Nosocomial malaria transmissions resolved by genomic analyses – a retrospective case report study in France (2007-2021)

Romain Coppée[1#], Véronique Sarrasin[2,3], Rizwana Zaffaroulah[2], Azza Bouzayene[2], Marc Thellier[4], Harold Noël[5], Jérôme Clain[2,3], Sandrine Houzé[2,3] and the Investigation Study Group*

[1] Université Paris Cité and Sorbonne Paris Nord, Inserm, IAME, F-75018 Paris, France.

[2] Centre National de Référence du Paludisme, AP-HP, Hôpital Bichat-Claude Bernard, F-75018 Paris, France.

[3] Université Paris Cité, IRD, MERIT, F-75006 Paris, France.

[4] Centre National de Référence du Paludisme, AP-HP, GHU Pitié-Salpêtrière, F-75013 Paris, France.

[5] Infectious diseases division, Santé Publique France, F-91410 Saint Maurice, France.

* The Investigation study group members are listed in the Acknowledgments.

[#] Corresponding author: Romain Coppée (romain.coppee@gmail.com), F-75018 Paris, France

Running title: Genomic analyses of nosocomial malaria

Summary: From 2007 to 2021 in France, we report six nosocomial malaria transmissions (four occurred during the COVID-19 pandemic) that were confirmed by epidemiological and clinical investigations, and parasite whole-genome and phylogenomic analyses based on single-nucleotide polymorphisms and short tandem repeats.

Abstract

Background: Exposure of blood to malaria parasites can lead to infection even in the absence of the anopheles mosquito vector. During a stay in a healthcare facility, accidental inoculation of the skin with blood from a malaria patient might occur, referred to as nosocomial malaria transmission.

Methods: Between 2007 and 2021, we identified six autochthonous malaria cases that occurred in six different French hospitals, originating from nosocomial transmission and imported malaria patients being the infection source. Four cases were observed during the COVID-19 pandemic. The genetic relatedness between source and nosocomial infections was evaluated by genome-wide short tandem repeats (STRs) and single-nucleotide polymorphisms (SNPs).

Results: None of the patients with autochthonous malaria had travel history to an endemic area nor had been transfused. For each case, both the source and recipient patients stayed a few hours in the same ward. After diagnosis, autochthonous cases were treated with antimalarials and all recovered except one. Genetically, each pair of matched source/nosocomial parasite infections showed less than 1% of different genome-wide STRs, and less than 6.9% (<1.5% for monoclonal infections) of different SNPs. Similar levels of genetic differences were obtained for parasite DNA samples that were independently sequenced twice as references of identical infections. Parasite phylogenomic results were consistent with travel information reported by the source patients.

Conclusions: Our study demonstrates that genomics analyses may resolve nosocomial malaria transmissions, despite the uncertainty regarding the modes of contamination. Nosocomial transmission of potentially life-threatening parasites should be taken into consideration in settings or occasions where compliance with universal precautions is not rigorous.

Keywords: malaria, nosocomial, autochthonous, short tandem repeat, single-nucleotide polymorphism, next-generation sequencing

Background

Although the incidence of malaria cases has decreased worldwide since 2010, the decline has stagnated and even reversed in some regions since 2014 [1]. The ongoing COVID-19 pandemic has contributed largely to a sharp increase in malaria-related deaths in 2020 (+12.5% compared to 2019) according to the latest malaria report of the World Health Organization [1]. Also, the increase in travel to malaria-endemic settings in recent decades means that imported malaria cases are becoming more common, as is the case in metropolitan France with approximately 5 570 cases in 2019 [2]. Most of these imported cases are acquired through anopheles mosquito bites during travel to malaria-endemic areas [2]. Rare cases of autochthonous malaria, defined as an individual with biologically-confirmed malaria that did not travel to an endemic area in the twelve months prior to clinical onset, have also been notified in recent years [2]. Autochthonous cases can be airport or luggage malaria [3], occurring when a *Plasmodium*-infected mosquito is imported from an endemic area in the hold of an aircraft, which should normally be disinsected before landing in a malaria-free region according to international sanitary regulation.

However, malaria transmission is not exclusive to the bite of *Plasmodium*-infected anopheles mosquitoes. In the absence of a compatible travel history, other modes of infection may be suspected including congenital malaria, blood transfusions, organ transplants and punctures [4–6]. These uncommon modes of malaria transmission can delay diagnosis and treatment due to the unexpected nature of the event, increasing the risk of developing the severe form of the disease.

