Original Article

Prevalence of imported malaria in Parma during 2005-2006

Simona Peruzzi, Chiara Gorrini, Giovanna Piccolo, Adriana Calderaro, Giuseppe Dettori, Carlo Chezzi

Department of Pathology and Laboratory Medicine, Section of Microbiology, University of Parma, Parma, Italy

Abstract. *Background and aim of the work:* Malaria is a protozoan infection caused by parasites of the genus *Plasmodium* (*P. falciparum, P. ovale, P. vivax, P. malariae*) that is transmitted from one human to another by female *Anopheles* mosquitoes. It can be considered a reemerging imported disease in our area because of increasing of movements from endemic countries, and nowadays it is the most common imported infection in Italy. This study describes the occurrence of imported malaria in our area between January 2005 and May 2006. *Methods:* During 17 months we analysed 170 blood samples belonging to 139 patients (95 foreigners and 44 Italians) with the clinical suspect of malaria. Samples were used to prepare orange acridine and Giemsa stained thin blood films for microscopic observation and to perform an immunochromatographic assay for the detection of specific plasmodia antigens. Molecular assays (nested-PCR and Real-time PCR) were also performed in order to confirm the diagnosis. *Results:* Thirty-six cases of malaria were diagnosed: 35 in foreigners coming from Africa and only one in an Italian who lived in Chad. Thirty-three patients were infected by *P. falciparum*, 1 by *P. ovale,* 1 by *P. vivax*, and a mixed infection by *P. falciparum*, *P. ovale* and *P. malariae* was also found. *Conclusions:* Malaria is usually associated with travels within areas where the infection is endemic and our data demonstrated that imported malaria in our area has a prevalence of 25.89%. (www.actabiomedica.it)

Key words: Malaria, diagnosis, Nested-PCR, Real-time PCR, prevalence

Introduction

Malaria has probably had a greater impact on world history than any other infectious disease (1).

Presently it is a re-emerging disease which is propagating to areas where it had been eradicated many years ago and is one of the most prevalent human infections worldwide (2).

Malaria is caused by four protozoan parasites of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*) and is transmitted from one human to another by female *Anopheles* mosquitoes (3). For the transmission of the infection it is indispensable that *Anopheles* mosquitoes survive and multiply and that malaria parasites complete their growth cycle in the mosquitoes. These premises can explain the particular geographic distribution of endemic malaria in tropical and subtropical areas (4-6).

Although malaria is usually associated with travels in countries where the infection is endemic it should be specified that there are some other situations which can result in infection, such as blood transfusion, the use of hypodermic needles that are contaminated by prior use, and possible congenital infection (1, 6).

Clinical symptoms of malaria include the classic paroxysm which consists in fever and sweating (even if many patients do not exhibit the typical fever pattern), splenomegaly, anaemia, lethargy, anorexia, nausea, vomiting, diarrhoea and headache (1, 4, 6, 7).

Presently malaria is the most common imported infectious disease in Italy: it has been demonstrated that in our country during 2000-2001, 29% of patients affected by malaria were Italian while 61% were foreigners, confirming that immigration is one of the most important causes of imported malaria in our country (4). The predominant *Plasmodium* species diagnosed was *P. falciparum* (more than 80% of the total cases), followed by *P. vivax* (9%), *P. ovale* (8%), and *P. malariae* (1.5%) (4). The majority of the infections were acquired in Africa, Central and South America, Asia, and Oceania (4). The higher number of imported infections observed is principally associated with the increase in population movements for tourism, migration, and trading to and from areas where malaria is endemic.

Considering the fact that no antimalarial chemoprophylaxis is universally effective and that vaccine development studies are still ongoing, the prevention of the infection is very difficult, thus laboratory diagnosis is critical in order to restore the patients to health. In particular, a patient with a diagnosed infection by *P. falciparum* should be considered as a medical emergency since the disease can be rapidly fatal and even if a low parasitemia is present on blood smears the patient could be faced with a life-threatening disease (1).

Microscopic examination of thin blood smears remains the gold standard for the diagnosis of malaria, but it presents limitations: it is time consuming and misdiagnosis is possible if the parasitemia is low or if the microscopist lacks experience (8, 9). Recently, genus- or species-specific molecular methods based on polymerase chain reaction have been developed and introduced in routine diagnosis to confirm microscopic observation, and to detect the presence of mixed infections that may be misdiagnosed by the microscopist (8, 9).

