# A RETROSPECTIVE EXAMINATION OF REINFECTION OF HUMANS WITH PLASMODIUM VIVAX

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*Abstract.* A retrospective examination was made of archival data collected between 1940 and 1963 to determine the impact of reinfection of patients with *Plasmodium vivax* with homologous and heterologous strains of the parasite. Following reinfection of 14 patients with a homologous strain, the geometric mean maximum parasite count was reduced from 9,101/ $\mu$ L during the primary infection to 998/ $\mu$ L and the geometric mean daily parasite count for the first 20 days was reduced from 923/ $\mu$ L to 16/ $\mu$ L. Following reinfection of 22 patients with heterologous strains of *P. vivax*, the geometric mean maximum parasite count was 8,460/ $\mu$ L during the primary infection versus a secondary level of 9,196/ $\mu$ L and the geometric mean daily parasite count was 8,460/ $\mu$ L during the primary infection versus a secondary level of 9,196/ $\mu$ L and the geometric mean daily parasite count decreased from 847/ $\mu$ L/day to 335/ $\mu$ L/day. Reductions in fever episodes  $\geq$  101°F and  $\geq$  104°F appeared to be a more sensitive measure of clinical immunity. Fever episodes  $\geq$  104°F in patients with homologous strain reinfections decreased from 1.92 episodes per week to 0.18 compared with 1.24 to 0.57 in patients with heterologous infections. Fever episodes  $\geq$  101°F decreased from 2.98 to 0.60 in the homologous strain compared with 2.08 to 1.07 for the heterologous infections. The average maximum fever temperature in the homologous group was 106°F during the primary infection versus 103.4°F for the secondary infection compared with 105.8°F during the primary infection versus 105.6°F for the secondary infection in the heterologous patients.

## INTRODUCTION

Studies are being made on the development of immunity to infection with different species of malaria parasites using archival data from patients infected between 1940 and 1963 for the treatment of neurosyphilis.<sup>1–15</sup> Sporozoite- and blood-induced infections of *Plasmodium vivax* were frequently used for these treatments, and at the direction of the medical staff, some patients were subsequently reinfected with homologous and heterologous species and strains of *Plasmodium*. An examination was made of data from 36 patients previously infected with the St. Elizabeth strain, 14 of whom were reinfected with the homologous St. Elizabeth strain and 22 with heterologous strains of *P. vivax*.

Reported here are the results of a retrospective examination of these archival data. The goal was to document the relationship between primary and secondary infection as regards 1) maximum parasite count, 2) mean daily parasite count, 3) episodes of fever  $\geq 101^{\circ}$ F and  $\geq 104^{\circ}$ F, and 4) maximum fever.

# MATERIALS AND METHODS

Patient management. Consent for whatever treatments the hospital staff determined necessary for treatment of the patient was granted by the families of the patients or the courts when patients were admitted to the hospital. The decision to infect a neurosyphilitic patient with a specific malaria was made as part of standard patient care by the medical staff of the South Carolina State Hospital. Patient care and evaluation of the clinical endpoints (e.g., fever) were the responsibilities of the medical staff. As previously reported,<sup>1</sup> during infection, temperature, pulse, and respiration were checked every four hours and every hour during paroxysms (fevers) by hospital personnel. During paroxysms, patients were treated symptomatically. Infections were terminated at the direction of the attending physician. The U.S. Public Health Service personnel provided the parasite for inoculation, monitored the daily parasite counts to determine the course of infection, and provided mosquitoes to be fed on the patients to transfer infections from one patient to another. All patients undergoing malaria therapy lived in screened wards of the hospital to prevent possible infection of local anophelines.

**Treatment.** Infections were terminated by treatment with various antimalarial drugs. Sporozoite-induced infections were treated with primaquine, as well as a schizonticidal drug such as chloroquine or pyrimethamine.

**Strains of** *P. vivax.* Primary infections in the patients were with the St. Elizabeth strain that was obtained in the 1930s from St. Elizabeth Hospital in Washington, DC. Its exact origin is unknown. The other strains such as Chesson, NG-1512, NG-V90, NG-V94, CBI-109, and Sicily were established from returning servicemen during World War II. Korean strains were isolated from returning servicemen during the Korean War. The Venezuela strain was isolated from a traveler returning from Venezuela.

**Parasitemia.** Patients were infected by the intravenous inoculation of parasitized erythrocytes or via sporozoite inoculation. Thick and thin peripheral blood films were made daily by the method of Earle and Perez,<sup>16</sup> stained with Giemsa, and examined microscopically for presence of parasites. The threshold for detection was approximately 10 parasites/µL. Asexual and sexual parasites were recorded per microliter of blood. Infections often persisted for many weeks.

