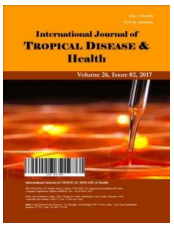

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Prevalence of *P. falciparum* Gametocyte Carrying between Two Sympatric Ethnic Groups Living in Seasonal Malaria Transmission Setting of Burkina Faso after Universal Bed Nets Coverage Campaigns

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SSS, TA, TY, SI, MD and SBS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HBN, SS, DA, KD, SSB managed the analyses of the study. Authors HBN, CBE, MV and ONI managed the literature searches. All authors read and approved the final version.

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ABSTRACT

Aims: This study aimed to compare the prevalence of *P. falciparum* gametocyte carriage in two sympatric ethnic groups living in seasonal malaria transmission setting in Burkina Faso.
Study Design: A cross-sectional survey was conducted from September to November 2017 in children aged from 2 to 12 years and living in Barkoundouba, avillage located at the Northeast part of Ouagadougou, capital city of Burkina Faso. The study participants were subject to clinical

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examination including axillary temperature. Blood samples were collected from finger pricks to performed RDT and blood smears for malaria diagnosis and on filter paper for molecular detection of the parasite. Any case of fever (temperature ≥ 37.5°C) with RDT positive was treated according to national guideline.

Methodology: We included 461 patients in this study. *P. falciparum* presence and densities were determined by microscopy using Giemsa-stained thick blood smears. The nested PCR was used to confirm the presence of the asexual parasites assessed by the microscopy.

Results: *P. falciparum* prevalence assessed by microscopy was 83 (32.55%) and 103 (50%) for Fulani and Mossi respectively, whereas the prevalence by nested PCR was 88 (39.11%) for Fulani and 121 (68.75%) for Mossi. The gametocyte carriage in the two ethnic groups was: 3.53% for Fulani and 11.65% for Mossi. The prevalence ratio for *P. falciparum* asymptomatic and gametocyte carriers was 1.5 and 3 in favor of Mossi group respectively.

Conclusion: This study showed that the Fulani have a lower prevalence of *P. falciparum* compared to the Mossi group despite the decrease of parasitemia and prevalence in both groups compared to previous studies.

Keywords: Malaria transmission; P. falciparum; gametocyte; Burkina Faso.

1. INTRODUCTION

Despite the recent effort, malaria still remains one of the most serious global public health problem, with a major presence throughout Asia, Africa, and South America, placing 3.2 billion people of the world's population at risk [1,2]. The number of malaria infections stands at 216 million cases increasing by 5 million from 2015 to 2016 indicating that the efforts directed towards eradicating the disease are coming to a halt [2].

In 2017, an estimate of 219 million cases of malaria occurred worldwide [3]. Children aged under 5 years are the most vulnerable group affected by malaria accounting for approximately 61% of all worldwide malaria deaths.

Considerable progress has been made in the last decade in reducing the burden of malaria by wide-scale deployment of insecticide-treated nets as well as efficacious artemisinin based combination therapy (ACT) as first-line antimalarial treatment [4]. Unfortunately, malaria transmission led by gametocytes still remains an important component of the disease control that needs to be well understood for appropriate actions [5].

Gametocytes, the precursors of male and female gametes, of malaria parasites are formed in the human host through the developmental switch from asexual replication in erythrocytes. Although, gametocytes are not responsible for clinical symptoms, they ensure the transmission of malaria from vectors to another new host. A large fraction of gametocyte-positive individuals living in malaria endemic settings are asymptomatic representing a considerable reservoir contributing to onward malaria transmission [6].

Previous studies have presented host genetic factors as factors that may play a role in this difference of susceptibility to malaria infection [7,8]. Indeed, in Burkina Faso, previous studies on different ethnic groups (Mossi, Rimaïbé, Fulani) indicated that the Fulani have lower parasite density (asexual stages and gametocytes), lower malaria incidence, and higher levels of anti-malaria humoral immune responses to a variety of malaria antigens [9, 10]despite the same level of transmission, based on entomologic data collected at the time of these studies [11]. Similar results have been reported between Fulani and Dogon in Mali [12]. But the relationship between *P. falciparum* gametocyte carriage and ethnicity and their relative contribution to malaria transmission remains less understood. In addition, it is not clearly known whether the temporal variation represents a factor that could play a role in the differential malaria susceptibility between the Mossi and the Fulani group.