Here, we clinically characterized six cases of nosocomial malaria, defined as patients who were infected by the pathogen during a stay in a healthcare facility, in France since 2007. These cases occurred in six different hospitals located in different cities (near Paris and its close provinces). Four of them were observed during the ongoing COVID-19 pandemic. Clinical investigations and parasite whole-genome and phylogenomic analyses based on single-nucleotide polymorphisms (SNPs) and short tandem

repeats (STRs) were conducted to prove the extreme genetic relatedness between the source and the nosocomial infections.

Methods

Sample collection

In France "autochthonous" or locally-acquired cases of malaria, defined as the presence of *Plasmodium* parasites on a blood smear with no reported travel to a malaria-endemic area during the twelve months preceding symptom onset, are notifiable. Physicians and microbiologists may report without delay any case fulfilling this sensitive case definition to the health agency of the residence region of the case, which prompts an epidemiological and clinical investigation to determine the origin of the case and prevent further transmission. The French National Malaria Reference Center received blood samples of patients with *Plasmodium* imported and autochthonous malaria from a network of French hospitals. Epidemiological data, clinical information and history of travel were systematically collected. Blood samples and DNA were banked until investigations.

Total DNA extraction

Total DNA was extracted from whole blood using the MagPurix® Blood DNA Extraction Kit 200 (Biosynex, France), then eluted using the elution buffer according to the manufacturer's recommendations. DNA extracts were quantified using the Qubit® dsDNA high sensitivity kit (Thermo Fisher Scientific, Waltham, USA).

STR genotyping by PCR

Five nuclear STR loci dispersed on five different *Plasmodium falciparum* chromosomes were genotyped by capillary gel electrophoresis [7]. Amplification of those five fragments was done by simplex or

duplex PCR. PCR reaction mixtures, cycling conditions and STR genotyping are described in **Supplementary Method S1**.

Whole-genome sequencing of parasite DNA

The selective whole-genome amplification strategy was used to enrich *P. falciparum* DNA from total DNA extracts prior to whole-genome sequencing (WGS) [8]. *P. falciparum* DNA-enriched samples were then used for library preparation then sequenced at 150 bp paired-end on a NextSeq Illumina system. The whole procedure is described in **Supplementary Method S2**.

Genome-wide SNP genotyping from genome sequences

Prior to any analysis, raw sequence data were subjected to standard Illumina QC procedures. Raw fastq files were aligned to the *P. falciparum* 3D7 reference genome (PlasmoDB release 39) using the bwa-mem algorithm from BWA (Burrows-Wheeler Aligner) [9]. Aligned reads were then sorted using SAMtools (http://samtools.sourceforge.net/) and indexed. Variant calling was performed independently for each sample, then merged together and filtered to retain only high-quality SNPs. The pipeline is fully described in **Supplementary Method S3**.

Genome-wide STR genotyping from genome sequences

We took advantage of WGS to analyze thousands of STRs. Genome-wide STR genotyping was performed with HipSTR (Version 0.6.2) using the haploid version (default parameters) [10]. We focused on the 6 768 STRs defined as high-quality loci selected by a multivariable logistic regression model from Han *et al.* [11]. Only samples with sufficient coverage depth were included.

Measure of parasite genetic distance between two infections

A source infection and a nosocomial infection from two patients who were in the same ward at the same time, and with identical parasite STRs genotyped by PCR, were considered as a matched infection pair. Other types of infection pairs were considered as unmatched infection pairs. As a

reference measure of high genetic relatedness, the nosocomial infection from case 6 was sequenced twice from two blood samples independently collected six hours apart (named 6h control pair); and three samples were independently sequenced twice (called resequencing control pairs). These control comparisons were grouped together as matched control pairs. Other comparisons between the controls were considered as unmatched control pairs.

For each sample, we first counted the number of SNPs that differed from the *P. falciparum* 3D7 reference genome. Then for each pair of samples, we retained only SNPs with no missing genotype in both samples as the number of SNPs investigated. Before counting the number of different SNPs within a pair, the genotypes were updated based on allele frequency: for a given genomic position, we considered the genotype identical between the two samples if the difference in major allele frequency was ≤30%. We then calculated the ratio of different SNPs among the total number of SNPs investigated within a pair.

The same procedure was applied for genome-wide STRs.

Characterization of within-host diversity

We applied the F_{ws} metric to determine whether each sample was monoclonal or polyclonal. Samples with F_{ws} <0.95 were considered polyclonal [12]. The F_{ws} statistic was calculated using the moimix (version 0.0.2.9001) R package [13].