**Statistical analysis.** Mean parasite counts and maximum fevers during the first versus secondary infections were compared using the Wilcoxon matched pairs signed rank test. Rate ratios of fever episodes were computed and compared using Poisson regression implementing generalized estimating equations to adjust for correlation between observations from the same individuals at different times.

### RESULTS

Fourteen patients previously infected with the St. Elizabeth strain of *P. vivax* were subsequently reinfected with the homologous strain (Table 1). The interval between the primary and the secondary infection ranged from 1 to 96 months. Long intervals between infection did not result in increased parasite counts during secondary infection. During the primary infection, the geometric mean maximum parasite count was 9,101/ $\mu$ L compared with 998/ $\mu$ L during the secondary infection

TABLE 1

Maximum parasite counts, mean daily parasite counts for first 20 days of patent parasitemia, number of fever episodes  $\geq 101$  and  $\geq 104^{\circ}$ F, and maximum fever recorded for 14 patients during primary and secondary infections with the St. Elizabeth (St. Eliz.) strain of *Plasmodium vivax*\*

| Patient | Strain    | Route | Parasite count/µL |       | Fever (°F) |       |            | Interval |           |       | Parasite count/µL |       | Fever (°F) |       |       |
|---------|-----------|-------|-------------------|-------|------------|-------|------------|----------|-----------|-------|-------------------|-------|------------|-------|-------|
|         |           |       | Max.              | Mean  | $\geq 101$ | Max.  | $\geq 104$ | (months) | Strain    | Route | Max.              | Mean  | $\geq 101$ | Max.  | ≥ 104 |
| S-629   | St. Eliz. | Sporo | 10,392            | 1,234 | 20         | 106.4 | 20         | 72       | St. Eliz. | Sporo | 50                | 0.7   | 0          | _     | 0     |
| S-745   | St. Eliz. | Sporo | 8,320             | 1,743 | 27         | 107.0 | 18         | 84       | St. Eliz. | Sporo | 54                | 1.3   | 0          | -     | 0     |
| S-768   | St. Eliz. | Sporo | 8,150             | 856   | 24         | 106.0 | 11         | 72       | St. Eliz. | Sporo | 140               | 1.8   | 2          | 103.2 | 0     |
| S-846   | St. Eliz. | Sporo | 10,240            | 1,936 | 22         | 106.4 | 12         | 96       | St. Eliz. | Blood | 660               | 3.9   | 2          | 103.0 | 0     |
| S-1167  | St. Eliz. | Blood | 9,580             | 1,482 | 20         | 107.2 | 14         | 9        | St. Eliz. | Sporo | 640               | 4.2   | 0          | -     | 0     |
| S-823   | St. Eliz. | Sporo | 11,055            | 2,481 | 21         | 106.4 | 20         | 59       | St. Eliz. | Blood | 600               | 4.3   | 1          | 102.0 | 0     |
| S-911   | St. Eliz. | Sporo | 21,140            | 2,402 | 23         | 106.0 | 14         | 20       | St. Eliz. | Sporo | 2,816             | 8.2   | 1          | 103.0 | 0     |
| S-1019  | St. Eliz. | Sporo | 4,300             | 1,995 | 17         | 106.2 | 6          | 78       | St. Eliz. | Sporo | 864               | 11.7  | 1          | 101.0 | 0     |
| S-1026  | St. Eliz. | Sporo | 752               | 5     | 3          | 103.8 | 0          | 1        | St. Eliz. | Blood | 816               | 14.0  | 1          | 103.4 | 0     |
| S-710   | St. Eliz. | Blood | 22,132            | 2,964 | 24         | 105.8 | 15         | 35       | St. Eliz. | Sporo | 2,752             | 34.9  | 1          | 104.0 | 1     |
| S-1314  | St. Eliz. | Blood | 9,957             | 120   | 7          | 106.0 | 6          | 2        | St. Eliz. | Blood | 3,060             | 44.1  | 6          | 104.0 | 1     |
| S-772   | St. Eliz. | Blood | 8,320             | 1,004 | 21         | 105.2 | 17         | 77       | St. Eliz. | Sporo | 3,030             | 113.6 | 6          | 104.0 | 1     |
| S-938   | St. Eliz. | Blood | 6,750             | 809   | 9          | 105.2 | 4          | 6        | St. Eliz. | Blood | 7,528             | 363.2 | 4          | 105.0 | 2     |
| S-1107  | St. Eliz. | Blood | 34,048            | 3,063 | 11         | 106.6 | 7          | 2        | St. Eliz. | Blood | 26,854            | 456.1 | 8          | 105.6 | 5     |

\* Max. = maximum; Sporo = sporozoites.