The aim of our study is to assess the prevalence of *Plasmodium falciparum* gametocyte carriage in two sympatric ethnic groups (Fulani and Mossi) in order to compare the relative contribution in malaria transmission.

2. MATERIALS AND METHODS

2.1 Ethics Statement

The study received approval (deliberation N°2017-000005/MS/SG/CNRFP/CIB) from the

Institution Ethical Committee of the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) and the Ethical Committee in Health Research of the Ministry of Health of Burkina Faso (deliberation N°2017-6-074).

2.2 Study Area and Populations

The study was carried out in Burkina Faso in Barkoundouba village organized in two separate quarters where the two ethnic groups are living separately; Barkoundouba Fulani where only the Fulani live and Barkoundouba Mossi with only the Mossi ethnic group. The two quarters are 3 km apart and situated at northeast at 35 km of the capital city Ouagadougou (Fig. 1). In this locality, Fulani and Mossi live together in sympatry. The parents or legally acceptable representative provided informed consent prior to any study specific procedure. Participants aged more than 7 years were provided with an additional assent at enrollment. In Burkina Faso, malaria transmission is hyper-endemic during the rainy season, which begins from June and lasts up to October. The annual entomological inoculation rate range from 10 to 500 infective bites per individual. *P. falciparum* is responsible for over 90% of malaria infections [13].

A total of 255 asymptomatic Fulani and 206 Mossi children aged between 2 and 12 years of age were enrolled in this study during cross sectional surveys.

2.3 Study Design and Epidemiological Surveys

A cross-sectional survey was conducted from September to November 2017. The study participants were examined by a physician for clinical signs and axillary temperature measurement. Blood samples were collected from finger pricks to perform RDT, blood smears for malaria diagnosis and filter paper for molecular detection. Any case detected with fever (temperature ≥ 37.5°C) associated with a positive RDT was treated according to national malaria treatment guideline. In parallel to the clinical data collection, data on the use of the bed nets and the door/window curtain were also collected.

2.4 Temperature Evaluation and the Use of Rapid Diagnosis Test (RDT)

Temperatures were taken using an electronic thermometer placed under the armpit. Hearing a signal from the thermometer indicates the end of the temperature reading. Fever was defined as an axillary temperature greater than or equal to 37.5ºC. In case of fever, the RDT was done and for any positive RDT, the patient was treated according to the national malaria treatment guideline.