Phylogenetic tree

To confirm the geographical origins of the parasites from the source and nosocomial infections, their genomic diversity was compared to those from worldwide infections (West, Central and East Africa, and Southeast Asia) that were previously sequenced [14]. We included 125 genomes sequenced by the MalariaGEN consortium (**Supplementary Table S1**). The final dataset contained 32 985 high-quality SNPs distributed across the 14 chromosomes of the parasites. A SNP-based phylogenetic tree was

produced by neighbor-joining with 100 bootstrap replicates using the *aboot* function from Poppr R package (version 2.7) [15], and plotted using the iTOL server [16].

Statistical analyses

Comparisons between matched and unmatched groups, based on genome-wide STRs or SNPs, were done using the non-parametric Mann-Whitney *U* test. Statistical analyses were performed using R statistical software (version 3.6.2).

Results

Description of nosocomial malaria cases

The cases occurred in six different general or university hospitals located in six cities (near Paris and its close provinces). For each autochthonous case, we identified an imported malaria case, defined as the infection source, admitted in the same ward at the same time. Medical staff or nurses who cared for the source malaria patients or autochthonous cases were almost identical. No medical act reported in the medical record was found to potentially explain the contamination between the patients.

Case 1. In October 2007, a 9-week-old child girl was admitted to the emergency ward in a tertiary-care pediatric hospital (hospital 1) for meningeal syndrome (fever and vomiting). She stayed seven hours in a single room and was then transferred to another hospital. Three hours after her admission in hospital 1, another child was admitted in the same care unit, to a single room located two rooms away, for a *P. falciparum* infection after returning from Cameroon, Central Africa. Eighteen days later, the girl was again admitted to emergency with fever (39°C), shock and severe anemia. A diagnostic of *P. falciparum* malaria was confirmed with an estimated parasitaemia at 4.5%.

Case 2. In mid-November 2019, a 75-year-old woman was admitted to the emergency ward in a general hospital (hospital 2) in altered general condition (anorexia, asthenia and cachexia). The patient was frail and homebound. After a few hours in the emergency ward, she was hospitalized for six days

in the internal medicine ward. Two days after her return home, she came back to the emergency ward because of a fever. Ten days after the first hospitalization, a diagnostic of *P. falciparum* malaria was performed incidentally by peripheral blood smear. Parasitaemia was estimated to be 16%. After an investigation tracing the patient's journey in the emergency ward, two patients have been admitted for imported malaria in the same care unit a few hours earlier before the woman's first admission. The patients were not related, and visited Gambia and Ivory Coast (West Africa) separately without having used prophylaxis. The patient from Ivory Coast later found to be at the origin of the contamination and the autochthonous case were admitted in two rooms side by side.

Case 3. In early June 2021, a 70-year-old man was admitted to the emergency ward for decompensated heart failure in a general hospital (hospital 3). After seven hours in the emergency ward, he was hospitalized in the cardiology ward for nine days. Upon returning home, the patient was bedridden and, eight days later, he was again admitted in the intensive care unit of the same hospital. He was declared positive to malaria with an estimated parasitaemia at 2.8%. An investigation found that eight hours before the man was admitted firstly to the emergency ward, another patient positive to *P. falciparum* malaria was hospitalized in the same emergency ward, after returning from Cameroon, Central Africa. This malaria patient was sent home for his treatment one hour after the admission of the old man in the emergency ward.

Case 4. On early July 2021, a 2-year-old gril was admitted to the emergency ward for acute gastroenteritis in the general hospital 4. She stayed four hours in this ward and then she was admitted in the pediatric ward for 24 hours. During her stay in the emergency ward, a boy with imported malaria from Cameroon was admitted in the same ward three hours before her admission, then he was transferred to a university hospital six hours after his arrival. After two weeks, the girl was admitted to the emergency ward of another university hospital (hospital 4 bis) with periodic fever, fatigue and shivers. Clinical symptoms led hospital staff to test for the possibility of malaria. The child was declared positive to the disease with a parasitaemia of 7.4%.

Case 5. In mid-September 2021, a 50-year-old woman was admitted in an intensive care unit of a university hospital (hospital 5). She exhibited fever, cough and fatigue. A SARS-CoV-2 PCR test confirmed that the woman was infected by the SARS-CoV-2 virus. Twenty days later, she was admitted again in the intensive care unit with no relation to a SARS-CoV-2 infection as evidenced by both antigenic and PCR tests. The woman was tested positive for severe malaria, with a *P. falciparum* parasitaemia reaching 7.5%. Another patient diagnosed with severe malaria was admitted in the same intensive care unit at the same time as the woman's first admission. The imported malaria case returned from Niger, West Africa.

