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Plasmodium vivax Infections over 3 Years in Duffy Blood Group Negative Malians in Bandiagara, Mali

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Abstract. *Plasmodium vivax* was thought to infect only the erythrocytes of Duffy blood group positive people. In the last decade, *P. vivax* has appeared throughout Africa, both in areas where Duffy positive and negative people live side by side as in Madagascar and Ethiopia and in areas where people are primarily Duffy negative, such as in western Kenya. We performed quantitative polymerase chain reaction on blood samples dried onto filter paper to determine the prevalence of *P. vivax* and *Plasmodium falciparum* in a cohort of 300 children (newborn to 6 years of age) in Bandiagara, a Sahelian area of Mali, west Africa, where the people are Duffy negative. We report 1–3 occurrences of *P. vivax* in each of 25 Duffy-negative children at six time points over two rainy seasons and the beginning of the third season. The prevalence of *P. vivax* infection was 2.0–2.5% at every time point (June 2009 to June 2010). All children with *P. vivax* infections were asymptomatic and afebrile, and parasite densities were extremely low. Anemia, however, was the main burden of infection. *Plasmodium vivax* could become a burden to sub-Saharan Africa, and the evidence of *P. vivax* existence needs to be taken into consideration in designing malaria control and elimination strategies in Africa.

INTRODUCTION

In the 1970s, Duffy blood group negative people were shown to be resistant to *Plasmodium vivax* infection in studies conducted on U.S. and Honduran volunteers and American soldiers.[1–3] From these studies, it was assumed that all Duffy-negative people were resistant to *P. vivax* infection. In Madagascar, Ethiopia, and Sudan where Duffy-positive and Duffy-negative people live side by side, infections of *P. vivax* were found and assumed to be the result of passage back and forth between Duffy positive and negative individuals, with the Duffy-positive population possibly maintaining the infection.[4–6] The *P. vivax* infections in Duffy-negative people showed lower parasite density and milder symptoms compared with *P. vivax* infections in Duffy-positive people, suggesting a less efficient infection.[4,5] However, *P. vivax* infections were also found in Duffy blood group negative people in a predominantly Duffy-negative area of western Kenya.[7] Menard and others[4] in Madagascar and Gunalan and others[8] in Ethiopia reported a large expansion of the gene-encoding Duffy-binding protein 1 (DBP1) in the *P. vivax* populations, especially in two patients in Ethiopia who were infected with *P. vivax* that had eight and three copies of the gene.[8] A recent study in southeast Asia found two to three copies of DBP1 in parasites in Duffy-positive populations[9]; the reason for the increase in DBP1 gene copy number in southeast Asia is unknown. However, this finding suggests the possibility that the *P. vivax* parasites that are able to infect Duffy-negative erythrocytes may have been selected for their ability to use multiple copies of DBP1 to invade Duffy-negative erythrocytes through an alternative receptor.

Plasmodium vivax has now been reported from all over Africa, Angola,[10] Benin,[11] Cameroon,[12,13] Ethiopia,[5] Equatorial Guinea,[10] Madagascar,[4] Mauritania,[14] Sudan,[6] and Mali,[15] raising the possibility that *P. vivax* was transmitted between Duffy-negative people as was first described in western Kenya.[7] In Mali, *P. vivax* has been found in the northern part of the country. In 1991, Doumbo and others described a case of *P. vivax* in the region of Kidal,[16] and subsequently a prevalence of 10–30% of *P. vivax* in febrile patients was reported in northern regions of Mali.[17,18] It was possible that *P. vivax* was transmitted from Duffy-positive people in the north Sahelian region to Duffy-negative people in the Bandiagara region. However, it is also possible that *P. vivax* was transmitted between Duffy-negative individuals, as is the case in western Kenya.

We searched for the presence of *P. vivax* in a cohort of 300 children (newborn to 6 years of age) in Bandiagara, a Duffy-negative area over a 3-year period. We identified 25 *P. vivax* infections in children who were all Duffy blood group negative determined by molecular techniques. This suggests that *P. vivax* in Duffy-negative people is widespread in Mali and perhaps in all of Africa.