(P < 0.001). There were 2.98 episodes of fever  $\ge 101^{\circ}$ F per person week of infection during the primary infection versus 0.60 during the secondary infection (rate ratio = 0.20, P =0.002). A total of 1.92 episodes of fever  $\ge 104^{\circ}$ F per person week of infection was noted initially compared with 0.18 during the secondary infection (rate ratio = 0.09, P = 0.003). The geometric mean daily parasite count for the first 20 days of patent parasitemia was 924/µL/day versus 16/µL/day during the secondary (P < 0.001).

Twenty-two patients previously infected with the St. Elizabeth strain of *P. vivax* were subsequently infected with heterologous strains of the parasite (Table 2). The interval be-

tween primary and secondary infection ranged from 1 to 164 months. There was no relationship between the interval between infections and the parasitologic outcome during the secondary infection. During the primary infection, the geometric mean maximum parasite count was  $8,460/\mu$ L compared with  $9,196/\mu$ L during the secondary infection. There were 2.08 episodes of fever  $\ge 101^{\circ}$ F per person week of infection versus 1.07 (rate ratio = 0.52, P = 0.007) and 1.24 episodes  $\ge 104^{\circ}$ F versus 0.57 (rate ratio = 0.45, P = 0.004). The geometric mean daily parasite count for the first 20 days of infection was  $847/\mu$ L/day versus  $336/\mu$ L/day following reinfection (P = 0.002).

TABLE 2

Maximum parasite counts, mean daily parasite counts for first 20 days of patent parasitemia, number of fever episodes  $\geq 101$  and  $\geq 104^{\circ}$ F, and maximum fever recorded for 22 patients during primary infection with the St. Elizabeth (St. Eliz.) strain of *Plasmodium vivax* and secondary infections with heterologous strains of the parasite\*

|         |           |       | Parasite $count/\mu L$ |       | Fever (°F) |       |       | Interval |           |       | Parasite $count/\mu L$ |       | Fever (°F) |       |       |
|---------|-----------|-------|------------------------|-------|------------|-------|-------|----------|-----------|-------|------------------------|-------|------------|-------|-------|
| Patient | Strain    | Route | Max.                   | Mean  | ≥ 101      | Max.  | ≥ 104 | (months) | Strain    | Route | Max.                   | Mean  | ≥ 101      | Max.  | ≥ 104 |
| S-1267  | St. Eliz. | Blood | 4,245                  | 29    | 4          | 105.0 | 3     | 5        | Chesson   | Sporo | 3,410                  | 14    | 0          | _     | 0     |
| S-921   | St. Eliz. | Blood | 704                    | 54    | 0          | -     | 0     | 1        | Chesson   | Blood | 310                    | 34    | 0          | -     | 0     |
| S-1005  | St. Eliz. | Blood | 16,640                 | 3,863 | 23         | 106.2 | 15    | 83       | Chesson   | Sporo | 7,425                  | 47    | 11         | 105.8 | 1     |
| S-1234  | St. Eliz. | Blood | 38,673                 | 941   | 24         | 106.0 | 24    | 51       | Chesson   | Blood | 21,780                 | 49    | 3          | 105.0 | 2     |
| S-1180  | St. Eliz. | Sporo | 3,540                  | 587   | 15         | 105.6 | 10    | 41       | Chesson   | Blood | 14,315                 | 76    | 5          | 104.4 | 2     |
| S-447   | St. Eliz. | Sporo | 9,400                  | 996   | 16         | 106.4 | 10    | 164      | Chesson   | Sporo | 2,760                  | 123   | 0          | -     | 0     |
| S-1017  | St. Eliz. | Sporo | 11,424                 | 2,495 | 21         | 106.6 | 15    | 21       | Korean    | Sporo | 5,208                  | 191   | 4          | 105.0 | 1     |
| S-1202  | St. Eliz. | Blood | 9,600                  | 2,224 | 23         | 106.2 | 13    | 32       | Chesson   | Blood | 9,806                  | 211   | 5          | 104.4 | 1     |
| S-599   | St. Eliz. | Blood | 3,350                  | 415   | 8          | 104.6 | 2     | 1        | NG-V90    | Sporo | 6,160                  | 324   | 7          | 105.4 | 3     |
| S-950   | St. Eliz. | Blood | 27,584                 | 1,640 | 18         | 105.4 | 9     | 6        | Chesson   | Blood | 13,241                 | 447   | 8          | 104.8 | 3     |
| S-753   | St. Eliz. | Blood | 12,320                 | 1,805 | 14         | 105.8 | 4     | 2        | NG-1512   | Blood | 9,120                  | 481   | 6          | 105.0 | 2     |
| S-1057  | St. Eliz. | Sporo | 5,856                  | 1,408 | 22         | 106.4 | 19    | 15       | Korean    | Sporo | 12,480                 | 503   | 6          | 106.2 | 5     |
| S-881   | St. Eliz. | Sporo | 2,456                  | 46    | 11         | 106.0 | 7     | 2        | Sicily    | Blood | 9,075                  | 565   | 10         | 106.6 | 4     |
| S-730   | St. Eliz. | Blood | 11,100                 | 714   | 10         | 105.2 | 6     | 3        | NG-1512   | Blood | 20,600                 | 684   | 8          | 106.0 | 5     |
| S-675   | St. Eliz. | Sporo | 12,350                 | 1,055 | 6          | 106.4 | 3     | 2        | NG-1512   | Sporo | 14,850                 | 735   | 7          | 106.4 | 2     |
| S-805   | St. Eliz. | Sporo | 1,600                  | 180   | 11         | 104.8 | 4     | 25       | Chesson   | Sporo | 9,240                  | 782   | 12         | 105.6 | 6     |
| S-867   | St. Eliz. | Blood | 10,857                 | 3,040 | 17         | 106.2 | 11    | 150      | Chesson   | Blood | 14,600                 | 860   | 6          | 106.8 | 4     |
| S-643   | St. Eliz. | Sporo | 35,072                 | 2,417 | 17         | 106.0 | 8     | 3        | NG-1512   | Blood | 16,550                 | 861   | 5          | 106.0 | 4     |
| S-689   | St. Eliz. | Blood | 14,425                 | 3,131 | 20         | 106.0 | 7     | 2        | CBI-109   | Blood | 5,725                  | 1,023 | 3          | 104.8 | 1     |
| S-554   | St. Eliz. | Blood | 12,500                 | 4,602 | 28         | 106.8 | 16    | 146      | Chesson   | Blood | 22,065                 | 1,198 | 7          | 106.4 | 6     |
| S-880   | St. Eliz. | Blood | 20,988                 | 2,730 | 20         | 106.4 | 15    | 5        | Venezuela | Blood | 32,633                 | 2,267 | 19         | 106.4 | 10    |
| S-592   | St. Eliz. | Sporo | 6,950                  | 426   | 2          | 103.8 | 0     | 121      | NG-V78    | Sporo | 19,350                 | 4,785 | 20         | 105.2 | 8     |