Case 6. In the first week of September 2021, a 72-year-old man was admitted in the emergency ward of a clinic (hospital 6) for a few hours with symptoms of lung infection and returned home with a medical treatment. Sixteen days later, he was admitted in another emergency ward of a university hospital (hospital 6 bis) for fever and nausea. The man was tested positive for *P. falciparum* malaria infection with a parasitaemia reaching 1.3%. During his stay at the clinic, a woman was diagnosed *P. falciparum* malaria positive (parasitaemia 5.7%) in the same emergency ward. The imported malaria case returned from Mali, West Africa and was sent home for her treatment.

All these *P. falciparum* malaria cases were cured by antimalarial treatments except for the case 3 who died despite appropriate management with artesunate monotherapy (**Table 1**). Before malaria clinical onset, none of the patients reported a travel to malaria-endemic settings in the prior twelve months. No return visits nor friends from malaria-endemic areas with a possible luggage carrying an infected mosquito was declared, and no neighbors nor families of the patient worked around an airport. No patient underwent transplantation or had been transfused. Altogether, these six cases were considered to be nosocomial malaria based on personal and medical histories.

Extreme genetic relatedness between the source and the nosocomial malaria cases

Nosocomial transmission would result in extreme genetic relatedness between the parasites from the source (or imported) infection and the associated nosocomial infection. First, we explored allele lengths at five independent chromosomal STR loci by PCR amplification. The same alleles were detected at all five PCR-amplified loci except for the case 2 for which only one of the two possible sources had the same alleles as the nosocomial infection (**Supplementary Table S2**). Both the source and the nosocomial infections from cases 1 and 4 were polyclonal, with up to two and three different alleles detected at four loci, respectively.

We then studied the genetic variation from Illumina-sequenced whole-genomes. Using the F_{ws} statistic, the infections from cases 1 and 4 were again scored as polyclonal with values of 0.887 and 0.558 respectively (**Table 2**). The infections (source and nosocomial) for the case 2 were scored as polyclonal (F_{ws} = 0.913), contrasting with the analysis based on the five PCR-amplified STRs. Each matched source/nosocomial infection pair had nearly identical F_{ws} statistics.

Next, we genotyped high-quality STRs from whole-genome sequencing data. Cases 1 and 2 were excluded from this analysis because genome coverage depths were too low for confident estimation (**Supplementary Table S3**). From 5 024 to 5 492 independent STRs were investigated for pairwise comparisons, depending on the per-sample depth of coverage. We observed that, within each pair, the source and nosocomial infections differed by less than 50 STRs (<1%), a value similar to the one observed for the 6h control pair (0.31%; p=1.00, Mann-Whitney U test; **Figure 1A**, **Table 2** and **Supplementary Table S4**). The pair of infections exhibiting the highest percentage of discordant STR genotypes was from case 4 (0.78%), a result that could be explained by the polyclonality. In contrast, pairwise comparisons of unmatched infections had from 1 329 to 2 011 (median: 1 664) different STR genotypes (25.11 to 37.38% of the total STRs genotyped, median: 31.60%) (**Figure 1A** and **Supplementary Table S4**). The number of discordant STRs in the group of unmatched infection pairs was statistically higher than in the group of matched infection pairs (p=0.006).

Finally, we studied genome-wide, high-quality SNPs for each matched and unmatched infection pair. From 3 133 to 26 875 (median: 13 312) SNPs (after excluding missing genotypes) differed from the *P. falciparum* 3D7 reference genome. From these SNPs was then counted the number of SNPs that differed within each pair. For the six pairs of matched infections, we detected from 73 to 571 (median: 99) different SNPs (0.61 to 6.93% of the total SNPs genotyped) (**Figure 1B** and **Table 2**). For monoclonal infections, these values were very similar to the ones obtained for the positive controls (from 76 to 104 SNPs, *i.e.* from 0.65 to 1.31% of the total SNPs genotyped). For polyclonal infections, most of the SNPs that differed within pairs were heterozygous, while only <90 SNPs were homozygous. No statistical difference in the count of SNPs within a pair was observed between the matched control pairs and the matched infection pairs (p=0.48; Mann-Whitney-*U* test). In contrast, the number of different SNPs ranged from 2 143 to 15 860 (51.01 to 73.60%) within unmatched infection pairs (**Figure 1B** and **Supplementary Table S5**). For both controls and cases, a significant difference in the count of SNPs differing within a pair was observed between the matched and unmatched groups (p<0.001). Altogether, the WGS analyses confirmed the high parasite genetic relatedness between each source/nosocomial infection.

