A TWELVE-YEAR EPIDEMIOLOGY REPORT OF IMPORTED MALARIA IN PARMA (ITALY)

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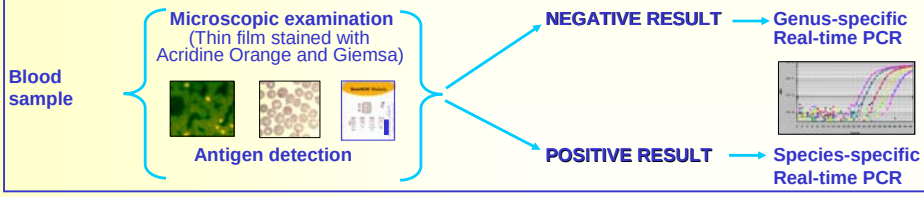
INTRODUCTION AND PURPOSE. Malaria is a major cause of morbidity and mortality in adults and children worldwide in endemic regions, but at present it is re-emerging as an imported disease in areas where it has been eradicated, such as Europe, due to the increasing amount of travellers and migratory flows from endemic countries. As a matter of fact, although in 1970 the World Health Organization (WHO) officially declared Italy malaria free, a surveillance system was established to prevent a possible return of malaria transmission and to monitor the epidemiology of imported cases. Malaria, nowadays, is the most commonly imported disease in Italy which has one of the highest rates of imported malaria among non-endemic countries, following France, United Kingdom, and Germany among European countries (1). Microscopic examination of blood smears remains the gold standard for the diagnosis of malaria and is required by guide-lines of both WHO and the Italian Ministry of Health, even if it presents limitations in sensitivity and/or specificity in cases of low parasitaemia and mixed infections. Furthermore, the routine use of PCR-based methods has transformed the epidemiology of malaria species by significantly identifying more *P. ovale* infections and mixed-infections than microscopy (1).

This study aimed to improve the diagnoses of malaria and to describe the occurrence in our area during the period January 2000 - October 2011 of different species of plasmodia involved in human disease [*Plasmodium falciparum* (Pf), *P. ovale* (Po), *P. vivax* (Pv), *P. malariae* (Pm)] focusing on patient clinical/epidemiological (origin of infection, nationality, administration of a chemoprophylaxis) information. To do this, data obtained with microscopic observation were compared to those obtained with the application of molecular assays (nested PCR and real-time PCR) in order to assess the usefulness of these assays in the diagnostic practice and to have a likely picture of the epidemiology of imported malaria in Parma where migratory flows and travelers from endemic areas are more and more increasing.

METHODS. From January 2000 to October 2011, blood samples from 1,002 patients with the suspicion of malaria were subjected to microscopy, to rapid assays based on plasmodial antigen detection, and to different nested- and Real-time PCR assays targeting plasmodial (Pf, Po, Pv, Pm) 18S-rDNA, alternatively used during the study period (2,3,4). The developed molecular methods were designed to detect also the species *P. knowlesi* (simian malaria agent recognized also as human malaria agent) at genus level (5), and to distinguish between the known polymorphisms in Po 18S-rRNA gene responsible for the existence of two variants in the species, named as *P. ovale curtisi* and *P. ovale wallikeri* (6,7). In our laboratory the diagnosis of malaria was performed on the basis of the following algorithm:



Prevalence of malaria in Parma in 12 years

MICROSCOPY 227/1,002 = 22.6%

PCRs 234/1,002 = 23.3%

Table 1. Imported malaria cases in Parma from January 2000 to October 2011 diagnosed by microscopic examination and by molecular methods (Nested- PCR or real-time PCR alternatively used)

	Microscopy	PCR
P. falciparum	189 (83.3%)	190 (81.2%)
P. ovale	12 (5.3%)	23 (9.8%)[a]
P. vivax	13 (5.7%)	9 (3.85%)
P. malariae	0	3 (1.3%)
Plasmodium spp.	10 (4.4%)	0
P. falciparum/ *Plasmodium* spp.	1 (0.4%)	0
Mixed infection	2 (0.9%)[b]	9 (3.85%)[c]
TOTAL	227	234

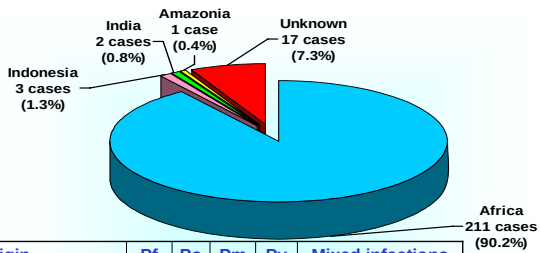
LEGEND:
a. Including 12 cases of Po *curtisi* and 11 cases of Po *wallikeri*
b. 2 Pf+Po
c. 2 Pf+Pm+Po; 4 Pf+Po; 3 Pf+Pm+Po

RESULTS. On the total of the blood samples analyzed in this study, belonging to 1,002 patients, 227 cases of malaria were diagnosed by microscopy, whilst 234 were diagnosed by PCRs, showing a prevalence of 22.6% and 23.3%, respectively. Plasmodia species detected in these blood samples by microscopic examination and by molecular methods are described in Table 1.