MATERIALS AND METHODS

Study site and ethical clearance. The study was conducted in Bandiagara, a town located in the central region of Mali and approximately 700 km from the capital city, Bamako. The Yame River, a tributary of the Niger River, passes through the town and constitutes an important breeding site for the malaria vector mosquitoes *Anopheles gambiae* and *Anopheles funestus* during 5 months of transmission each year. The landscape is of the Sahelian type on a rocky plateau. The population of Bandiagara is around 13,364 and the economic activity is mainly agropastoral with some tourism.[19] The climate is characterized by a rainy season from June to October,

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with an average rainfall of 600 mm/year and a longer dry season from November to May. Malaria transmission is seasonal with transmission from June or July to November or December. The peak incidence of clinical malaria is observed in August–September with more than 60 infected bites per person per month.[19] The incidence of clinical malaria was 1.7 episodes per transmission season in children less than 10 years of age, and *P. falciparum* was the most frequent species with 97% of the malaria infection, *Plasmodium malariae* was 3% and *Plasmodium ovale* was rarely identified by microscopy, while *P. vivax* was not detected.[19,20]

The study was reviewed and approved by the institutional review board of the University of Maryland, Baltimore, MD, and the Institutional Ethical Review Committee of the Faculty of Medicine, Pharmacy and Odonto-Stomatology at the University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali. The study protocol and risks and benefits of participation were explained in local languages to the population and community permission was obtained. Individual written informed consent was obtained from the parents or legal guardians of children before inclusion in the study. An identification code was assigned to each participant and personal identity was kept confidential.

Population and study design. As part of a prospective cohort study of malaria incidence in Bandiagara, blood samples were collected during monthly scheduled visits from 300 children aged < 1 to 6 years. Blood was collected using Whatman 3MM chromatography filter paper (GE Healthcare, Buckinghamshire, UK). Dried blood spots were sealed in individual plastic bags with desiccant and kept in a dry area at room temperature. *Plasmodium vivax* sampling was done at time points in June, September, and December 2009, February and June 2010, and June 2011. Prevalence at each time point (except June 2011) was calculated based on the number of positive infections among the total samples analyzed.

Blood smear preparation. Thick smears were performed on blood samples of each participant for active surveillance of malaria regardless of the fever or other malaria symptoms. The smears were air-dried and not fixed with methanol. The thick smears were stained using 5% Giemsa solution for 45 minutes. The number of parasites in fields containing 1,000 leukocytes was determined. The parasite count was determined assuming 8,000 leukocytes/μL.

Molecular detection of *P. falciparum* and *P. vivax* infection. Molecular screening of *P. falciparum* and *P. vivax* was carried out using genomic DNA extracted from dried blood spot filter papers of 300 samples over five time points (June, September, December 2009, February, June 2010, and June 2011) from the beginning of one transmission season every 2–3 months to the beginning of the second season and for only *P. vivax* in the beginning of the third season (June 2011). DNA isolation was performed using QIAamp 96 DNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *Plasmodium* parasite DNA was qualitatively detected using a quantitative real-time polymerase chain reaction (qPCR) assay (LightCycler® 480, Roche, Basel, Switzerland) based on primers targeting the 18S small subunit ribosomal RNA (18S rRNA) gene as described.[21] A two-plex *P. falciparum* and *P. vivax* PCR was performed to determine the presences of these two species in the children's specimens. The assay consists of a combination of two different forward primers, two different TaqMan probes each labeled with a different fluorophore, and one reverse primer that amplifies and detects varied 18S rRNA regions specific to either *P. falciparum* or *P. vivax*. Detection of the appropriate fluorophore will indicate the presence of *P. falciparum* and *P. vivax*. The PCR amplification was performed using the following oligonucleotide primers and probes sequences: Forward *P. falciparum* primer: 5′-CCGACTAGGTGTTGGATGAAAGTGTTAA-3′; Forward *P. vivax* primer: 5′-CCGACTAGGCTTTGGATGAAAGAT-TTTA-3′; Reverse *Plasmodium* primer: 5′-AACCCAAAGACT TTGATTTCTCATAA-3′; TaqMan *P. falciparum* probe: 5′-(Cy5)-AGCAATCTAAAAGTCACCTCGAAAGATGACT-(BHQ-2)-3′; TaqMan *P. vivax* probe: 5′-(TAMRA)-AGCAATCTAAGAA-TAAACTCCGAAGAGAAAATTCT-(BHQ-2)-3′.[21]

The PCR amplification was performed using 5 μL of template DNA added to 20 μL of reaction master mix consisting of 0.5 μL of each individual primer at 45 μM, 0.5 μL of each probe at 0.25 μM, and 12.5 μL of 2× concentration of TaqMan master mix (Applied Biosystem, Foster City, CA). A final reaction volume of 25 μL was run with the following thermal cycling conditions: 50°C for 2 minutes followed by 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. *Plasmodium falciparum* 3D7 DNA and *P. vivax* DNA were used as positive controls for each species and the PCR reaction master mix with no template DNA was used as a negative control.