In a previous report from our laboratory published in 2004, among 122 analysed blood samples, 43 *P. falciparum*, 11 *P. vivax*, 3 *P. ovale,* 2 *P. malariae,* 1 *Plasmodium* spp., and 1 *P. vivax/P.falciparum* were detected at microscopic observation*;* with Nested-PCR 42 *P. falciparum,* 8 *P. ovale,* 8 *P. vivax*, *and* 2 *P. malariae* were detected; with Real-time PCR 42 *P. falciparum*, 8 *P. ovale*, and 8 *P. vivax* were observed (8). Moreover, 2 mixed infections by *P. falciparum* and *P. ovale* (by Real-time PCR), one by *P. falciparum* and *P. ovale* (by Nested-PCR), and one by *P. falciparum, P. ovale* and *P. malariae* (by Nested-PCR) were also found (8).

Our study aimed to describe the occurrence of imported malaria in our area during the 2005-2006 period, comparing the data obtained with microscopic observation and molecular methods (Nested-PCR and Real-time PCR).

Material and methods

During 17 months (from January 2005 to May 2006) we analysed 170 blood samples belonging to 139 patients with the clinical suspicion of malaria. Each sample was taken when the patient presented a bout of fever, in order to be certain to find parasites in the blood.

Each time we received a blood sample for the research of plasmodia we contacted the clinician to know some information about the patient (presence of fever at the time of blood sampling, nationality, recent travel through malaria endemic areas, eventual prophylaxis, relevant specific clinical symptoms and signs). Each sample, containing at least five millilitres of blood drawn into sterile tubes with EDTA, was used to prepare thin blood films for microscopic observation and to perform an immunochromatographic rapid assay for the detection of specific *Plasmodium* antigens (I.C.T. binax®). The blood smears were stained with orange acridine (10) and then with 1% Giemsa stain in phosphate-buffered saline (pH 7.0) (5) and examined under the microscope at 1,000 X magnification for the research of plasmodia: thin blood films were defined as negative if no parasites were observed in 300 oil immersion fields. The parasite concentration, when necessary, was calculated by counting the number of parasitized red blood cells seen in 10,000 erythrocytes and expressed as a percentage, as described for areas of non endemicity (6). Aliquots of each blood sample were stored at 4° C or –20° C and then used to perform PCR assays in order to confirm the microscopic diagnosis. DNA from blood samples was extracted using "High Pure PCR Template Preparation Kit" (Roche), according to the manufacturer's instructions (8, 9). To perform the Ne-

sted-PCR, DNA templates were used for the amplification in a DNA thermal cycler (Gene Amp 2400 PE) using a genus-specific primer set for the first amplification and four species-specific primer sets for the second amplification, as previously described (8, 10). For the Real-time PCR primers and probes were designed to specifically amplify the *P. falciparum*, *P. vivax*, and *P. ovale* 18S rRNA gene, according to the previously described method (8, 11).

Results

During 17 months we analysed 170 blood samples belonging to 139 patients (95 foreigners and 44 Italians) with the clinical suspect of malaria. Thirty-six cases (25.89%) of malaria were diagnosed: 35 in foreigners coming from Africa and one in an Italian who lived in Chad. Patients were 13 females and 23 males, 3 children and 33 adults (Table 1).

Table 1. Data available for the patients analysed in this study

Patient code	Age	Sex	Country of origin	Travels abroad
1	25	Male	Cameroon	Cameroon
2	36	Male	Ghana	Ghana
3	45	Female	Nigeria	Nigeria
4	33	Female	Nigeria	Nigeria
5	37	Male	Ghana	Ghana
6	19	Male	Burkina Faso	Burkina Faso
7	27	Female	Africa	Not known
8	37	Male	Nigeria	Nigeria
9	2	Male	Nigeria	Nigeria
10	41	Female	Nigeria	Nigeria
11	41	Female	Ivory Coast	Ivory Coast
12	31	Male	Ivory Coast	Ivory Coast
13	32	Male	Ivory Coast	Ivory Coast
14	40	Female	Benin	Benin
15	12	Male	Nigeria	Nigeria
16	8	Male	Ghana	Ghana
17	33	Male	Ghana	Ghana
18	42	Male	Ghana	Ghana
19	39	Female	Guinea	Guinea
20	30	Male	Senegal	Senegal
21	20	Female	Cameroon	Cameroon
22	11	Male	Nigeria	Nigeria
23	18	Male	Senegal	Senegal
24	35	Male	Nigeria	Nigeria
25	34	Female	Nigeria	Nigeria
26	40	Male	Ghana	Ghana
27	40	Male	Cameroon	Cameroon+Kenya
28	40	Female	Ivory Coast	Ivory Coast
29	25	Male	Eritrea	Eritrea
30	18	Female	Ivory Coast	Ivory Coast
31	49	Male	Italy	Chad
32	31	Male	Cameroon	Cameroon
33	43	Female	Ghana	Ghana
34	46	Male	Ghana	Ghana
35	41	Female	Ghana	Ghana
36	41	Male	Ghana	Ghana

Thirty-three patients were infected by *P. falciparum*, 1 by *P. ovale,* 1 by *P. vivax* and a mixed infection by *P. falciparum*, *P. ovale* and *P. malariae* was also found (Table 2).