Geographical origin of the parasites

We finally checked the geographical origin of the parasites based on parasite genetics and on the travel history reported by the source patients (**Table 1**). For that, we compared the genome-wide SNPs from our samples with those from 125 samples previously genotyped as part of the MalariaGEN consortium (**Supplementary Table S1**). The neighbor-joining phylogenetic tree confirmed that the parasites from nosocomial transmission of cases 1, 3 and 4 were originated from Central Africa, and likely from Cameroon (**Figure 2**). The parasites from nosocomial transmission of cases 2, 5 and 6 clustered with West African parasites. Altogether, the results were fully congruent with the travel history of the source patients.

Discussion

The anopheles mosquito vector is not always necessary for transmission of the malaria parasite. Accidental inoculation of the skin with blood from a malaria patient, although a very rare event, was sporadically reported in the literature. Four cases have been described in Spain between 1978 and 2011 [17]. According to investigation results, patients were hospitalized for a few hours in the same room or unit of the initial imported malaria cases and with the same nurses, but no risky actions from hospital workers were reported. One nosocomial transmission was confirmed in a tertiary care setting in the United States because of improper use of saline flush syringes [18]. Finally, between 2016 and 2018, isolated nosocomial transmissions were observed in Greece, Italia, Spain and Germany, but inquiries did not permit to understand the exact causes of these transmissions [19].

Here, we report six distinct nosocomial transmissions in metropolitan France, four of which occurring during the COVID-19 pandemic. The natural transmission of malaria by a mosquito between the source cases and the nosocomial infections was incompatible with the generation times observed between the matched infections. Indeed, the maturation cycle from gametocyte ingestion in the blood meal to the infective sporozoite stage in the salivary glands of the mosquito requires about 10 to 20 days [20], and at least one additional week to have clinical signs in humans after the infecting bite. Furthermore, if it is assumed that an anopheles fed on patients with imported malaria, it is unlikely that it would then bite patients with nosocomial malaria 10 days later since the average lifespan of a mosquito is seven days. This reasoning also makes it possible to exclude the hypothesis of airport malaria [21]. Direct transmission of parasitized blood by another arthropod vector than *Anopheles* mosquito could be possible, but no such transmission has been reported to date. By exploring the whole-genome of the parasites for each pair of source and hospital-acquired cases, we observed that the percentages of different STRs and SNPs were very low. The results were similar to those from some samples independently amplified and sequenced twice, and those from relapses attributed to *P. vivax* [22,23].

Considering that it was notified that the patient pairs were admitted in the same care unit at the same time, we thus concluded the malaria infections as nosocomial.

The causes of these nosocomial transmissions were not fully understood. Considering the increasing number of nosocomial transmissions during the COVID-19 pandemic, we cannot exclude that the pressure on hospitals caused by the large number of SARS-CoV-2-positive patients resulted in hospital practitioners not sufficiently trained to risky actions (especially junior nurses) or with a decreased attention to such gestures. As examples, we can cite improper use of blood glucometers, multi dose heparin vials, contaminated catheters, reused saline flush syringes containing invisible traces of infected blood, successive infusions by the same caregiver, or even sharing contaminated multiple-use infusion sets [4–6,24]. Importantly, a very low quantity of infected blood with a very low parasite density is sufficient to transmit malaria. For example, one nanoliter of blood with a *P. falciparum* parasitemia of 0.1% contains approximately five parasites. A direct contact of this volume to uninfected blood through blood transfusion or any clinical material (including catheters, glucometers) may lead to malaria infection. However, investigating contaminated materials is often difficult because they are thrown away or undergo new washings in the case of reuse, between exposure to parasites and diagnosis of malaria. Consequently, understanding the way of transmission is mainly limited to interviews with patients and healthcare workers.

To our knowledge, no other nosocomial transmission of infections such as HIV or hepatitis virus was reported in parallel to these nosocomial malaria in the concerned hospitals. Nevertheless, these possibilities could occur but were not diagnosed according to the time between the contamination and the first clinical symptoms. And even if these events occurred, it will be very difficult to confirm the nosocomial origin of these pathologies.

In conclusion, these nosocomial malaria transmission events should serve as a reminder of the need for strict adherence to universal precautions in healthcare facilities. These multiple transmissions of a rather uncommon blood infection suggest that nosocomial transmission via blood of more common

pathogens, in particular bacteria, may be frequent. According to the European Center for Disease Prevention and Control, about 8.9 million healthcare-associated infections are estimated to occur every year in European hospitals [25].