Duffy blood group genotyping. The absence of Duffy antigen receptor on the surface of the erythrocytes is due to the inability of the GATA1 transcription factor to bind to the upstream promotor region of Duffy blood group antigen, which is due to the mutation at 33rd nucleotide position upstream of the transcription initiation site. Hence, no Duffy blood group antigen is expressed on the surface of the erythrocyte.[22] In this study, the Duffy blood group genotyping was performed using PCR amplification of the human Duffy antigen/chemokine receptor gene followed by sequencing. The primary PCR amplification (Nest 1) of a fragment of 997 basepair (bp) was performed using primer pairs (ExtForward 5′-GTGGGGT-AAGGCTTCCTGAT-3′ and ExtReverse 5′ CAGAGCTGC-GAGTGCTACCT-3′) in a reaction mixture of 26 μL containing 22.5 μL of Platinum PCR SuperMix (Invitrogen, Carlsbad, CA), 1 μL of each forward and reverse primer at 10 μM concentration, and 1.5 μL of DNA template as described.[4] Nest 2 PCR amplification was performed using primer pairs (ExtForward 5′-GTGGGGTAAGGCTTCCTGAT-3′ and InterReverse 5′-CAAACAGCAGGGGAAATGAG-3) to determine a single-nucleotide polymorphism in the Duffy gene (223 bp fragment) which is the GATA-1 transcription factor–binding site in the promoter region that governs the erythrocyte Duffy negativity. Reaction mixture for PCR amplifications included 22.5 μL of Platinum PCR SuperMix (Invitrogen, Carlsbad, CA), 1 μL of each forward and reverse primer for each primer at 10 μM concentration, and 1.5 μL of primary PCR product in a final volume of 26 μL. Two known Duffy-positive controls were used.

PCR products were purified by filtration using MultiScreen PCR Plates for DNA cleanup (Merck Millipore, Billerica, MA). Sequencing reactions were performed for each strand using the ABI PRISM BigDye Terminator cycle sequencing ready-to-use reaction kit run on a 3730XL automatic sequencer (Applied Biosystems, Foster City, CA). Electrophoregrams were visualized and analyzed with Sequencher® version 5.4.1 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI).

RESULTS

We searched for *P. vivax* infections in samples collected at six time points over 2 years among 300 children in Bandiagara. We identified a total of 25 *P. vivax* (Figures 1 and 2) and 109 *P. falciparum* (Figure 1) first-time infections in each individual in the cohort while the samples collected at the beginning of the third transmission season (June 2011) were not tested for *P. falciparum*. The prevalence of *P. vivax* was between 2.0% and 2.5% (Figure 1). The prevalence of *P. falciparum* was