Thirty-five samples were positive by microscopy: 33 *P. falciparum* (91.66%), 1 *P. ovale* (2.77%) and 1 *P. vivax* (2.77%). Only one blood smear resulted negative at microscopic observation (Table 2). In the 33 smears that were positive for *P. falciparum*, parasitemia was included between 0.001% and 2.13%; in the cases of infections by *P. ovale* and *P. vivax* the parasitemia was 0.07% and 0.1%, respectively. The major parasitemia (2.13%) observed was counted in the blood smears belonging to the only Italian patient infected by *P. falciparum.*

The results of immunochromatographic assay agreed with all the results of microscopy, except in the case of the infection by *P. ovale*, which resulted negative (Table 2).

The results obtained by Nested-PCR agreed with microscopic observation in 20 cases, while they were

Table 2. Plasmodia identified in blood samples of patients with imported malaria in Parma

Patient code	Plasmodia identified			
	Microscopy	Nested-PCR	Real-time PCR	ICT
1	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
2	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
3	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
4	*P. falciparum*	Not performed	*P. falciparum*	*P. falciparum*
5	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
6	*P. falciparum*	*P. falciparum*+ *P. ovale*+ *P. malariae*	*P. falciparum*	*P. falciparum*
7	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
8	Negative	*P. falciparum*	Not performed	Negative
9	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
10	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
11	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
12	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
13	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
14	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
15	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
16	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
17	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
18	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
19	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
20	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
21	*P. ovale*	*P. ovale*	Not performed	Negative
22	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
23	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
24	*P. falciparum*	Negative	*P. falciparum*	*P. falciparum*
25	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
26	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
27	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
28	*P. falciparum*	Not performed	*P. falciparum*	*P. falciparum*
29	*P. vivax*	Positive	Not performed	*P. vivax*
30	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
31	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
32	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
33	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
34	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
35	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*
36	*P. falciparum*	*P. falciparum*	Not performed	*P. falciparum*

discordant in 14 cases and the Nested-PCR assay was not performed in 2 cases (Table 2).

Moreover the results by microscopy were confirmed by Real-time PCR in 15 cases while in 21 cases this molecular assay was not performed.

In the case of the mixed infection by *P. falciparum*, *P. ovale*, and *P. malariae* microscopy revealed only the presence of *P. falciparum*, the Nested-PCR detected the DNA of *P. falciparum*, *P. ovale*, and *P. malariae*, while the Real-time PCR detected only the DNA of *P. falciparum* (Table 2).

Conclusions

During 17 months (January 2005-May 2006) among 139 patients (95 foreigners and 44 Italians) we diagnosed 36 cases of imported malaria (35 in foreigners and 1 in an Italian male).

Thirty-four foreign patients came from African countries (10 from Ghana, 9 from Nigeria, 5 from the Ivory Coast, 4 from Cameroon, 2 from Senegal, 1 from Burkina Faso, 1 from Benin, 1 from Guinea, 1 from Eritrea) while for one patient the nationality was unknown, even if he originated from Africa. For all of these patients the clinical suspect for malaria was formulated on the basis of symptoms that are indicative of the infection, such as fever with associated headache, abdominal pain, and diarrhoea (8).

The only Italian patient lived in Chad and was admitted to the hospital some days after his return to Italy. At the time of blood sampling he presented fever (41°C).

Among all the patients we found some with very interesting clinical findings. In particular, an 18 years old pregnant woman (22 weeks) who came from the Ivory Coast but had lived for 2 years in Italy. She suffered from severe anaemia (haemoglobin 6,8 g/dl and red blood cells 3420000/mm^3), never had fever, and was in an apparently good healthy state. Although she was not able to communicate in Italian, she referred to have suffered from malaria some years before when she lived in her country. The blood sample was positive for *P. falciparum* and parasitemia was <0.001%. The therapy promptly restored the patient to health. In malaria endemic areas pregnant women are more susceptible to the infection than the general population and it can cause serious pregnancy complications with high maternal and foetal mortality, as reported in the literature (7, 12, 13), thus a prompt diagnosis is absolutely mandatory.

