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Adherence to Chemoprophylaxis and *Plasmodium falciparum* Anti-Circumsporozoite Seroconversion in a Prospective Cohort Study of Dutch Short-Term Travelers

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**Abstract**

**Background:** We conducted a prospective study in a cohort of short-term travelers assessing the incidence rate of anti-circumsporozoite seroconversion, adherence to chemoprophylaxis, symptoms of malaria during travel, and malaria treatment abroad.

**Methods:** Adults were recruited from the travel clinic of the Public Health Service Amsterdam. They kept a structured daily travel diary and donated blood samples before and after travel. Blood samples were serologically tested for the presence of *Plasmodium falciparum* anti-circumsporozoite antibodies.

**Results:** Overall, the incidence rate (IR) of anti-circumsporozoite seroconversion was 0.8 per 100 person-months. Of 945 travelers, 620 (66%) visited high-endemic areas and were advised about both chemoprophylaxis and preventive measures against mosquito bites. Most subjects (520/620 = 84%) took at least 75% of recommended prophylaxis during travel. Travel to Africa, use of mefloquine, travel duration of 14–29 days in endemic areas, and concurrent use of DEET (N,N-diethyl-meta-toluamide) were associated with good adherence practices. Four travelers without fever seroconverted, becoming anti-circumsporozoite antibody-positive. All four had been adherent to chemoprophylaxis; two visited Africa, one Suriname, one India. Ten subjects with fever were tested for malaria while abroad and of these, three received treatment. All three were adherent to chemoprophylaxis and tested negative for anti-circumsporozoite antibodies.

**Conclusion:** Travel to Africa, using mefloquine, travel duration of 14–29 days in endemic areas, and use of DEET were associated with good adherence to chemoprophylaxis. The combination of chemoprophylaxis and other preventive measures were sufficient to protect seroconverting travelers from clinical malaria. Travelers who were treated for malaria abroad did not seroconvert.

**Introduction**

Half the world’s population is at risk of malaria [1,2]. An estimated 216 million malaria cases, and 655,000 deaths, occurred in 2010, mostly among the local population in malaria-endemic regions [2]. Malaria is also a threat to the approximately 80–90 million travelers who visit the 106 endemic countries annually. Travelers can protect themselves against malaria by using antimalarial chemoprophylaxis and preventive measures against mosquito bites. Recommendations for these preventive measures are based on the anticipated infection rate and drug resistance in *Plasmodium falciparum* [3–5], and differ by country.

Risk estimates for malaria infection among travelers from nonendemic countries are usually based on transmission rates in endemic populations and reports of infections in returned tourists. Neither estimate correlates well with the risk for travelers [6,7]. Studies that estimate incidence based on these reports lack information on how many travelers were protected by chemoprophylaxis and how many were treated for malaria abroad. They are, however, valid to estimate trends. In the Netherlands, the incidence of imported *falciparum* malaria among travelers declined from 10.0/10,000 in the year 2000 to 3.4/10,000 travelers in 2007, whereas the proportion of travelers who did not use chemoprophylaxis rose from 47% to 52% [8]. Since most of the malaria cases occur in travelers who fail to use – or adhere to – the
appropriate chemoprophylaxis [5,9–11], adherence to chemopro-
phylaxis is associated with protection against malaria. Prospective
studies among travelers are more valid to estimate risks [12]. Most
prospective studies are incomplete in their assessment of adher-
ence to chemoprophylaxis combined with serological testing for \emph{P. falciparum} infection [13–20]; in fact there is only one study, from
1991–1992, which examined both adherence to chemoprophylaxis in
relation to serological testing.

In order to contribute to a more definitive assessment of risks for
travelers, we conducted a comprehensive prospective study as to
the incidence rate of \emph{Plasmodium falciparum} anti-circumsporozoite
seroconversion, adherence to chemoprophylaxis, symptoms of
malaria during travel, and malaria treatment abroad.