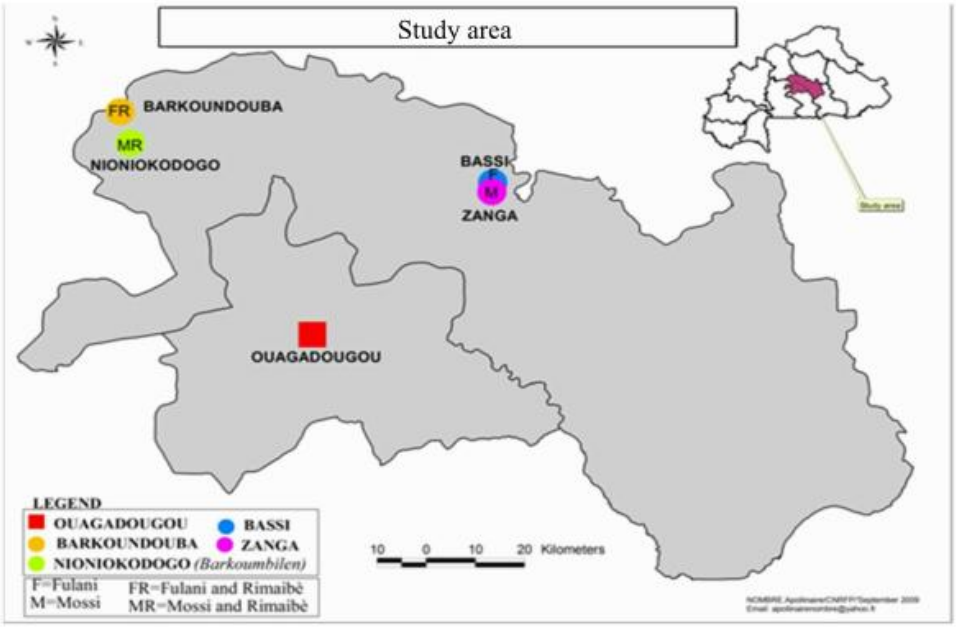

Fig. 1. Study area in Burkina Faso

2.5 Hematological Parameters

The hemoglobin level was determined by the spectrophotometric method using the portable battery-powered HemoCueHb201*whose principle is as follows: the reaction in the microcuvette is a modified azidemethemoglobin reaction. The erythrocyte membranes are disintegrated by sodium deoxycholate, releasing the hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with azide to form azidemethemoglobin. The absorption was measured at wavelengths of 750 and 880 nm.

2.6 Malaria Diagnosis by Microscopy

The thick and thin smear collected were air dried and stained with 5% Giemsa for 35 minutes. One hundred high power fields per thick smear were examined for malaria parasites by two skilled microscopy specialists independently and the mean density was considered. A third reading was requested in case of discrepancy between the 2 readers (qualitatively or when the difference between the two first readers exceeded 30%). In this case, the mean of the two closest densities was used as final result. Trophozoïte and gametocyte densities were assessed by counting against respectively 200 leukocytes and 1000 leukocytes of blood and converted into counts per microliter by assuming a standard count of 8000 leukocytes/µl blood. A slide was considered negative if no asexual parasite stages were found after examination of 100 power fields.

2.7 Asexual Parasite Detection by PCR

In the previous studies carried out in the same population, malaria was diagnosed using microscopy detection of the parasite which has a limit sensitivity compared to other DNA amplification technique. In order to check for a very low sub-microscopy parasitemia, a nested polymerase chain reaction (PCR) analysis of the asexual forms was done on the collected samples in the present study. Briefly, DNA was extracted from filter paper by QIAGEN kit according to manufacturer's protocol [14]. Plasmodium species were then determined by nested PCR methods as previously described [15], with minor modifications. Briefly, Plasmodium genus were detected using the outer genus-specific primers (rPLU 5 & 6) targeting sequences of their small subunit ribosomal (ssrRNA) genes. The initial outer reaction contained 4 mM of MgCl2, 200 µM DNTPs, 0.0625 µM of each primer and one unit of Taq DNA polymerase (Sigma-Aldrich, USA). The PCR cycling conditions for the primary reaction were an initial denaturation at 94°C for 5 min, denaturation for 1 min at 94°C and annealing at 58°C for 2 min (all for 30 cycles). Extension was at 72°C for 2 min, a final annealing at 58°C for 2 min and final extension at 72°C for 10 min. The inner PCR reaction was used for the detection of all Plasmodium species as described in [16]. The cycling conditions and number of PCR cycles were similar as above except for annealing condition which was set at 55°C for 2 min. All PCR reactions were performed using a GeneAmp PCR System 2700 (Applied Biosystems Incorporated, US).

Due to the storage condition of the filter paper, the extracted DNA from the 30 first participants of each ethnic group was not amplifiable. The PCR analysis was then carried out on 225 and 176 subjects from Fulani and Mossi group, respectively.

2.8 Calculating Prevalence Ratio (PR) and Prevalence Odd Ratio (POR)

The odds ratio (OR) (also called close relative risk) is a statistical measure, often used in epidemiology, expressing the degree of dependence between qualitative random variables. It is used in Bayesian inference and logistic regression, and allows the effect of a factor to be measured.

In the present study it is defined as the ratio of the odds of *P. falciparum* malaria infection occurring to a Mossi and Fulani Ethnic group of individuals.