\* Max. = maximum; Sporo = sporozoites.

### DISCUSSION

Primary infection with the St. Elizabeth strain of P. vivax in 14 patients resulted in geometric mean maximum parasite counts of 9,101/µL and a geometric mean daily parasite count during the first 20 days of patent parasitemia of 924/µL/day. Maximum fevers ranged from 103.8°F to 107.2°F with a median of 106.1°F. Patients averaged 1.92 episodes fever per person week of infection. Maximum parasite counts varied following homologous reinfection with the St. Elizabeth strain, but the geometric mean maximum parasite count was one-tenth of that seen during the primary infection  $(9,101/\mu L)$ versus 998/µL). The greatest evidence of immunity to reinfection with the homologous parasite was shown by the decrease in mean daily parasite count from  $923/\mu L/day$  to 16/  $\mu$ L/day and in fever ( $\geq 101^{\circ}$ F, from 2.98 episodes/person week infection to 0.60/person week infection;  $\geq 104^{\circ}$ F, from 1.09 episodes/person week infection to 0.18 episodes/person week infection).

When patients were reinfected with heterologous strains of *P. vivax*, the protection was less obvious, although present. Maximum parasite counts were actually higher (9,196 versus 8,460/ $\mu$ L) following reinfection with heterologous parasites, most of which were from the South Pacific region. This suggested that there was either little or no cross-immunity or that these parasites were more adapted to produce high density parasite counts. However, an examination of the geometric mean daily parasite counts for the first 20 days of patent parasitemia showed a reduction from 847/ $\mu$ L/day to 335/ $\mu$ L/day. More evident was a reduction in fever episodes ( $\geq 101^{\circ}$ F, 2.1 to 1.07 episodes/person week; and  $\geq 104^{\circ}$ F, 1.24 to 0.57 episodes/person week).

The development of vaccines against malaria is based somewhat on the premise that previous infection confers a level of measurable immunity and that immunization should mimic or add to this immunity. An examination of these data suggests that vaccines developed against the blood stages of a particular strain of *P. vivax* may be effective against the homologous strain of the parasite, but less so against heterologous strains.

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