\textbf{Methods}

\textbf{Ethics Statement}

Study protocol was approved by the Medical Ethics Committee
of the Academic Medical Center Amsterdam (MEC 06/016). Partic-
ipants were included only with informed and written consent.

\textbf{Study Population}

A prospective study was performed among persons attending the
travel clinic of the Public Health Service Amsterdam from
October 2006 to October 2007. All persons 18 years and older
were eligible if they were planning to travel for 1 to 13 weeks to
one or more malaria-endemic countries. Countries were grouped
in continents according to the composition of macro geographical
(continental) regions described by the United Nations Statistics
Division [21]. All participants were seen by a doctor or nurse
specialized in travel medicine. Based on Dutch national guidelines
for travelers’ health advice [22], participants traveling to low-risk
malaria-endemic areas (‘low-endemic areas’) were advised about
strict preventive measures against mosquito bites without chemo-
 prophylaxis; participants traveling to intermediate- and/or high-
risk malaria-endemic areas (‘high-endemic areas’), were advised
about strict preventive measures against mosquito bites and
antimalarial chemoprophylaxis. Depending on travel destination
and travelers’ characteristics, atovaquone-proguanil, mefloquine,
doxycycline, or proguanil were recommended in the Netherlands
as chemoprophylaxis against infection with \emph{P. falciparum} malaria
[22]. Travelers taking mefloquine were advised to start 3 weeks

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
& \textbf{Total} & \textbf{High-endemic area\textsuperscript{a}} & \textbf{Low-endemic area\textsuperscript{b}} \\
\hline
\textbf{Sex} & & & & \\
\hline
Male & 945 & 620 & 325 \\
\hline
Female & 545 & 355 & 190 \\
\hline
\textbf{Age group, years} & & & & \\
\hline
18–30 & 312 & 207 & 105 \\
\hline
31–45 & 304 & 197 & 107 \\
\hline
46–59 & 223 & 155 & 68 \\
\hline
>&=60 & 106 & 61 & 45 \\
\hline
\textbf{Country of birth} & & & & \\
\hline
Western country & 879 & 581 & 298 \\
\hline
Non-Western country & 66 & 39 & 27 \\
\hline
\textbf{Primary purpose of travel} & & & & \\
\hline
Tourism & 815 & 519 & 296 \\
\hline
Visiting friends and/or relatives & 59 & 42 & 17 \\
\hline
Work or education & 71 & 59 & 12 \\
\hline
\textbf{Previous travel to a tropical/subtropical country} & & & & \\
\hline
0 & 162 & 91 & 71 \\
\hline
1–6 times & 546 & 357 & 189 \\
\hline
6 times or more & 237 & 172 & 65 \\
\hline
\textbf{Length of stay in endemic area} & & & & \\
\hline
<&=13 days & 529 & 302 & 227 \\
\hline
14–28 days & 333 & 249 & 84 \\
\hline
>&=29 days & 83 & 69 & 14 \\
\hline
\textbf{Travel destination} & & & & \\
\hline
Africa & 285 & 279 & 6 \\
\hline
Asia & 454 & 202 & 252 \\
\hline
Latin America & 206 & 139 & 67 \\
\hline
\end{tabular}
\caption{Characteristics of a prospective cohort of short-term travelers from the Netherlands who visited a malaria-endemic area, October 2006–October 2007.}
\end{table}

\textbf{Table 1. Characteristics of a prospective cohort of short-term travelers from the Netherlands who visited a malaria-endemic area, October 2006–October 2007.}
prior to arrival in high-endemic areas, in case of atovaquone-proguanil one day. Travelers taking proguanil or doxycycline start on the day of arrival. Travelers were advised to continue mefloquine, proguanil, and doxycycline 4 weeks after leaving high-endemic areas, and atovaquone-proguanil for 7 days. Participants received a prescription for the appropriate antimalarial chemoprophylaxis, oral and written information about malaria, the use of chemoprophylaxis, and preventive measures against mosquito bites.

