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Plasmodium ovale in a highly malaria endemic area of Senegal

Farba B. K. Faye[1], Lassana Konaté[2], Christophe Rogier[3,4] and Jean-François Trape[1] [1]*Laboratoire de Paludologie, ORSTOM, B.P. 1386, Dakar, Sénégal;* [2]*Département de Biologie Animale, Université de Dakar, Dakar, Sénégal;* [3]*Service d'Epidémiologie, Institut Pasteur de Dakar, Dakar, Sénégal;* [4]*IMTSSA, Le Pharo, Marseille, France*

Abstract

During 4 months, from June to September 1990, the population of Dielmo village, Senegal, an area of intense and perennial malaria transmission, was enrolled in a follow-up study including daily clinical surveillance and bi-weekly malaria parasitaemia monitoring. Thick blood film examinations indicated that 48·5% of children (49/101) and 32·4% of adults (34/105) were infected at least once by *Plasmodium ovale* during the study period; 148 distinct episodes of patent parasitaemia were observed, with estimated maximum durations of 3–115 d. The mean duration at first decreased significantly with age, from 11·4 d in children under 5 years old to 4·2 d in adults aged 40–59 years, but then increased in older adults to 7·0 d. In all age groups, most infections were asymptomatic. Only high parasitaemias were significantly associated with fever; 3 clinical malaria attacks due to *P. ovale* were seen during the study period.

Keywords: malaria, *Plasmodium ovale*, epidemiology, incidence, morbidity, Senegal

Introduction

Plasmodium ovale, the rarest of the 4 human malaria parasites, is practically confined to tropical Africa and limited areas of several islands of the western Pacific region (LACAN, 1963; GARNHAM, 1966; LYSENKO & BELJAEV, 1969; BAIRD *et al.*, 1990). In tropical Africa, its prevalence is usually lower than 5% in children and 1% in adults; however, prevalences reaching 5–10% in children are not rare (BRAY, 1957; GARNHAM, 1966; LACAN, 1967; TRAPE, 1987 and unpublished data). Clinically, *P. ovale* malaria has a mild, tertian course in non-immune subjects; paroxysms of high fever may be incapacitating, but complications are infrequent and almost never have a fatal outcome (JAMES *et al.*, 1949; GARNHAM, 1966; ZUIDEMA & MEUWISSEN, 1966; BAUFINE-DUCROCQ *et al.*, 1969; VAN DER VYNCKT, 1979; FACER & ROUSE, 1991). In endemic populations, *P. ovale* is rarely reported as a cause of morbidity, even in children. However, it is not clear whether the rarity of clinical attacks is due to under-diagnosing, low transmission, rapidly acquired species-specific immunity, or partial cross-immunity with the much more prevalent *P. falciparum*. This paper reports a series of parasitological and clinical observations on *P. ovale* collected during 4 months of close monitoring of a rural Senegalese community.

Population and Methods

Background

The village of Dielmo, Senegal, is in an area of intense and perennial malaria transmission where the entomological inoculation rate averages 200 infective bites per person per year, yet is subject to marked annual and seasonal fluctuations (KONATÉ *et al.*, 1994; FONTENILLE *et al.*, 1997). From 1 June to 30 September 1990, the whole population was involved in a prospective study described elsewhere (TRAPE *et al.*, 1994). Briefly, in order to identify all episodes of fever, the 247 inhabitants were kept under daily medical surveillance. Systematic observations included thick blood film examinations twice a week and temperature readings every alternate day. Supplementary thick blood films, medical examinations, and other tests were made when fever or symptoms were present. Malaria transmission was monitored during the study period and the number of infective bites per person was estimated at 74·3, 14·6 and 6·6 for *P. falciparum*, *P. malariae* and *P. ovale*, respectively (TRAPE *et al.*, 1994).

Address for correspondence: Dr J. F. Trape, ORSTOM, B.P. 1386, Dakar, Sénégal; phone +221 832 09 62, fax +221 832 16 75, e-mail trape@belair.orstom.sn

Study population

For the present study, we took into account the results of 206 people (101 children <15 years and 105 adults) who continued to live in Dielmo during the follow-up period (maximum absence 10 d) and met the following criteria: (i) at least 50% of their life since birth spent in Dielmo or an area of high malaria endemicity (95–100% of their life for most adults and nearly all children); (ii) continued presence in the village or a maximum absence of 30 d during the 6 months preceding the study period; and (iii) a return to Dielmo at least 2 years before the study for people who had lived for more than a year in areas of low malaria endemicity.