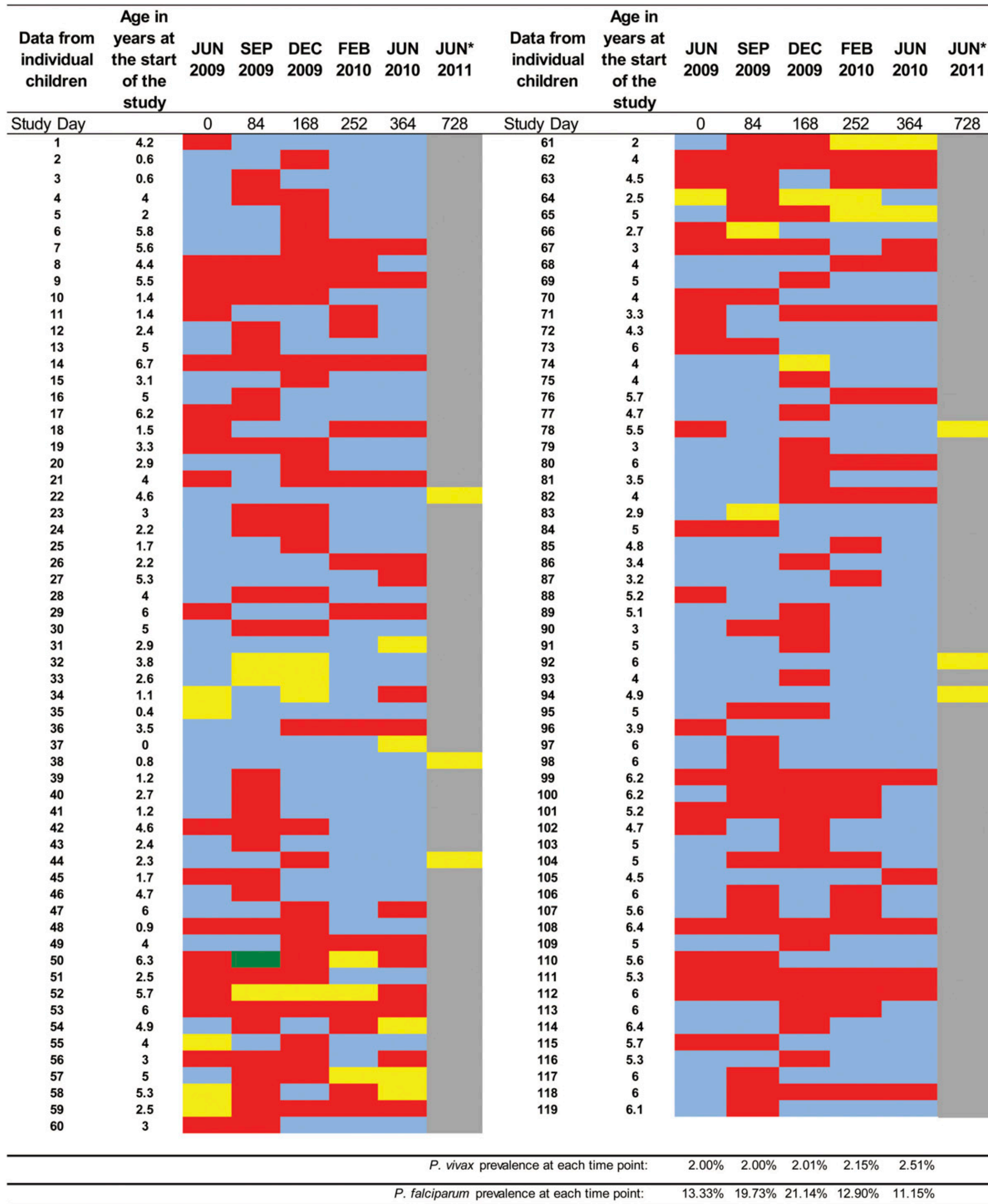

FIGURE 1. *Plasmodium vivax* and *Plasmodium falciparum* infected children from June 2009 to June 2010. Only six *P. vivax* infected children were identified on June 2011. This figure appears in color at www.ajtmh.org.