As this study is designed as a cross-sectional study, the OR is referred to as the Prevalence OR (POR) and the Prevalence Ratio (PR) is calculated with the following interpretation.
If the odds ratio is:

- close to 1, the *P. falciparum* malaria infection is independent of ethnic the group;
- greater than 1, the *P. falciparum* malaria infection is more frequent in Mossi group than in Fulani ethnic group;
- less than 1, the disease is less frequent in Mossi group than in Fulani ethnic group;

- close to zero, the disease is much less frequent in Mossi group than in Fulani ethnic group.

2.9 Data Analysis

Data were double entered and were compared for typing errors. Statistical analyses were carried out using STATA (Version 13.0) according to an a priori Statistical Analysis Plan [17,18]. Briefly, we determined prevalence and densities of sexual and asexual parasites at enrolment in the two ethnics groups. The calculation of odds ratio and prevalence odds ratio permitted us to evaluate the degree of dependence of *P. falciparum* malaria infection in the different ethnic groups. The difference in proportions and frequencies were compared using Pearson's Chi-squared test. The Student t-test was used for comparison of means. When the theoretical number was less than 5 for comparison of proportions, the Fischer exact test was used. The tests were considered significant at $p < .05$.

In addition to the demographic, biology and clinical data, we also assessed from in the study participants, the use of impregnated nets (bed nets, doors and or windows curtains) as factors that can influence the mosquito bites and consequently malaria transmission.

3. RESULTS

3.1 Demographic Characteristics of Study Participants

A total of 461 subjects were enrolled: 255 (55.31 %) were from Fulani ethnic group with 116 (45.49%) female and 139 (54.51 %) male; 206 (44.69%) were from Mossi ethnic group with 100 (48.54%) female and 106 (51.46 %) male. The mean age (years) between Fulani (7.61 ± 3.27) and Mossi (7.79 ± 3.26) ethnic group was not statically different ($p > 0.05$) (Table 1) The means temperature and hemoglobin rate estimated at 36.7°C ± 0.55 and 10.32 g/dl ± 1.61;36.8°C ± 0.43 and 11.24°C ± 1.42 in Fulani and in Mossi ethnic group, respectively were statistically comparable ($p > 0.05$) (Table1).

3.2 *P. falciparum* Prevalence Assessed by Microscopy and PCR

The prevalence of *P. falciparum* asexual forms (trophozoites) carriage assessed by microscopy, was significantly lower ($p < 0.001$) in Fulani group (32.55 %) compared to Mossi group (50 %). The same trends were observed in the gametocyte carriage assessed by microscopy with the prevalence being statistically lower ($p < 0.001$) in Fulani (3.53 %) compared to Mossi (11.65 %) ethnic group (Table 2). However, the geometric mean of asexual and sexual parasite density was significantly higher among the Fulani than the Mossi ($p<.05$) ethnic group (Table 2).

The presence of gametocytes and trophozoites of *P. falciparum* (Fig. 2) was observed in 33 and 186 patients respectively in both ethnic group during the study. While the *P. falciparum* trophozoite carriers were comparable between the two ethnic groups (Fulani 55 (49.11 %), and Mossi 57 (50.89%) in September; the gametocytes carriage showed a prevalence in both November and October in Mossi compared to Fulani ethnic group [5 (27.78%) in Fulani and 13 (72.22%) Mossi in September; 2 (16.67%) Fulani and 10 (83.33%) Mossi in October].

The PCR amplification of *P. falciparum* asexual forms showed a prevalence of 39.11 % (88/225) and 68.75 % (121/176) in Fulani and Mossi group, respectively. Even though, the PCR prevalence is higher compared to microscopy results in both ethnic group, the difference of parasite carriage remains statistically higher in Mossi than Fulani group ($p < 0.001$) (Table 2).

3.3 Calculating Prevalence Ratio (PR) and Prevalence Odd Ratio (POR)

The prevalence of *P. falciparum* trophozoites in Fulani and Mossi group were respectively 0.33 and 0.5. The odds ratio calculation showed OR of 1.5 in favor of Mossi group indicating that the Mossi ethnic group is 1.5 time likely to carry *P. falciparum* trophozoites. In the same time, gametocytes prevalences were 0.04 in Fulani and 0.12 in Mossi group with 3 as odds ratio in Mossi favor meaning Mossi ethnic group is 3 times likely to carry gametocytes.