Thick blood film examination and parasitological data analysis

All thick blood film examinations were standardized (TRAPE, 1985; TRAPE *et al.*, 1994). Two hundred oil immersion microscope fields (about 0·5 μL of blood) were examined on each slide. The ratio of trophozoites to leucocytes was established separately for each *Plasmodium* species, either by counting the trophozoites until 200 leucocytes had been observed (ratio ≥0·01) or from the total number of trophozoites seen in the 200 fields and an estimation of the average number of leucocytes per field (ratio <0·01). Gametocytes of each species were recorded separately. Some parasitological findings, including variations in *P. ovale* parasite density with age in the study population, have been presented in detail elsewhere (TRAPE *et al.*, 1994).

The presumed duration of each episode of patent *P. ovale* infection was calculated as follows: (i) the number of days between the preparation of the first to the last blood films showing parasites was counted and 2 d were arbitrarily added to this total; (ii) a minimum duration of 3 d was attributed to each infection, when only one of the bi-weekly thick films revealed parasites; (iii) successive *P. ovale* infections in the same individual were counted as separate only if at least 3 thick blood films over a 10 d period during the interval between these infections failed to reveal parasites (because of the low parasite density of most infections, when only one or 2 intermediate thick films failed to reveal parasites they were presumed to be false negatives and were scored as positive).

Similar rules were used for calculating the presumed duration of each episode of patent gametocytaemia. We also calculated the time interval between the beginning of patent parasitaemia and the beginning of the first (or only) episode of patent gametocytaemia; when sexual and asexual forms were simultaneously observed for the first time in the same blood film, we arbitrarily attributed a time interval of one day.

For each age group, the daily recovery rate (r) was derived from the average duration of infections ($1/r$). We also derived the daily incidence rate (h) and the time interval between consecutive infections ($1/h$) from the slide positivity rate (SPR), assuming that, for each age group, $SPR=h/(h+r)$.

Relationship between parasitaemia and fever

To investigate the relationship between *P. ovale* parasitaemia and fever we used the same definition of case and control observations that we had previously used for investigating the relationships between *P. falciparum* parasitaemia and fever in this population during the same period (ROGIER *et al.*, 1996). In brief, individual observations were considered to be fever cases if the axillary temperature was 38°C or higher, or the rectal temperature was 38·5°C or higher. Two febrile episodes were regarded as separate if they were separated by more than 72 h without fever or associated symptoms. Individual observations were considered to be asymptomatic controls if the axillary or rectal temperature was 37·9°C or 38·4°C respectively, or lower, and if no symptom associated with fever was recorded during the 72 h preceding or following the preparation of the thick blood films. All observations following antimalarial treatment were excluded. Data collected from pregnant women (6 persons) were also excluded. In all, 5076 simultaneous measurements of parasitaemia and temperature in 200 individuals aged from one month to 83 years were taken into account for this analysis.

Results

The total number of thick blood films examined either systematically or during febrile episodes was 7036, an average of 34·2 per person; 439 films (6·2%) contained *P. ovale* alone or associated with *P. falciparum* and/or *P. malariae*. The slide positivity rate was maximal in children 5–9 years old, when it reached 14·3%, and minimal in adults 20–39 years old, when it was 1·3%. Of the 206 subjects, 83 (40·3%) were found to be infected by *P. ovale* at least once during the study period (Table 1). In all, 148 distinct episodes of patent parasitaemia were identified, of which 72·3% occurred in children.

with age between 40–59 years and ≥60 years: $P<0·03$ by the Kruskal–Wallis test). Parasites were usually detected on all blood films between the first and the last positive films in each infection. The proportion of infections with one or more negative intermediate thick films was only 15% (16/107) in children under 15 years old and 5% (2/41) in adults. Antimalarial drugs given for treatment of clinical attacks (due to *P. ovale* or *P. falciparum*) reduced the duration of current *P. ovale* infections in 6 cases (4 in subjects aged 0–4 years and one each in those aged 5–9 and 15–19 years).

Estimates of the incidence rate are presented in Table 1. The estimated mean duration of the time interval between consecutive infections varied from 44 d for children 5–9 years old to 425 d for adults 20–39 years old (Fig. 2).