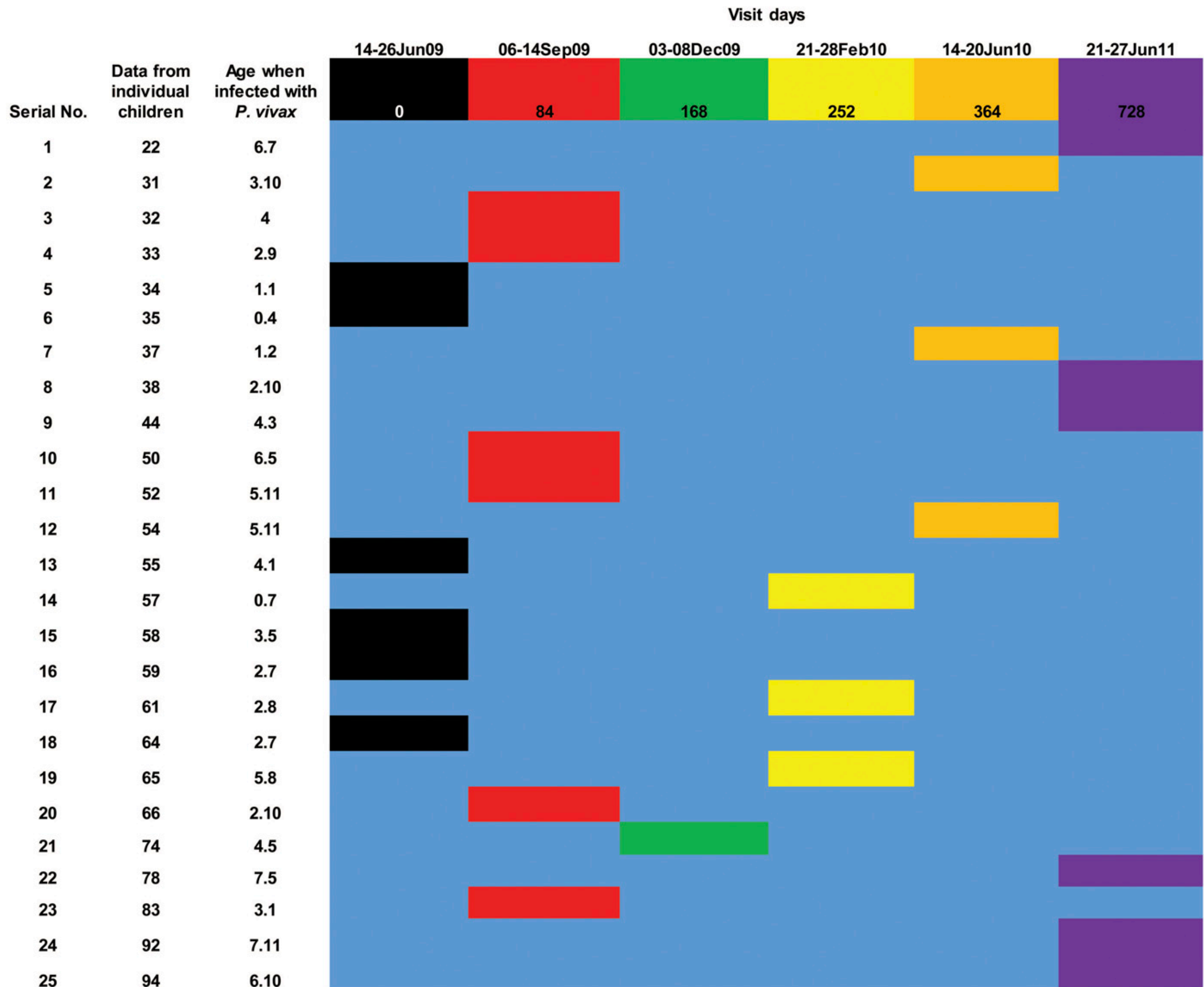

FIGURE 2. The first time the children were infected with *Plasmodium vivax* from Figure 1. In total, there were 25 *P. vivax* infections in the 300 children. The date in the table shows the first-time *P. vivax* infection was observed in a child. The color coding marks each collection period and is used in the map in Figure 4. This figure appears in color at www.ajtmh.org.

between 11% and 21% during the same periods (Figure 1). One child was infected with both *P. falciparum* and *P. vivax* (Figure 1). All cases of *P. vivax* were asymptomatic. None had fever defined by axillary temperatures equal or above 37.5°C. Of *P. vivax* cases (Figure 1), two had 37.0°C and one had 37.4°C; the rest had below 37.0°C. None of the children had a history of fever and chills, headache, or muscle ache, symptoms that may be associated with malaria. Three children had respiratory symptoms and two had rhinorrhea or nasal congestion. The children were not treated for *P. vivax* as they did not have fever and no parasites were seen on the smear.

The thick smears of all the *P. vivax* PCR-positive children were read as negative in the field. Slides from 24 of the 25 children who were PCR positive for *P. vivax* were re-read by two experts. One slide was PCR positive for both *P. vivax* and *P. falciparum* and was excluded because of the problem of identifying the difference between *P. falciparum* and *P. vivax* rings on a thick smear. Thirteen of the 23 slides were read as negative by two readers. Of the remaining 10 slides, the parasite density was between eight and 157 parasites/μL (88.7 ± 53.4 parasites/μL), calculated from the number of white blood cells assuming 8,000 white blood cells/μL. *Plasmodium vivax* parasites from the thick blood smears are shown in Figure 3. These results indicate an extremely low parasite density, indicating the importance of molecular tests for *P. vivax* infections. The location of the children infected with *P. vivax* was spread throughout the area of study (Figure 4).