**Survey Methods**

A standard questionnaire in Dutch or English was used before departure to collect data on sociodemographics, travel history, and purpose of travel (tourism, work or education, or visiting friends).
and/or relatives [VFR]). Participants were given a thermometer and were asked to take their temperature if they felt feverish. They were also asked to keep a structured travel diary, recording itinerary, use of antimalarial chemoprophylaxis, use of preventive measures against mosquito bites such as a mosquito net and/or an insect repellent containing N,N-diethyl-meta-toluamide (DEET) and/or sleeping in air conditioned rooms, signs of disease (fever), doctor visits, and [self]treatment. Participants made daily diary entries from the day they arrived at their destination to 2 weeks after their return, to encompass the incubation period of *Plasmodium falciparum* malaria and to register adherence to chemoprophylaxis after return. After travel, a nurse checked the diary in the presence of the participant. Participants were then asked if they had taken the advised chemoprophylaxis prior to travel – if applicable.

### Case Definitions

The number of days spent in malaria-endemic areas was defined as ‘exposure time’. Participants were considered ‘adherent’ to chemoprophylaxis if they took at least 75% of their recommended tablets as prescribed during their stay in high-endemic areas. Adherence before and after travel were separately described. The use of DEET, use of a mosquito net, and sleeping in an air-conditioned room were quantified in percentages by dividing the number of days the preventive measure was used by the number of days spent in a malaria-endemic area; and we dichotomized these variables by using the mean of this proportion in the total study population as the cut-off. Fever was self-reported (feeling feverish or thermometer confirmed fever with a temperature of 38°C or higher) starting 1 week after arriving in a malaria-endemic area.

### Laboratory Methods

Before departure and 2 to 6 weeks after return, participants donated venous blood samples for serology. All blood samples were immediately stored at 6°C. Blood samples for serologic testing were centrifuged (Hettich Rotixa 50 S, see APP/407: program 1, 10 min. 3000 rpm (210 g)) and frozen at −80°C within 24 hours until use. The CSP-ELISA (circumsporozoite protein enzyme-linked immunosorbent assay) was used to test for the presence of *P. falciparum* anti-circumsporozoite antibodies according to standard protocols [23]. The optical density (OD) was read at 450 nm (reference filter 620 nm). Positive-control pooled plasma samples from Tanzanian individuals and negative-control plasma samples from Dutch individuals were included in each plate. The threshold for positivity for anti-circumsporozoite antibodies was calculated as the mean OD of the 7 negative control plasma samples plus 3 standard deviations. If the (OD) in serum samples after travel tested positive and showed a ≥2-fold increase compared to the OD value pretravel, these were defined as anti-circumsporozoite antibody seroconversion. This anti-circumsporozoite antibody seroconversion was considered indicative of exposure to *P. falciparum* during present travel.

### Data Analysis

Data analysis was performed with SPSS version 19.0.0.1 (2010, IBM, Somers, USA). Attack rates (ARs) and incidence rates (IRs) were based on positive anti-circumsporozoite antibody seroconversion indicative for exposure to *P. falciparum*. ARs were calculated by dividing the number of study subjects displaying seroconversion in the CSP-ELISA by the total number of participants at risk. IRs per 100 person-months were calculated by dividing the number of travelers with exposure to *P. falciparum* by our calculation of their exposure time. If a traveler was exposed to *P. falciparum*, we used half of their travel duration in endemic area as their exposure time; for travelers without exposure to *P. falciparum*, we used their total travel duration. Pearson chi-square tests of association were used to compare categorical variables between any two groups. Independent determinants for adherence to chemoprophylaxis and for use of DEET were identified by multiple logistic regression analysis and expressed as odds ratios with 95% confidence intervals. A p-value <0.05 was considered statistically significant.