Gametocytes—always associated with trophozoites and/or schizonts—were seen in 28 of 148 infections (18·9%), of which 78·6% (22/28) occurred in children 0–14 years old (Table 2). The mean time interval between the beginning of patent parasitaemia and the beginning of patent gametocytaemia was 4·5 d. The mean duration of the episodes of patent gametocytaemia was 4·7 d. The average gametocyte count on positive slides was 6·7/µL of blood and the maximum gametocyte density observed was 256/µL (in 2 children aged 4 and 8 years).

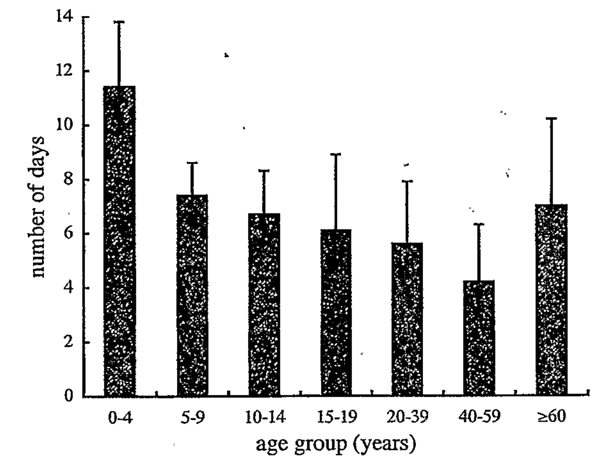

Fig. 1. The mean duration of patent *Plasmodium ovale* infections in Dielmo, Senegal.

Table 1. Characteristics of *Plasmodium ovale* infections according to age group in 206 permanent residents of Dielmo, Senegal, June–September 1990

Age group (years)	No. of subjects	No. of blood films	Slide positivity rate[a]	Cumulative prevalence[b]	No. of infections	Duration of infections (d)[c]	Daily incidence rate	Daily recovery rate
0–4	46	1454	8·9% (130)	32·6% (15)	32	3–66 (11·4)	0·0086	0·0877
5–9	32	1096	14·3% (157)	62·5% (20)	48	3–115 (7·4)	0·0225	0·1351
10–14	23	819	8·7% (71)	60·9% (14)	27	3–45 (6·9)	0·0142	0·1493
15–19	20	707	3·3% (23)	35·0% (7)	10	3–21 (6·1)	0·0056	0·1639
20–39	46	1598	1·3% (20)	23·9% (11)	12	3–10 (5·6)	0·0024	0·1786
40–59	23	806	1·9% (15)	34·8% (8)	9	3–17 (4·2)	0·0046	0·2381
≥60	16	556	4·1% (23)	50·0% (8)	10	3–10 (7·0)	0·0061	0·1429
Total	206	7036	6·2% (439)	40·3% (83)	148	3–115 (7·4)	0·0060	0·0901

[a]Number of positive slides in parentheses.
[b]Number of positive subjects in parentheses.
[c]Range (geometric mean in parentheses).

The total presumed duration of patent infections varied from 3 to 115 d (observed duration 1–113 d); the maximum was in a child 7 years old. The duration exceeded 10 d in only 40% (32/80) of infections among children aged under 10 years and in 20·6% (14/68) of infections among older children and adults. The mean duration of patent infections at first decreased with age, from 11·4 d in children 0–4 years old to 4·2 d in adults 40–59 years old, but then increased in older adults to 7 d (Fig. 1). Both variations were statistically significant (decrease with age for the 7 age groups from 0–4 years to ≥60 years: $P<0·02$ by Spearman's rank test; increase

The relationship between *P. ovale* parasitaemia and the occurrence of fever is presented in Table 3. Of 140 episodes of fever, 16 (11·4%) occurred in patients infected with *P. ovale*. Of 4936 observations on asymptomatic persons, 312 (6·3%) involved subjects infected with *P. ovale*. Most *P. ovale* infections were mixed with

Table 2. Characteristics of *Plasmodium ovale* gametocytaemia according to age group