The hemoglobin (Hb) concentration for all the children was measured during each visit. As anemia is associated with malaria, we looked for *P. vivax*-associated anemia in these children (Figure 5). Children (32, 33, 34, 38, 59, 66, and 94) highlighted in red and bold were infected only with *P. vivax* and observed to have decrease in Hb concentration to the anemic level. Child 32 had 10.7 g/dL Hb when infected with *P. vivax* and the concentration decreased to 9.6 g/dL a month later and started to increase to 11.9 g/dL in around 50 days and no

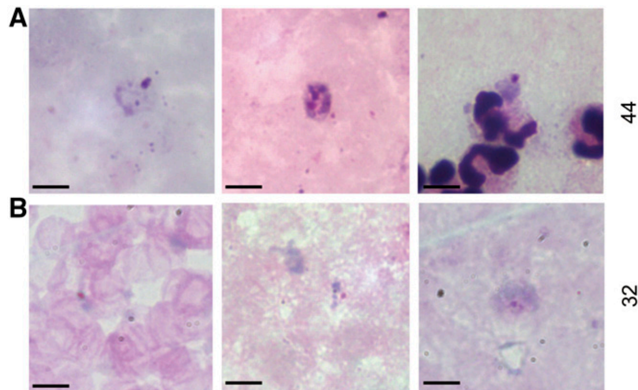

FIGURE 3. *Plasmodium vivax* infections in children from Bandiagara. The blood smears containing *P. vivax* parasites from two children in Bandiagara, Mali. Images of different stages of blood stage *P. vivax* parasites are shown from (**A**) child 44 and (**B**) child 32. Bars indicate 5 μm. This figure appears in color at www.ajtmh.org.

P. vivax and *P. falciparum* was found in subsequent time points. Others such as child 33 had a marked reduction from 9.6 to 7.7 g/dL the month after *P. vivax* infection. We do not know the cause of the decrease of Hb to 7.7 g/dL, although untreated *P. vivax* could have resulted in the anemia. Another marked fall in Hb in child 94 was from 13.1 during *P. vivax* infection to 9.4 g/dL that decreased a month after *P. vivax* and returned to 13.2 a month later. Again, as the *P. vivax* was untreated, the anemia may have been caused by *P. vivax*. A similar effect was also observed in children 34, 38, and 66.

All *P. vivax* were found in Duffy blood group negative children with the mutation from T to C in the GATA1-binding area 5′ to the open reading frame.[22] The location of the children in Bandiagara during each study period is shown in color related to time of their first infection (Figure 4). The 2–3 episodes of *P. vivax* infection occurrence in some of the Duffy-negative children suggest a possible reinfection (new infection) by mosquito bites with a different *P. vivax* strain or due to relapse from hypnozoites in the liver. The children 32/33 and 58/59 were siblings living in the same house and each pair was infected during the same study period (Figures 2 and 4), suggesting that the infection occurred within their respective houses. Infections occurred in most areas where the study children lived. In Bandiagara, the rainy season starts in May and continues through October. The six infections in February 2010 (in yellow, Figure 1) were unusual in that they occurred during the dry season,

FIGURE 4. *Plasmodium vivax* infected children's locations in the Bandiagara Map. The figure shows the map of Bandiagara and the locations of each patient with *P. vivax* infections. The unique colors to the children are based on the first time a child had a *P. vivax* infection during the specific collection dates as shown in Figure 2. The river Yame passes through Bandiagara which is colored in blue as well as the water ponds. This figure appears in color at www.ajtmh.org.

