings of the American Society of Tropical Medicine and Hygiene, Atlanta, Ga., USA, October 1968.

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RÉSUMÉ

PALUDISME À *PLASMODIUM OVALE* CONTRACTÉ AU VIET-NAM

Durant la période de 39 mois s'étendant de janvier 1966 à mars 1969, quatre cas de paludisme à *Plasmodium ovale* ont été diagnostiqués chez des membres des forces armées des Etats-Unis d'Amérique en garnison au Viet-Nam. Aucun de ces hommes n'avait servi dans d'autres territoires d'endémicité paludéenne et l'étude de leurs déplacements et de leurs antécédents donne à penser que l'infection leur a été transmise par des moustiques. Ces cas ne représentent que 0,066% du total de 6036 infections paludéennes décelées pendant ce laps de temps chez

des sujets rentrant du Viet-Nam; sur 3686 étalements de sang trouvés positifs au Laboratoire du paludisme du Centre national des Maladies transmissibles d'Atlanta (Etats-Unis d'Amérique), 0,11% seulement renfermaient *P. ovale.*

La technique des anticorps fluorescents a permis de confirmer le diagnostic de paludisme à *P. ovale* dans un cas. Dans les trois autres cas, l'identification précise du parasite en cause n'a pas été possible, les titres d'anticorps étant identiques pour *P. vivax* et *P. ovale.* Un seul des sujets infectés a signalé une atteinte précédente de paludisme à *P. vivax.*

La plupart des formes présentes dans les étalements de sang étaient des trophozoïtes en développement et des schizontes non mûrs; les formes annulaires et les gamétocytes étaient peu fréquents; on n'a pas observé de schizontes mûrs. La morphologie du parasite était celle de *P. ovale* typique et plus de 50% des érythrocytes infectés présentaient un contour effrangé et/ou une forme ovalaire.

Les auteurs mentionnent brièvement quatre autres cas de paludisme à *P. ovale* dont deux diagnostiqués chez des soldats américains cantonnés au Viet-Nam et deux chez des Thaïlandais; ils confirment l'existence du parasite dans la partie continentale de l'Asie du sud-est.

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