Age group (years)	Slide positivity rate[a]	Cumulative prevalence[b]	No. of infections with gametocytes	No. of distinct episodes of gametocytaemia	Mean gametocytaemia (per μL)[c]	Mean duration of gametocytaemia (d)[d]	Mean delay (d)[e]
0–4	1·6% (23)	13·0% (6)	8	11	8·2 (2–256)	6·0 (3–17)	4·3 (3–10)
5–9	1·8% (19)	15·6% (5)	11	11	6·6 (2–256)	4·8 (3–11)	5·6 (1–105)
≥10	0·3% (14)	6·3% (8)	9	10	4·9 (2–66)	3·6 (3–7)	3·7 (1–38)
Total	0·8% (56)	9·2% (19)	28	32	6·7 (2–256)	4·7 (3–17)	4·5 (1–105)

[a]Number of positive slides in parentheses.
[b]Number of positive subjects in parentheses.
[c]Geometric mean of positive slides only (range in parentheses).
[d]Geometric mean duration of distinct episodes (range in parentheses).
[e]Geometric mean period between onset of patent parasitaemia and first appearance of gametocytes (range in parentheses).

Table 3. Relationship between *Plasmodium ovale* parasitaemia and risk of fever in the presence or absence of *P. falciparum* and/or *P. malariae*

P. ovale parasitaemia[a]	Subjects Asymptomatic	Symptomatic	Total	Odds ratio[b]	*P*[c]
Mixed infections					
0	2862	115	2977	1	–
<0·01	229	9	238	0·98 (0·46–2·02)	0·95
0·01–<0·1	39	3	42	1·91 (0·37–6·16)	0·22
0·1–<1	6	1	7	4·15 (0·09–34·6)	0·22
1–<2	0	1	1	∞	<0·05
≥2	0	2	2	∞	<0·01
Total	3136	131	3267	–	–
P. ovale only					
0	1762	9	1771	–	–
<0·01	31	0	31	–	–
0·01–<0·1	6	0	6	–	–
0·1–<1	1	0	1	–	–
1–<2	0	0	0	–	–
≥2	0	0	0	–	–
Total	1800	9	1809	–	–

[a]Parasite/leucocyte ratio.
[b]95% Confidence interval in parentheses.
[c]Fisher's exact test.

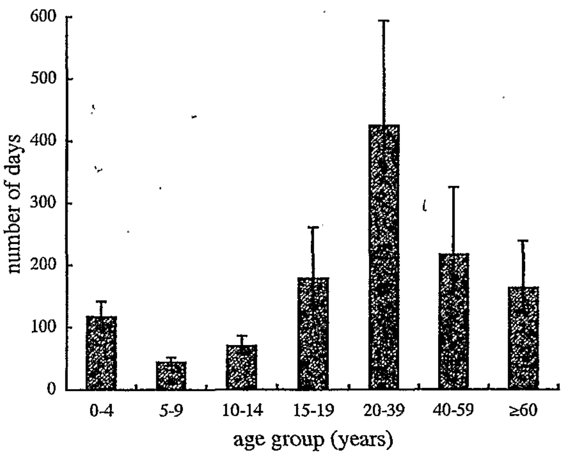

Fig. 2. The mean time interval between consecutive patent *Plasmodium ovale* infections in Dielmo, Senegal.

other *Plasmodium* species. Since species associations could possibly modify the relationships between parasite density and fever risk, we analysed separately observations in which *P. falciparum* and/or *P. malariae* were present and observations in which these 2 species were absent (Table 3). During the study period, infections with *P. ovale* alone were always low grade and asymptomatic. When *P. ovale* was mixed with other species, only high *P. ovale* parasitaemias (parasite/leucocyte ratio ≥1) were significantly associated with fever.

Discussion

P. ovale is generally considered to be a rare species. In Dielmo, its average prevalence was 11% in children and 2% in adults. However, a very different picture is revealed by the longitudinal study, with 49% of children and 32% of adults presenting at least one infection over a period of 4 months. Most of these infections had very low parasite densities and only careful examination of the thick blood film allowed their detection. This suggests that *P. ovale* could be much more frequent than previously believed in many parts of tropical Africa. As suggested by a recent study in Guinea Bissau, a more sensitive technique such as the polymerase chain reaction could be very useful in assessing the true prevalence and incidence of this species (SNOUNOU *et al.*, 1993).