Serial No.	Data from individual children	Hemoglobin (g/dL) level before *P. vivax* infection. Date, Hb	*P. vivax* infection and Hemoglobin (g/dL) level. Date, Hb	Hemoglobin (g/dL) level after *P. vivax* infection. Date, Hb
1	22	5/22/2011, 10.9	6/22/2011, 11	7/20/2011, 11.4
2	31	5/18/2010, 11.5	6/15/2010, 11.7	7/15/2010, 11.7
3	32	8/11/2009, 11.4	9/8/2009, 11.4	10/6/2009, 12.2
4	**32**	**11/4/2009, 11.8**	**12/4/2009, 10.7**	**12/27/2009, 9.6** **1/26/2010, 11.9**
5	33	8/11/2009, 8.7	9/8/2009, 9.2	10/6/2009, 9.9
6	**33**	**11/4/2009, 9.6**	**12/4/2009, 9.7**	**12/27/2009, 7.7** **1/26/2010, 10.9**
7	34	NA	6/16/2009, 9.2	7/14/2009, 8.8
8	**34**	**11/4/2009, 9.1**	**12/5/2009, 7.9**	**12/29/2009, 8.4** **2/23/2010, 9.6**
9	35	NA	6/16/2009, 11.3	7/14/2009, 11.1
10	37	5/18/2010, 9	6/15/2010, 9.7	7/15/2010, 9.2
11	**38**	**5/23/2011, 11.6**	**6/22/2011, 10.9**	**7/22/2011, 11.9**
12	44	5/23/2011, 8.5	6/22/2011, 8.3	7/22/2011, 9.5
13	50	8/13/2009, 11.2	9/10/2009, 10.6	10/8/2009, 11.8
14	50	1/28/2010, 9.2	2/24/2010, 10.3	3/25/2010, 10
15	52	8/14/2009, 13.1	9/11/2009, 11.6	10/9/2009, 10.8
16	52	11/7/2009, 12.1	12/5/2009, 11.4	1/3/2010, 13.2
17	52	1/29/2010, 11.9	2/25/2010, 12.2	3/26/2010, 12.8
18	54	5/21/2010, 11.2	6/18/2010, 10.9	7/17/2010, 11.4
19	55	NA	6/19/2009, 10.6	7/17/2009, 10.8
20	57	1/29/2010, 10	2/25/2010, 10.4	3/26/2010, 11
21	57	5/21/2010, 9.5	6/18/2010, 9.8	7/17/2010, 11.9
22	58	NA	6/19/2009, 12.1	7/17/2009, 13.7
23	58	5/21/2010, 13.1	6/18/2010, 11.9	7/17/2010, 13
24	**59**	**NA**	**6/19/2009, 12.3**	**7/17/2009, 11.4** **8/14/2009, 10.9**
25	61	1/29/2010, 12.4	2/25/2010, 10.8	3/26/2010, 12
26	61	5/21/2010, 11.4	6/19/2010, 12	7/15/2010, 13.4
27	64	NA	6/19/2009, 11.3	7/17/2009, 10.9
28	64	11/7/2009, 10.2	12/6/2009, 11.6	1/3/2010, 11.6
29	64	1/29/2010, 11.2	2/25/2010, 10.7	3/26/2010, 11.8
30	65	1/29/2010, 12.4	2/25/2010, 11.1	3/26/2010, 13.3
31	65	5/21/2010, 11.7	6/18/2010, 11.3	7/17/2010, 12.3
32	**66**	**8/14/2009, 11.8**	**9/11/2009, 11.6**	**10/9/2009, 10.4** **11/7/2009, 11.5**
33	74	11/7/2009, 11.1	12/6/2009, 11	1/3/2010, 11.1
34	78	5/25/2011, 12.5	6/25/2011, 12.5	7/23/2011, 12.3
35	83	8/15/2009, 11.2	9/13/2009, 11.5	10/10/2009, 11
36	92	5/26/2011, 11.4	6/26/2011, 12.7	7/25/2011, 11.3
37	**94**	**5/26/2011, 13.2**	**6/26/2011, 13.1**	**7/25/2011, 9.4** **8/21/2011, 13.2**

FIGURE 5. *Plasmodium vivax* and anemia in Malian children. The table shows the data of hemoglobin concentration in 25 *P. vivax* infected children. The data points highlighted in bold are the children only infected by *P. vivax.* The hemoglobin concentration was markedly reduced during *P. vivax* infection and returned to normal within 30–60 days. This figure appears in color at www.ajtmh.org.

which could be due to relapse. However, during the dry season, the Yame River that runs through Bandiagara dries up, but the local people block the river for water to grow crops such as tomatoes and onions. We know that *P. falciparum* infection persists at low incidence during the dry season in Bandiagara.[20] We speculate that *P. vivax* transmission may have continued through February by mosquitoes breeding in the dammed river.

DISCUSSION

We describe here a longitudinal study of *P. vivax* infection in a Duffy-negative population of 300 children spanning across three rainy seasons in Bandiagara. At every time point except June 2011, *P. vivax* was detected in about 2% of the children, and over the course of the study 8.3% of the children experienced *P. vivax* infection at least once. The prevalence data highlight that *P. vivax* infection occurs throughout the year irrespective of the rainy season, further suggesting a chronic low level infection or relapses. Approximately 36% of the children were infected with *P. falciparum* during the study period. It has been shown that there is an association between *P. vivax* relapse after *P. falciparum* infection and treatment.[23–25] Although none of the *P. vivax* infected children were symptomatic, however, 10 of 25 children were treated with the malaria drug at different time points during June 2009 to June 2012. Of the 10 children, only three children were treated with malaria drug before *P. vivax* infection (Supplemental Figure 1), the other seven were treated with malarial drugs after *P. vivax* infection. Hence, from this observation we cannot conclude whether *P. falciparum* infection and its treatment with malarial drugs would have led to triggering of *P. vivax* hypnozoites.