Declarations

Author's contributions

SH and RC conceived and coordinated the study. HN coordinated the exploration of nosocomial cases. MT managed the database of the French National Reference Center for Malaria. VS, RZ and AB genotyped STRs by PCR and capillary gel electrophoresis. RC performed genome assembly, genome-wide SNP and genome-wide STR analyses. RC drafted the manuscript. RC, HN, JC and SH participated in the editing and final preparation of the manuscript. All authors read and approved the final manuscript.

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Ethics approval

No specific consent from patients were required since clinical and biological data were collected from the French National Reference Center for Malaria database in accordance with the common public health mission of all National Reference Centers in France, in coordination with the Santé Publique France organization for malaria surveillance and care. The study was considered as non-interventional research according to article L1221–1.1 of the public health code in France and only requires the non-opposition of the patient (per article L1211–2 of the public health code). All data were anonymized before use.

Consent for publication

There are no case presentations that require disclosure of respondent's confidential data/information in this study.

Availability of data and materials

Next-generation sequence files of the *P. falciparum*-imported and nosocomial infections, and resequencing controls are accessible on the European Nucleotide Archive (ENA) under the accession numbers ERS11622153 to ERS11622172 (project: PRJEB52198).

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Competing interests

The authors declare that they have no competing interests with the current work.

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Tables

Table 1. Clinical characteristics and history of the source and nosocomial malaria cases.

Case	Type	Hospital	Age (year)	Sex	Date of first admission	Reason for first admission	Country of contamination	Care unit of first admission	Days between the first and second admission	Second admission	% par.	Nosocomial transmission	Status
1	Autochthonous	1	9w	F	10-2007	Meningeal syndrome	—	Emergency	18	Emergency (hospital 1)	4.5	Confirmed	Recovered
	Imported malaria	1	4	M	10-2007	*P. falciparum* imported malaria	Cameroon	Emergency	—	—	0.78		Recovered
2	Autochthonous	2	75	F	11-2019	Altered general condition	—	Emergency	10	Emergency (hospital 2)	16	Confirmed	Recovered
	Imported malaria 1	2	35	F	11-2019	*P. falciparum* imported malaria	Ivory Coast	Emergency	—	—	1.6		Recovered
	Imported malaria 2	2	69	M	11-2019	*P. falciparum* imported malaria	Gambia	Emergency	—	—	0.55	Not confirmed	Recovered
3	Autochthonous	3	70	M	06-2021	Decompensated heart failure	—	Emergency	18	Emergency (hospital 3)	2.8	Confirmed	Died
	Imported malaria	3	35	M	06-2021	*P. falciparum* imported malaria	Cameroon	Emergency	—	—	ND		Recovered
4	Autochthonous	4	2	F	07-2021	Acute gastroenteritis	—	Emergency	14	Emergency (hospital 4 bis)	7.4	Confirmed	Recovered
	Imported malaria	4	5m	M	07-2021	*P. falciparum* imported malaria	Cameroon	Emergency	—	—	12		Recovered
5	Autochthonous	5	50	F	09-2021	SARS-CoV-2	—	Intensive care unit	20	Intensive care unit (hospital 5)	7.5	Confirmed	Recovered
	Imported malaria	5	45	M	09-2021	*P. falciparum* imported malaria	Niger	Intensive care unit	—	—	1.4		Recovered
6	Autochthonous	6	72	M	09-2021	Lung infection	—	Emergency	16	Emergency (hospital 6 bis)	1.3	Confirmed	Recovered
	Imported malaria	6	50	F	09-2021	*P. falciparum* imported malaria	Mali	Emergency	—	—	5.7		Recovered

Note – % par., parasitaemia in percentage. The "autochthonous" case 1 was 9 weeks of age; the imported case 4 was 5 months of age. ND, not determined.

Table 2. Parasite genetic comparison for the different cases and positive controls