3.4 *P. falciparum* Gametocyte and Trophozoïte Carriers According to the Study Period (months)

The presence of trophozoïtes and gametocytes of *P. falciparum* was assessed in 33 and 186 participants respectively in both group during the study (Fig. 2). The prevalences of trophozoïtes and gametocytes carriers in Mossi (50.89% and 72.22% respectively) were statistically higher compared to the fulani group (34.92% and 27.78%) respectively in October.

Table 1. Demographic characteristics of Fulani and Mossi ethnic group

	Fulani	Mossi	p-value
Number of children examined (N=461)	255 (55.31 %)	206 (44.69 %)	NS
Use ofbednet (%)	100	100	NS
Use of door/window curtain (%)	< 1	12.62	p < 0.001
Gender			
Female	116 (45.49 %)	100 (48.54 %)	NS
Male	139 (54.51 %)	106 (51.46 %)	NS
Any age (mean year)	7.62 ± 3.26	7.78 ± 3.27	NS
[2-6[	3.83 ±1.16	3.88 ±1.15	NS
[6-12]	9.55 ±2.08	9.75 ±1.60	NS
Temperature (°C) ± SD	36.7 ± 0.55	36.8 ± 0.43	NS
Hb mean rate (g/dl) ± SD	10.32 ± 1.61	11.24 ± 1.42	NS

Table 2. Prevalence of *P. falciparum* asexual (trophozoites) and sexual (gametocytes) forms

	Fulani	Mossi	p-value
Asexual (N/%)	83 (32.55)	103 (50.0)	< 0.001
Sexual (N/%)	9 (03.53)	24 (11.65)	0.001
Geometric mean parasite density excluding zeroes by age group			
***P.falciparum* asexual parasite (Mean-[95%])**			
Any age	1645 [1152-2349]	1321 [939-1859]	0.004
[2-6[	3465 [1407-8533]	1558 [477-5093]	0.003
[6-12]	1358 [926-1990]	1279 [902-1814]	0.01
***P.falciparum* gametocytes (Mean-[95%])**			
Any age	32 [14-71]	29 [18-47]	NS
[2-6[	30 [0.3-2819]	55 [9-326]	NS
[6-12]	33 [17-63]	26 [16-43]	NS
Prevalence by Nested-PCR (%)	39.11 (88/225)	68.75 (121/176)	< 0.0001

NS= Not Significant; PCR= Polymerase Chain Reaction; N= Number

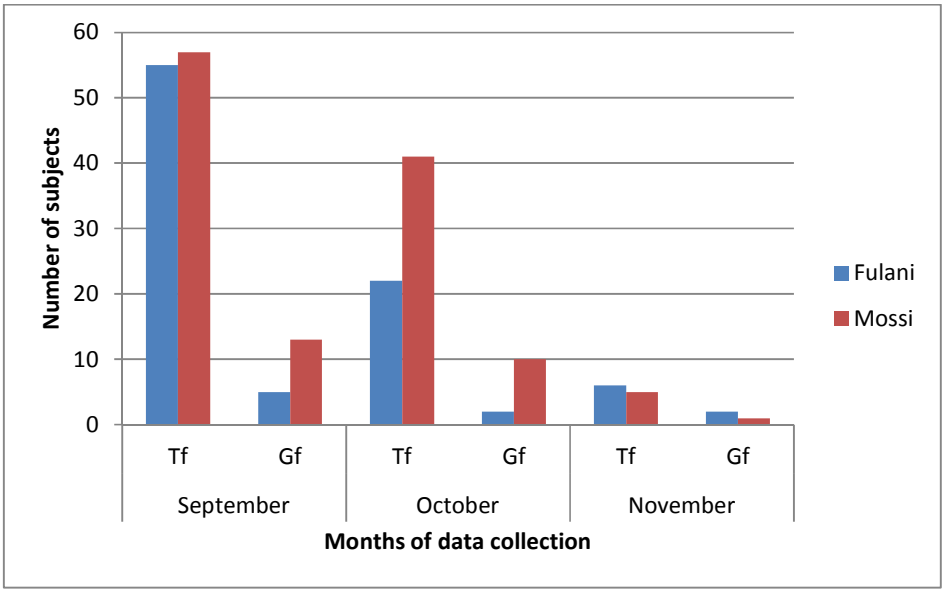

Fig. 2. Variation of *P. falciparum* (trophozoïte and gametocyte) prevalence according to the month of data collection