Even though microscopy remains the most important diagnostic method, we observed that in some cases molecular assays are indispensable to perform a correct diagnosis, in particular to detect mixed infections. In the only case in which we detected a mixed infection, microscopic observation allowed to detect only trophozoites of *P. falciparum* (<0.001%) while Nested-PCR detected DNA specific for *P. falciparum*, *P. ovale*, and *P. malariae.*

We found only one infection by *P. ovale* and one by *P. vivax*. The first case was a 20 years old female, coming from Cameroons and speaking only French, who presented fever and sweating from few days: at the microscopic observation *P. ovale* trophozoites (parasitemia 0.07%) were detected and the diagnosis was confirmed by Nested-PCR. The other case was a male coming from Eritrea, who had arrived for the first time in Italy 3 months before the admission to the hospital for the suspect of meningitis with fever (38°C), headache, and vomit. Microscopic examination revealed the presence of *P. vivax* schizontes (parasitemia 0.1%), successively confirmed by molecular methods.

Malaria has always had an important impact on human life and even if it is not endemic in our country since 1970 (4), it is one of the most important imported diseases in Italy. The data obtained in our laboratory demonstrated that the most relevant factor that incides on the prevalence of this infection is immigration from or travel to areas where malaria is endemic.

According to our previously published reports the prevalence of imported malaria in our area strongly underlines that *P. falciparum* infections remain the most prevalent (91.66%), *P. vivax*, *P. ovale* and mixed infections represent the 2.77% respectively.

This study also confirmed that molecular methods (such as PCR) are not only an aid in performing an accurate diagnosis of malaria but also that they are powerful tools in obtaining knowledge on the epidemiology of imported malaria in our area.

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References

1. Garcia LS, Bruckner DA. Diagnostic medical parasitology. 1997 Third edition American Society for Microbiology, 1325 Massachusetts Avenue, N.W. Washington, DC 2005.
2. Suh KN, Kain KC, Keystone JS. Malaria. *CMAJ* 2004; 170: 1693-702.
3. Carter R, Mendis KN. Evolutionary and Historical Aspects of the Burden of Malaria. *Clin Microbiol Rev* 2002: 564-94.
4. Romi R, Boccolini D, D'Amato S, Caraffa De Stefano D, Majori G. La malaria in Italia nel 2000-01. *BEN- Notiziario ISS* 2002, 15: 7-8.
5. Warhurst DC, Williams JE. Laboratory diagnosis of malaria: ACO broadsheet no. 148. *J Clin Pathol* 1996; 49: 533-8.
6. Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev* 2002; 15: 66-78.
7. Guerin PJ, Olliaro P, Nosten F, et al. Malaria: current status of control, diagnosis, treatment and a proposed agenda for research and development. *Lancet Infect Dis* 2002; 2: 564-73.
8. Perandin F., Manca N., Calderaro A. et al. Development of a Real-time PCR assay for detection of *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* for routine clinical diagnosis. *J Clin Microbiol* 2004; 42: 1214-9.
9. Calderaro A, Piccolo G, Zuelli C, et al. Evaluation of a new plate hybridization assay for the laboratory diagnosis of imported malaria in Italy. *The New Microbiologica* 2004; 27: 163-72.
10. Kawamoto F, Liu Q, Ferreira MU, Tantular IS. How prevalent are *Plasmodium ovale* and *P. malariae* in East Asia? *Parasitol Today* 1999; 15: 422-6.
11. Snounou G, Viriyakosol S, Zhu XP, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol Biochem Parasitol* 1993; 61: 315-20.
12. Mutabingwa TK. Malaria and pregnancy: epidemiology, pathophysiology and control options. *Acta Trop* 1994; 57: 239-54.
13. Dicko A, Mantel C, Aly Thera M, et al. Risk factors for malaria infection and anemia for pregnant women in the Sahel area of Bandiagara, Mali. *Acta Trop* 2003; 89: 17-23.

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Correspondence: Prof. Adriana Calderaro MD, PhD
Associate Professor of Microbiology and
Clinical Microbiology,
Department of Pathology and Laboratory Medicine
Section of Microbiology University of Parma
Viale A. Gramsci, 14
43100 Parma, Italy
Tel. +39-0521-988885 / 988877
Fax +39-0521-993620
E-mail address: adriana.calderaro@unipr.it; www.actabiomedica.it