Sample [a]	F_{ws} statistic [b]	Clonality	Identical PCR-derived STRs [c]	Number of different STRs (%) [d]	Number of different SNPs (%) [e]
Case 1 – Source	0.886	Polyclonal	5/5	ND	571 (6.93)
Case 1 – Nosocomial	0.814	Polyclonal			
Case 2 – Source 1	0.913	Polyclonal	5/5	ND	95 (1.29)
Case 2 – Nosocomial	0.913	Polyclonal			
Case 2 – Source 2	0.941	Polyclonal	0/5	ND	6 162 (67.08)
Case 2 - Nosocomial	0.913	Polyclonal			
Case 3 – Source	0.989	Monoclonal	5/5	16 (0.31)	73 (0.61)
Case 3 – Nosocomial	0.989	Monoclonal			
Case 4 – Source	0.552	Polyclonal	5/5	42 (0.78)	513 (2.22)
Case 4 – Nosocomial	0.562	Polyclonal			
Case 5 – Source	0.992	Monoclonal	5/5	41 (0.76)	103 (0.78)
Case 5 – Nosocomial	0.992	Monoclonal			
Case 6 – Source	0.991	Monoclonal	3/3	ND	56 (1.50)
Case 6 – Nosocomial	0.992	Monoclonal			
Case 6 – Nosocomial	0.992	Monoclonal	NA	17 (0.32)	87 (0.65)
Case 6 – Nosocomial (6h control)	0.992	Monoclonal			
Resequencing control 1.1	0.971	Monoclonal	NA	ND	104 (1.31)
Resequencing control 1.2	0.961	Monoclonal			
Resequencing control 2.1	0.976	Monoclonal	NA	ND	76 (0.86)
Resequencing control 2.2	0.974	Monoclonal			
Resequencing control 3.1	0.977	Monoclonal	NA	ND	85 (1.05)
Resequencing control 3.2	0.977	Monoclonal			

[a] Three positive controls consisted of three different samples independently amplified and sequenced twice (resequencing controls). Another positive control compared two samples of the nosocomial infection from case 6, collected 6 hours apart (6h control).

[b] The F_{ws} statistic was calculated using the genomic diversity of our samples, together with 125 other genomes retrieved from MalariaGEN.

[c] The exact lengths of PCR-derived STRs are available in Supplementary Table S1. NA, not applicable.

[d] Genome-wide STRs were only investigated for a pair of samples that were deeply covered. The percentage indicated the proportion of STRs that differed from the total of STRs genotyped within the pair. ND, not determined.

[e] The percentage indicated the proportion of SNPs that differed from the total of SNPs genotyped within the pair.

Figure legends

Figure 1. Comparison of genome-wide STRs and SNPs across matched and unmatched infection and control pairs. (**A**) Proportion of genome-wide STRs that differed within pairwise comparisons. The control consisted of two samples independently collected 6 hours apart from the case 6. (**B**) Proportion of genome-wide SNPs that differed within pairwise comparisons. In addition to the two samples from the nosocomial case 6 collected 6 hours apart, three other controls were included consisting of monoclonal *P. falciparum* infections from patients, independently amplified and sequenced twice. Box boundaries represent the 25th and 75th percentiles and the length of whiskers correspond to 1.5 times the interquartile range.

Figure 2. Phylogenetic tree of the parasites from the imported and nosocomial infections, together with 125 African and Asian parasites retrieved from MalariaGEN. Countries of collection of the samples are Benin, Burkina-Faso, Gambia, Guinea and Ivory Coast for West Africa (blue); Cameroon, Congo and Gabon for Central Africa (orange); Kenya, Tanzania and Uganda for East Africa (red); and Cambodia, Thailand and Vietnam for Southeast Asia (green). The phylogenetic tree was inferred by neighbor-joining using 32 985 SNPs. Isolates marked with a star are the pairs of imported/nosocomial infections. The two-letter code on the maps identifies each malaria-endemic country included in the analysis.