Among the 234 cases, 213 (91%) were foreigners and 21 (9%) were Italian travelling for tourism, business or humanitarian mission. The majority of the patients presented with fever (about 90%) and had no correct anti-malarial chemoprophylaxis (65%); the analysis of all the cases showed that the majority of patients (90.2%) became infected in Africa, most of them in Countries located in West Africa, such as Ghana (20%), Nigeria (21.8%), Ivory Coast (15%), Cameroon (10.2%), and Senegal (8.1%). Origin or travelling areas of patients with malaria diagnosed by molecular methods in our laboratory are reported in Figure 1.

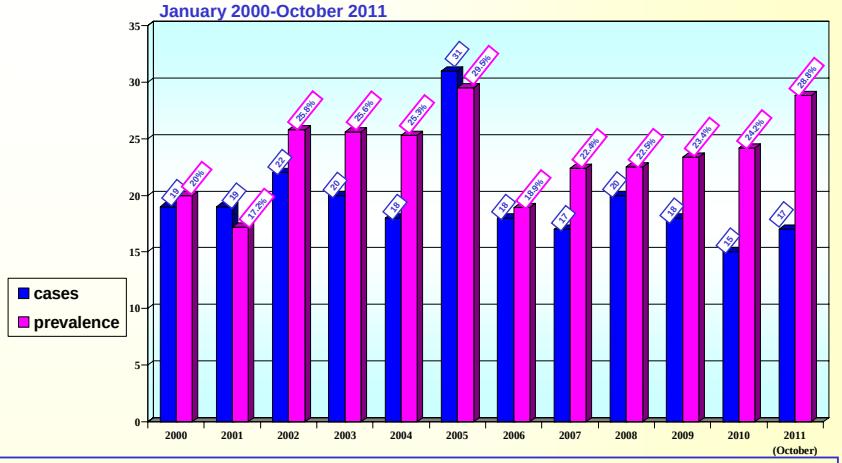
The number/year of imported malaria cases revealed by PCR assays in Parma during January 2000-October 2011 was mean 19.5, as detailed in Figure 2.

Figure 1. Origin or travelling areas of patients with malaria diagnosed by PCRs in our laboratory



Origin	Pf	Po	Pm	Pv	Mixed infections
Africa	175	21	3	3	9 (see table 1)
Indonesia	1			2	0
India				2	0
Amazonia				1	0
Unknown	14	2		1	0

Figure 2. Number of cases and prevalence of imported malaria in Parma during January 2000-October 2011



CONCLUSIONS. This epidemiological study is of interest because of the high prevalence (23.3%) of malaria described in Parma as an uncommon finding in a non-endemic country, but in accordance with other reports describing malaria as one of the most important imported diseases in Europe.

The number of malaria cases diagnosed in our laboratory was stable during 2002 to 2004 (mean value 19.6), grew in 2005 (31 cases), and showed a reduction in 2006 (18 cases) and 2007 (12 cases). Reasons explaining this trend are not assessed even if variable flow of immigration could play an important role. According to the national trend, in the area of Parma imported malaria is more frequent among immigrants from areas where malaria is endemic, in particular, from West Africa, explaining our growing prevalence of *P. ovale* cases (data not shown) among non-*P. falciparum* infections, prevalent in those countries. The most of malaria cases in our area as well as in Italy were imported from Africa and due to *Pf*, followed by *Po* and *Pv*.

Most of the African immigrants who contracted malaria live in Italy and usually underestimate the risk they take visiting their native lands after a long period of stay in a nonendemic country. In fact, in our study, most of the patients had no correct antimalarial chemoprophylaxis. Therefore, making accurate information on prophylaxis available to immigrants and Italian travelers may further reduce malaria imported cases, and an accurate diagnosis (microscopy supported by PCR-based methods) may allow the administration of a correct therapy.

Despite microscopy remains the reference diagnostic method, in our experience molecular assays were the only ones allowing a correct diagnosis of malaria, particularly in cases of infections by species other than *Pf* and mixed infections, reflecting in a reliable description of the epidemiological picture of imported malaria in our area. PCR assays were able to detect 6 single and 1 mixed infections missed by microscopy, revealing 5 single and 1 mixed infections incorrectly diagnosed by microscopy and giving speciation in 11 cases in which microscopy had limited the result to genus identification. Furthermore, only one PCR assay developed by us showed the higher accuracy in *Po* detection due to specific primer design done to recognise all the variants in *Po* 18S-rRNA gene. In our experience a rapid and accurate diagnosis of malaria allowed to administer a prompt and targeted therapy with positive impact on the clinical management of the patients also in terms of days of hospitalisation.

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22nd European Congress of Clinical Microbiology and Infectious Diseases (London, 31 March-3 April 2012)

With the contribution of FONDAZIONE CARIPARMA