Tf= Trophozoïte of P. falciparum; Gf= Gametocyte of P. falciparum

4. DISCUSSION

The prevalence of *P. falciparum* carriage assessed by microscopy in our study revealed a decrease in both of the Fulani (32%) and Mossi (50%) ethnic group compared to the previous data [19]. Indeed the prevalence assessed by microscopy in the previous study and carried out in the same locality was estimated at 60% and 85% in Fulani and Mossi group respectively [19]. This reduction of *P. falciparum* prevalence could mostly be explained by the increased of community-level bednet coverage which appears to provide additional protection against malaria transmission. However, the prompt management of malaria cases at the community level with ACT provided by the NMCP could also be an additional explanation to the reduction of *P. falciparum* carriage. However, even the Seasonal Malaria Chemoprevention (SMC) implementation can indirectly affected the parasite prevalence, the SMC was not targeting our study age group.

It is well known that the gametocyte stage of *P. falciparum* is essential for malaria transmission. This is well characterized from previous studies which showed that despite a low proportion, gametocytes carriage may lead to effective transmission of malaria [20,21].

In addition, a number of factors were known to influence the gametocyte carriage, such as the parasite load or the simultaneous presence of multiple parasite clones in a single infected individual among others [22,23] as well as the immunological response. The cellular and humoral immune responses were not assessed during our study. However, the lower prevalence and lower parasite density observed in the Fulani group compared to the Mossi could be due to the stronger cellular and humoral responses observed in the Mossi group as described in previous studies carried out in Burkina Faso [24,25].

In our study, we conducted a three-month cross-sectional survey (September to November). Parasitological results revealed that the prevalence of asymptomatic subjects with *P. falciparum* decreased from September to November. The same was true for gametocyte carrying, regardless of ethnic group (Fig. 2). A high prevalence was observed at the beginning of the study (September), and statistically significant decrease was observed in October ($p< 0.002$). This could be due to the scarcity of rainfall during these periods and which practically announces the end of the rainy seasons.

In our study, we observed that Mossi group was 1.5 times likely of being carriers of *P. falciparum* and 3 times the risk of being gametocyte carriers, then 3 time more to ensure malaria transmission compared to Fulani ethnic group. The current study has confirmed the earlier findings in other communities in Burkina Faso that the Fulani have less patent *P. falciparum* infections of malaria than the Mossi [26-28]. One other study conducted in Mali has reported similar results between the Fulani and Dogon ethnic group [12].

However, our study showed that the Fulani group presented higher asexual parasites densities compared to the Mossi group in any age (Table 2). These results disagree the earlier results from the previous studies [11,19]. Indeed, the Fulani people are traditionally nomadic pastoral people with probably more exposition to mosquito. Several studies have demonstrated that the Fulani are less parasitized, have a higher incidence of spleen enlargement [11,19,29] and are less affected by malaria sickness, despite the fact that people in these areas are exposed to malaria at the same level. This high parasitemia density in the Fulani could be due to the lack of usage of door and window curtains. Besides, less than 1% of Fulani used door curtains or windows (Table 1).

5. CONCLUSION

Our study showed that the Fulani have a lower prevalence of *P. falciparum* compared to the Mossi while they presented some significantly higher parasites densities. The Mossi ethnic was more likely to be infected by *P. falciparum* and more probably competent to ensure malaria transmission as they are three (3) time gametocytes carriers compared to the Fulani ethnic group. Further investigations to explore whether the difference in gametocyte carrying observed in the two groups has an impact on mosquito infectivity and subsequently on malaria transmission.

CONSENT

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

The study received approval (deliberation N°2017-000005/MS/SG/CNRFP/CIB) from the Institution Ethical Committee of the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) and the Ethical Committee in Health Research of the Ministry of Health of Burkina Faso (deliberation N°2017-6-074).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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