The average duration of patent *P. ovale* infections was very short in Dielmo villagers, confirming previous observations in République du Congo (TRAPE, 1993). The total duration of patent infections rarely exceeded 10 d, even in children. Since only very few infections were treated and only a limited number of reinfections may have been confused with the previous infection, it is unlikely that bias would have had a significant effect on the overall duration of infection. Much longer average durations were reported from Nigeria by MOLINEAUX & GRAMICCIA (1980); however, these estimates were derived from transition frequencies (negative to positive and positive to negative) between consecutive surveys made at intervals of 10 weeks using a reversible catalytic model (BEKESSY *et al.*, 1976). With this model, the greater the time interval between surveys, the more the risk increases that 2 consecutive samples from the same person do not reflect the true course of parasitaemia during that period, since infections may develop and disappear without being detected. We tested this model in Dielmo and major discrepancies appeared when we introduced transition rates corresponding to time intervals between surveys of 14 d or more (data not shown).

Clinical monitoring and the analysis of the relationship between *P. ovale* parasitaemia and fever risk indicated that only high parasitaemias caused clinical malaria. Similar observations were made previously for *P. falciparum* in the same population, and the findings provided evidence for an age-dependent pyrogenic threshold of *P. falciparum* parasitaemia (ROGIER *et al.*, 1996). The low number of fever cases associated with *P. ovale* did not allow us to investigate the possible existence of a threshold effect. Although not significant, the increasing odds ratios associated with fever cases with parasite/leucocyte ratios ranging from 0·01 to one led us to reconsider all the biological and clinical data available for the 4 corresponding fever episodes. In each case, a diagnosis other than *P. ovale* malaria was clearly established. Thus, of 148 patent infections seen during 4 months in our study population, only 3 resulted in a clinical attack, in patients aged one, 4 and 18 years. In addition, a report of asthenia (without other symptoms) was clearly associated with a peak of medium-grade *P. ovale* parasitaemia (parasite/leucocyte ratio 0·45) in a 3 years old child, but temperature records failed to reveal fever.

We conclude that *P. ovale* infections have a high incidence in all age groups, but that they are usually rapidly controlled and the maximum parasitaemia rarely reaches a level sufficient to induce a clinical attack of malaria. In order to explore the relationships between previous exposure to *P. ovale* and disease occurrence, we are now investigating prospectively the incidence and development of *P. ovale* infections in a cohort of infants and young children from Dielmo who were enrolled at birth.

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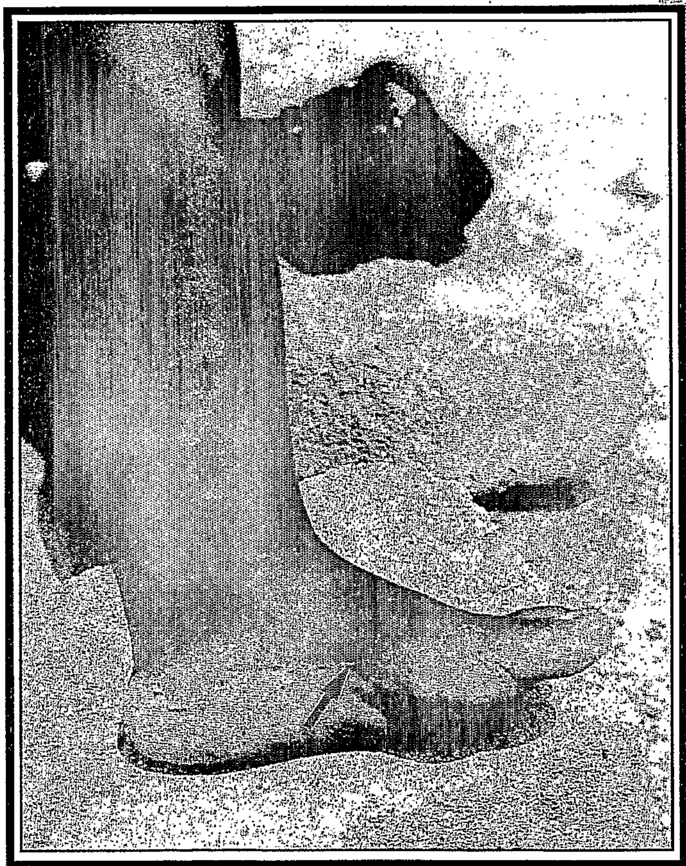

Royal Society of Tropical Medicine and Hygiene,
Manson House, 26 Portland Place,
London, W1N 4EY, UK
Telephone: +44 (0)171 580 2127
Fax: +44 (0)171 436 1389
e-mail: mail@rstmh.org
Web site: http://www.rstmh.org

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