PREVALENCE OF IMPORTED *Plasmodium ovale curtisi* AND *P. ovale wallikeri* MALARIA IN PARMA (ITALY)

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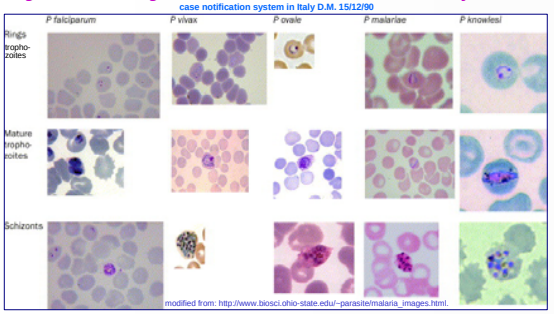
INTRODUCTION AND PURPOSE. ***Plasmodium ovale*** **is the third species, in case number, among the agents causing human malaria, reported all over the subtropical countries and commonly found in tropical Africa, New Guinea, Indonesia and Philippines.** ***P. ovale*** **microscopic diagnosis is difficult (Fig. 1) and even molecular analysis often failed because of the presence of a polymorphism within the** ***P. ovale*** **genomic sequences (Fig. 2, 3). Species identification by 18S rRNA gene sequencing split the samples positive for** ***P. ovale*** **into 2 groups,** ***P. ovale curtisi*** **(*Poc*) and** ***P. ovale wallikeri*** **(*Pow*), and further investigations confirmed that this dimorphism extend to other genes (including those encoding the lactate dehydrogenase and the ookinete surface protein). Herein we report the prevalence of imported malaria cases by these 2 species, as detected by a newly designed Real-time PCR assay (*Poc-Pow* Real-time PCR), among the overall 259 imported malaria cases diagnosed at our University Hospital.**

METHODS. From January 2000 to October 2012, blood samples collected on hospital admission from 1,069 patients presenting with symptoms consistent with malaria were subjected to microscopy and to different nested- and Real-time PCR assays, alternatively used during the study period. The 18S rRNA was the target of all the molecular assays.

Fig. 1. MICROSCOPY:
the gold standard diagnostic method for malaria for over a century and till now
case notification system in Italy D.M. 15/12/90



PITFALLS
- **sensitivity (50 p/μl) including acridine orange and Giemsa stains (Calderaro A., 2012);**
- **failure in distinguishing species in mixed infections;**
- **failure in distinguishing between *P. malariae* and *P. knowlesi*;**
- **experienced microscopist required.**

Fig. 2. MOLECULAR METHODS: NESTED-PCR ASSAYS

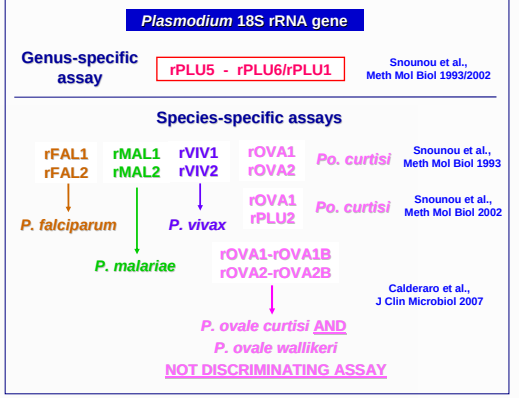
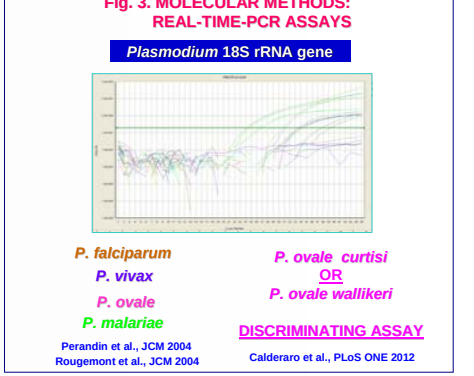
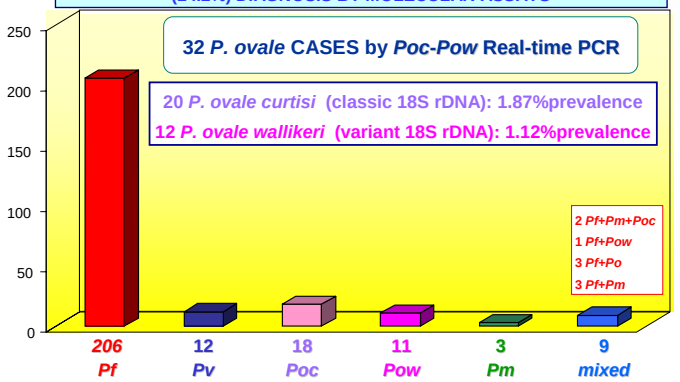