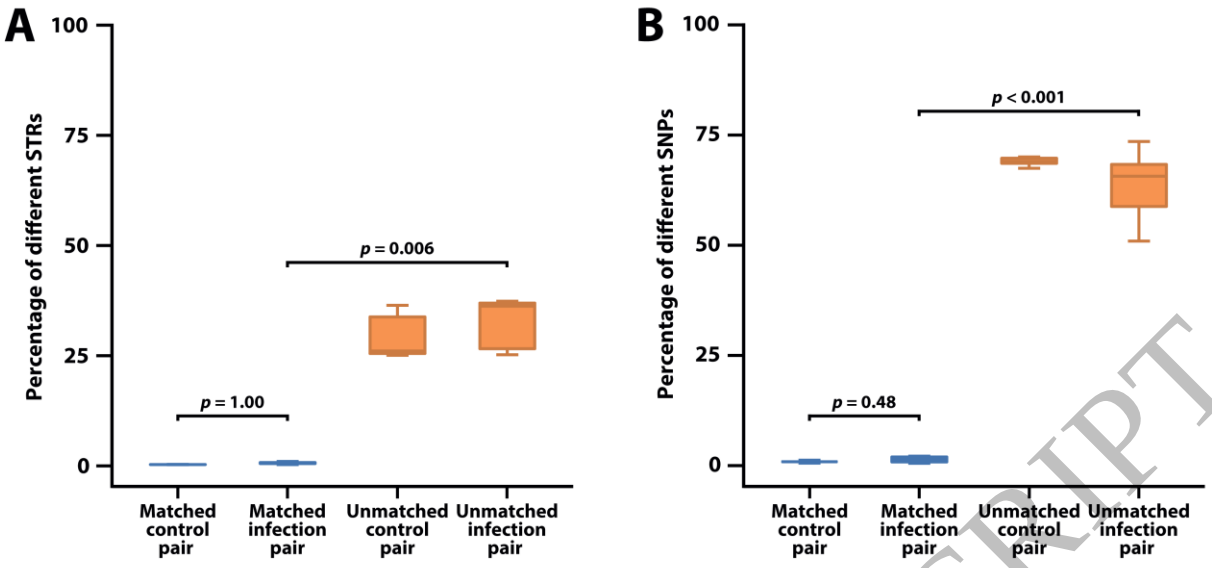

Figure 1
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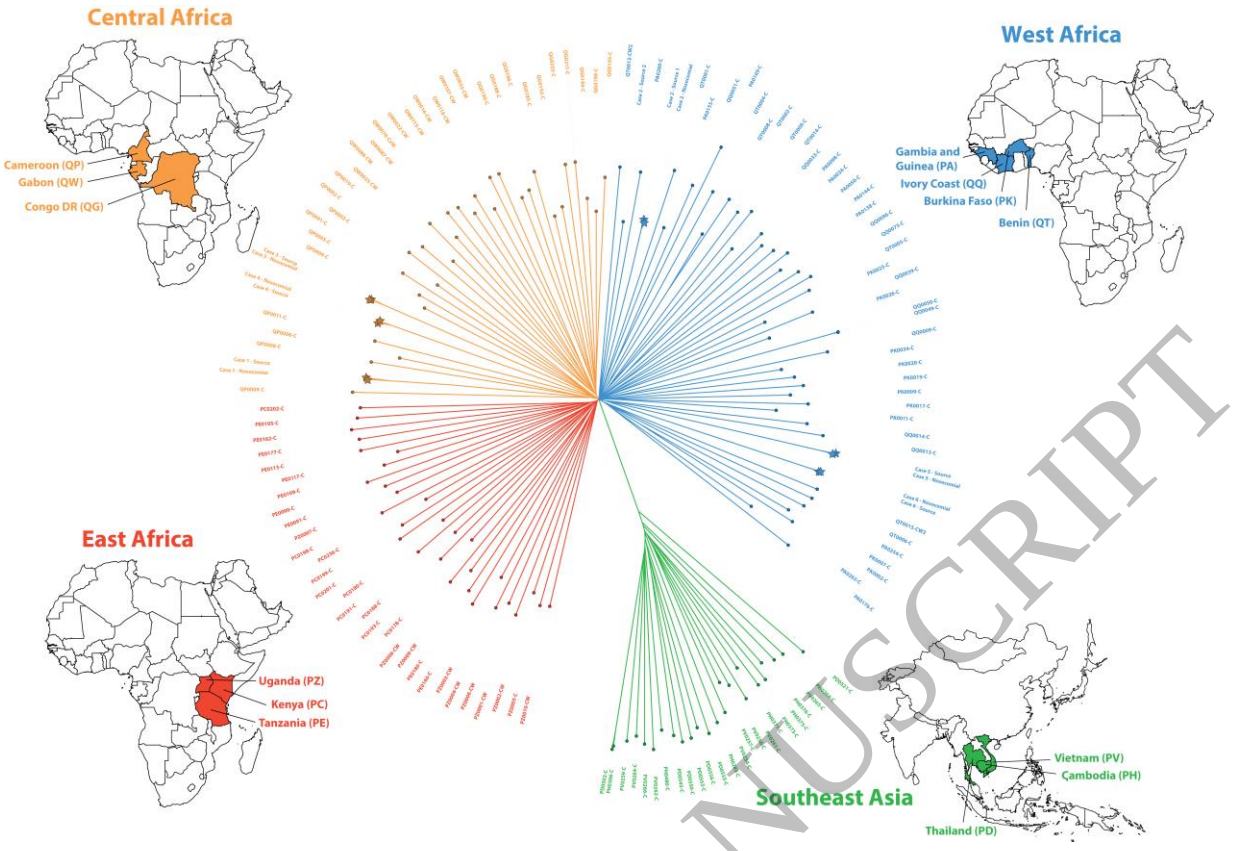

Figure 2
160x111 mm (6.2 x DPI)