### Results

#### Study Population

A total of 945 travelers to malaria-endemic countries were recruited. Of these, 400 (42%) were male (Table 1). The majority (616, 65%) was under 45 years of age, most (783, 83%) had visited tropical or subtropical countries before, and 879 (93%) were born in a Western country; 815 (86%) traveled for holiday; 71 (8%) traveled for work or education, and 59 (6%) were VFR. More than half (529, 56%) stayed less than 14 days in malaria-endemic areas. The most frequently visited continent was Asia (454, 48%); 285 (30%) traveled to Africa and 206 (22%) to Latin America. Of the 945 travelers, 620 (66%) were advised of both chemoprophylaxis and preventive measures against mosquito bites. The other 325 participants traveled to low-endemic areas.

### Adherence to Chemoprophylaxis among Travelers to High-endemic Areas

Of the 620 travelers to high-endemic areas who were prescribed chemoprophylaxis, 449 were prescribed atovaquone-proguanil (48%), 91 proguanil (10%), 70 mefloquine (8%), and 10 (2%) other types of chemoprophylaxis, such as minocyclin, doxycycline, or a combination of chemoprophylaxis.

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**Table 3.** Adherence to the most-prescribed antimalarial chemoprophylaxis among travelers who started with recommended chemoprophylaxis.

<table>
<thead>
<tr>
<th>Advised</th>
<th>Started*</th>
<th>Prior to reaching endemic area(s)</th>
<th>While in endemic area(s)</th>
<th>After leaving endemic area(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n1</td>
<td>n1/N</td>
<td>n2</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>70</td>
<td>69</td>
<td>99%</td>
<td>63</td>
</tr>
<tr>
<td>Atovaquone-proguanil</td>
<td>449</td>
<td>396</td>
<td>88%</td>
<td>331</td>
</tr>
<tr>
<td>Proguanil</td>
<td>91</td>
<td>76</td>
<td>84%</td>
<td>NA</td>
</tr>
</tbody>
</table>

* NA, not applicable. doi:10.1371/journal.pone.0056863.t003
Of 620 travelers, 520 (84%) took at least 75% of the recommended tablets during their stay in high-endemic areas (Table 2). In multivariable analysis, travelers to Africa were significantly more adherent to chemoprophylaxis than travelers to Asia or Latin America (OR 3.5 [95% CI 1.9–6.5] and 2.7 [95% CI 1.4–5.3] respectively); travelers who spent 14–29 days in endemic areas were significantly more adherent compared to travelers who spent either ≤13 days or ≥29 days in endemic areas (OR 2.2 [95% CI 1.2–3.8] and 2.5 [95% CI 1.1–5.6] respectively); travelers who used DEET as an additional preventive measure in more than 50% of days spent in high-endemic areas were significantly more adherent compared to those who did not use DEET (OR 2.6 [95% CI 1.4–4.8]; and travelers using mefloquine were significantly more adherent compared to travelers using atovaquone-proguanil or proguanil (OR 5.3 [95% CI 1.2–23.1] and 6.0 [95% CI 1.3–27.5] respectively); Of 620 travelers, 466 (75%) took 100% of the recommended advised tablets during their stay in high-endemic areas, with the same determinants as for 75% adherence.

Table 3 shows the adherence to mefloquine, atovaquone-proguanil and proguanil prior to, during, and after stay in endemic area(s) among travelers who started with prophylaxis. The highest adherence percentage for all categories was found among travelers using mefloquine.

Antimosquito Preventive Measures

Of 945 travelers, 791 (84%) used DEET, 465 (49%) used a mosquito net, and 544 (58%) slept in an air-conditioned room at least once.

Multivariable analysis showed that travelers born in a non-Western country used significantly less DEET and also slept significantly less often under a mosquito net than travelers born in a Western country. Female travelers used significantly more DEET than male travelers. Older travelers used significantly less DEET or mosquito nets than younger travelers. Spending more days in endemic areas was an independent determinant for using less DEET and sleeping less often in an air-conditioned room. Travelers to high-endemic areas in general used a mosquito net significantly more often and slept less often in an air-conditioned room compared to travelers to low-endemic areas. Travelers to Africa or Latin America used a mosquito net significantly more often and slept less often in an air-conditioned room compared to travelers to Asia.