Ten of the 25 children who experienced *P. vivax* were infected more than once (range 2–3 infections), sometimes at consecutive time points, and sometimes separated by the absence of detectable *P. vivax*. The *P. vivax*-infected children were observed in different parts of the Bandiagara study area (Figure 4). Two siblings in two families were infected with *P. vivax* during the same study period, indicating that the infections were occurring in their home. All infections were seen in Duffy-negative children and not in Duffy positive, indicating that the *P. vivax* infections were similar to what was observed in western Kenya[7] but different from those in Madagascar[4,26] and Ethiopia[5,8] where Duffy-positive *P. vivax* infections can serve as a source of infection for the Duffy blood group negative people.

What was the source of *P. vivax* in Bandiagara? It is possible that *P. vivax* could be introduced from the Sahara desert in the north where the Tuareg people may be Duffy positive,[17,18] but the continuous infection for 3 years raises the possibility that *P. vivax* infection is maintained by infections from Duffy-negative children. The other possibility is tourists visiting Bandiagara who are Duffy positive could interact with Duffy negatives, but the area of the study is not near the tourist area.

The first evidence that *P. vivax* could exist in a Duffy blood group negative population was in western Kenya in 2006[7] where the evidence for Duffy negative was defined by the negativity with anti-Fy6 and anti-Fy3. In other areas such as in Cameroon, two studies found *P. vivax* in Duffy-negative people, presumably in different areas.[12,13] These Duffy negative infections were identified by the mutation of T to C in the GATA1-binding domain 5′ to the open reading frame.[22] Other areas of Africa had *P. vivax*, but the Duffy type was not determined.[27,28]

It is likely that *P. vivax* selected for the refractory Duffy-negative phenotype in Africa. The selection is accelerated by the protection of the heterozygote[29] and may have occurred in 500 generations over 10,000 years, depending on the selective advantage of the homozygous null and partial dominance of the heterozygote. Anemia due to *P. vivax* has been well documented.[30–32] Our data in this study suggest that low-level *P. vivax* parasite density may have caused anemia in the children. Thus, the diagnosis and the treatment of *P. vivax* might benefit the children in Bandiagara.

Plasmodium vivax varies greatly in virulence with the Madagascar *P. vivax* isolate studied by James and others[33] associated with marked virulence and mortality of 10–14% if left untreated. It has been presumed that *P. vivax* was responsible for the mortality in the Thames estuary over many centuries, preventing people from working in the zone.[34] Today various reports describe the severity of *P. vivax*.[30,35,36] *Plasmodium vivax* can cause severe disease and selected for the loss of the erythrocyte receptor, the Duffy blood group, to prevent the infection with *P. vivax*.

In recent years, with advanced molecular and cellular techniques, including conventional PCR, real-time PCR, and flow cytometry, we are able to detect *P. vivax* infection with little or no clinical symptoms, which could have gone unnoticed and undetected for an unknown period in Duffy-negative individuals. This further suggests that *P. vivax* is maintained in the Duffy-negative population in Bandiagara without any malarial disease (fever and chills). In addition, it is not clear whether the observed persistent infections in Bandiagara are due to new infections or relapses due to reactivation of dormant liver stage hypnozoites. It was shown that 70% and ∼96% of *P. vivax* infections in Papua New Guinea (PNG) and Thailand, respectively, are due to reactivation of hypnozoites, and this difference in PNG and Thailand is due to primaquine treatment failure in PNG.[37]

We are now faced with an enigma. In the 1970s, Duffy-negative people were shown to be refractory to *P. vivax* infection. The present data indicate that at some time in the past, *P. vivax* adapted to infect Duffy-negative people throughout Africa. The mutations in *P. vivax* that permitted infection may be the expansion of the DBP locus[8] or other unknown mutations that may upregulate or change invasion ligands on merozoites that permit invasion.[38–40] Studies from Ethiopia and Madagascar show that the infection with *P. vivax* results in a mild disease. With continued mutations, will *P. vivax* become a more virulent infection in Duffy-negative populations as seen in Duffy-positive populations? For example, the Duffy blood group antigen in Saimiri and Aotus monkey erythrocytes are highly similar. However, the DBP1 of *P. vivax* Salvador I does not bind Saimiri monkey erythrocytes, although it binds to the erythrocytes of Aotus monkey. Yet the infection of Saimiri and Aotus monkeys is similar to the Salvador I *P. vivax* infection.[8,41,42] As we learn more about *P. vivax* in Africa, the mutations in ligands that affect specificity or expression levels in the parasite that have allowed its spread throughout Africa today will be defined.

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