Fig. 3. MOLECULAR METHODS: REAL-TIME-PCR ASSAYS



RESULTS. 259 cases (24.2%) of malaria were diagnosed (Fig. 4): 206 *P. falciparum*, 12 *P. vivax*, 29 *P. ovale*, 3 *P. malariae* and 9 mixed infections, including 2 *Poc* and 1 *Pow* (in 3 *Pf+Po* cases, *Poc-Pow* Real-time PCR was not performed due to the unavailability of residual sample). In particular, by *Poc-Pow* Real-time PCR it was found that among the 32 *P. ovale* infections 20 were due to *Poc* and 12 to *Pow*.

Fig. 4. IMPORTED MALARIA CASES IN PARMA 2000-2012: 259/1,069 (24.2%) DIAGNOSIS BY MOLECULAR ASSAYS



CONCLUSION. Our results confirm, as already reported by us in 2007, that *P. ovale wallikeri* is not confined to Southeast Asia, since the majority of the patients analyzed in this study had acquired malaria in Africa and that the 2 *P. ovale* species are sympatric in the countries where they occur. It is interesting that in this study 9 of the 32 patients presented to the hospital between 3 months and 2 years after their arrival to Italy. Thus, it is likely that the samples from these patients contained parasites from relapses (parasite blood stages originating from the activation of a subset of the hypnozoites in the liver) indicating that relapses seem to occur in both species. The ability to detect and distinguish the 2 *P. ovale* species using the *Poc-Pow* Real-time PCR opens the way to epidemiological investigations of these parasites. As a matter of fact, *P. ovale* is one of the least studied among the *Plasmodium* species that infect humans. In the context of the goal of malaria control and eventual eradication, it becomes important to investigate *P. ovale*, given that its prevalence is likely to be substantially higher than previously thought also among imported malaria cases and that it can persists as chronic infection itself in the human host as a result of its ability to produce liver hypnozoites. Any meaningful investigations of the true epidemiology and biology of the 2 *P. ovale* species, whose infections lead to only relatively scanty parasitaemias even in primary infections, will necessitate the application of sensitive and specific molecular methods of detection.

REFERENCES. (1) Calderaro A et al. (2008). An 8 years-survey on the occurrence of imported malaria in a non-endemic area by comparing microscopy and molecular assays. *Diagn. Microbiol. Infect. Dis*. 61(4):434-9; (2) Rougemont M et al. (2004). Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J. Clin. Microbiol*. 42:5636-43; (3) Perandin F et al. (2004). Development of a Real-time PCR assay for detection of *Plasmodium falciparum*, *P. vivax* and *P. ovale* for routine clinical diagnosis. *J. Clin. Microbiol*. 42:1214-9; (4) Calderaro A et al. (2012). A new Real-time PCR for the detection of *Plasmodium ovale wallikeri*. PLOS One. 7(10): e48033; (5) Calderaro A et al. (2007). Genetic polymorphisms influence *P. ovale* PCR detection accuracy (2010). *J. Clin. Microbiol*. 45:1624-7; (6) Sutherland CJ et al. Two non recombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *J. Infect. Dis*. 1544-1550.

With the contribution of FONDAZIONE CARIPARMA

23rd European Congress of Clinical Microbiology and Infectious Diseases (Berlin, 27th - 30th April 2013)