Anti-circumsporozoite Antibodies

Of 945 travelers, only 938 serum samples were tested using the CSP-ELISA method because the sample was insufficient to test in 7 cases. Of the 938 samples, 4 (AR 0.4% [95% CI 0.1–1.0]) showed an anti-circumsporozoite antibody seroconversion. All 4 travelers were born in the Netherlands, had been 100% adherent to their chemoprophylaxis during travel, and none reported fever or had visited a doctor (Table 4). Of the 4 travelers, 2 stayed in a high-endemic area up to 14 days and 2 between 14 and 29 days; 2 visited Africa, 1 India and 1 Suriname. The overall IR of anti-circumsporozoite antibodies was 0.8 per 100 person-months (95% CI 0.3–2.0).

Fever

Of the 945 travelers, 74 (8%) reported fever after a median period of 17 days [IQR 12–26] in endemic areas. Of these 74, 67 (91%) used the provided thermometer and recorded fever with a median temperature of 38.7°C [IQR 38.2°C–39.6°C]. Of the 74, 24 (32%) had visited low-endemic areas and 50 (68%) high-endemic areas. Of 24 travelers who reported fever in low-
endemic areas, 13 (54%) sought medical attention abroad, 2 of whom were tested negative for malaria. Of 50 travelers to high-endemic areas, 17 (34%) consulted a doctor abroad, of whom 8 were tested for malaria and 3 were actually treated for malaria. These last 3 travelers had been fully adherent to chemoprophylaxis during travel and tested negative for anti-circumsporozoite antibodies.

Discussion

In this prospective study with short-term travelers to malaria-endemic countries, self reported adherence to chemoprophylaxis was good. Best adherence was found among travelers to Africa, travelers using mefloquine as chemoprophylaxis, travelers who spent 14–29 days in endemic areas and travelers who were more adherent to use of DEET. Based on seroconversions for anti-circumsporozoite antibodies, we found an overall attack rate (AR) for \( P. falciparum \) of 0.4% and an overall incidence rate (IR) of 0.8 per 100 person-months.

Adherence to chemoprophylaxis found in our study is in agreement with self reported treatment adherence found in other studies, which ranged from 70%–89% for atovaquone-proguanil and 72%–95% for mefloquine [14,15,24,25]. Travelers to Africa were more adherent to chemoprophylaxis, which is also consistent with other studies [14,20,26]. Even though in our travel consultation we do not communicate differences in risk between different high-endemic continents, it is possible that travelers know that malaria risk in Africa is higher than in other continents and therefore are more cautious. This may also be the reason that travelers to high-endemic areas used a mosquito net more often than in other areas. Further, we found good adherence to DEET to be independently related to good adherence to chemoprophylaxis, suggesting that those travelers who were more adherent to one preventive measure were also more likely to follow other preventive measures. These results are in agreement with previous reports [27,28]. The high level of adherence to mefloquine in our study suggests that this group of travelers experienced minimal side effects, which is supported by data in the daily diaries. One could argue that travelers who did not tolerate mefloquine during previous travel due to side effects chose to use alternative chemoprophylaxis, such as atovaquone-proguanil or doxycycline. However, previous travel experience was univariably not associated with better adherence to mefloquine, atovaquone-proguanil, or doxycycline.

As in our study, previous studies found longer travel duration to be a determinant of nonadherence [24,29].

Determinants of nonadherence to chemoprophylaxis found in previous studies were visiting friends and/or relatives (VFR), younger age, extensive travel experience, adventurous travel, and (assumed) adverse reactions [20,24,26,29,30]. We did not find VFR to be less adherent than other groups of travelers. This is probably because Lobel et al and Ropers et al studied travelers flying back from destinations, whereas VFR in our study (only 7% of the total study population) were recruited in a pretravel clinic and are therefore not representative for VFR in general. The VFR in our study were probably more aware of the risk, therefore attended our pre-travel clinic and were also more likely to follow our recommended chemoprophylaxis. Indeed, imported malaria is mostly seen in VFR who did not seek pre-travel health advice and who never intended to use chemoprophylaxis, or who used chemoprophylaxis inadequately [4,31,32].

Furthermore, we did not find a relation between travel experience and adherence, but we did find less adherence to chemoprophylaxis in younger travelers, although not significantly.

Studies have shown that detecting anti-circumsporozoite antibodies can be used in nonimmune travelers using chemoprophylaxis as a measure of \( P. falciparum \) infection [3,13,17,19,33,34]. In our study population, 0.4% had a recent infection with \( P. falciparum \) sporozoites. All 4 travelers were infected in high-endemic areas, none reported fever or malaria treatment, and all had been adherent to chemoprophylaxis. This suggests that the use of chemoprophylaxis protected these 4 travelers from clinical malaria. There are a few other prospective studies on malaria infection using anti-circumsporozoite antibody tests in nonimmune travelers using chemoprophylaxis [13,17,19]. The AR of 0.4% we found was lower than the AR found in 1991–1992 by Cobelens et al [1.3%), but their IR of 1.7 per 100 person-months is comparable to our IR (IR 0.8; 95% CI 0.3–2.0). Nothdurft et al published in 1999 an AR of 4.96% and Knappik et al found in 1999 an AR of 0.95%. Differences in ARs could be due to changes in incidences [8,35,36], but also to differences in group characteristics, countries visited, or the use of different ELISA tests. Therefore the studies cannot be compared.

Our study is one of the few comprehensive studies that combined \( P. falciparum \) anti-circumsporozoite seroconversion, adherence to chemoprophylaxis, and collection of clinical malaria data among travelers. The prospective nature of this study with blood samples pre- and post-travel allowed estimating the AR and IR of exposure to \( P. falciparum \) malaria. The daily diary entries, which minimized recall bias, provided a good record of the use of chemoprophylaxis and antimosquito preventive measures, fever, and treatment for malaria during travel.

Interpretation of our study results may be influenced by a number of shortcomings. First, the daily diary entries could have served as a reminder for travelers to take their chemoprophylaxis and therefore lead to better adherence to treatment. Second, because of the study design, the end date for travelers to fill in their diaries was 2 weeks after return, so we do not know if travelers using mefloquine or proguanil continued chemoprophylaxis after the 2 weeks. Treatment adherence during stay in endemic areas, however, remains most important in protection against infection with \( P. falciparum \). Finally, measurement of malaria exposure by anti-CSP seroconversion has limitations due to the methodology. The sensitivity is limited due to small numbers of inoculated sporozoites by infected mosquitoes and their short life span [37]. Development of a detectable anti-CSP response is dose related and requires multiple inoculations [34,30]. Preliminary data from controlled human malaria infections in malaria naive Dutch volunteers [39] show that anti-CSP seroconversion occurs in 30% of the volunteers after a single infection with 5 infected mosquitoes (Sauerwein, unpublished). Therefore, the actual number of travelers exposed to sporozoites may be higher for all endemic areas, and our AR and IR likely represent underestimations. The 3 seronegative travelers treated for malaria may have been exposed and infected.

In conclusion, travel to Africa, using mefloquine, travel duration of 14–29 days in endemic areas, and good adherence to DEET were associated with good adherence to chemoprophylaxis. Adherence to chemoprophylaxis in combination with other preventive measures was good enough to protect the travelers who seroconverted from clinical malaria. None of the travelers to low-endemic areas where chemoprophylaxis is not recommended contracted malaria, so there is no reason to adapt the Dutch national guidelines. Similar prospective studies with larger numbers to specific destinations are needed to make more specific destination-dependent advice about the use of chemoprophylaxis.
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Author Contributions

Commented on the manuscript: WR RS. Conceived and designed the experiments: AvdH GS. Analyzed the data: SB. Contributed reagents/materials/analysis tools: WR RS. Wrote the paper: SB AH GS.

References