Performance of real-time PCR in the diagnosis of imported malaria: comparison with microscopy and RDT, and impact on submicroscopic malaria.

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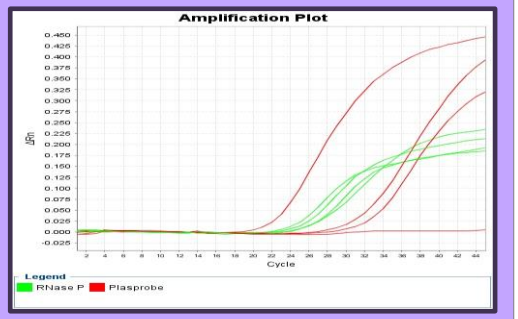
OBJECTIVES

For more than a century microscopy has been the reference standard for malaria diagnosis. Rapid diagnostic tests (RDT) have made easier the diagnosis but problems about sensitivity and specificity have arisen. These problems are especially relevant in malaria infections with parasite densities below the detection threshold of either microscopy or RDT. PCR-based diagnostic methods have surpassed microscopy methods but they are only performed in reference centers. The aim of this study is to know the utility of molecular methods in the routine diagnosis of malaria in a large sample of returned travelers with suspicion of malaria.

MATERIALS AND METHODS

Between December 2010 and June 2013 whole blood samples from patients that came to the Tropical Medicine Unit with suspicion of malaria were studied. Front-line diagnosis was performed by microscopic examination and a RDT. In addition, a pan-species quantitative real-time PCR (qPCR)[1] was performed in all cases and positive samples were further confirmed by a multiplex species-specific qPCR[2]. Molecular techniques were considered the gold standard. Biochemical, haematological and epidemiological data were also recorded.



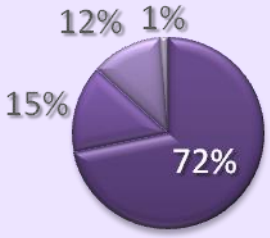
Pan-species quantitative real-time PCR

CONCLUSIONS

- **qPCR** was the technique which showed the **best performance** in terms of **sensitivity and specificity** to identify malaria infections compared to microscopy and RDT.
- **Submicroscopic malaria** is not a rare event and it was related with **haematological and biochemical alterations**.
- qPCR should be included as a **diagnostic tool in low-density infections** as in travellers who had an inadequate anti-malaria prophylaxis.

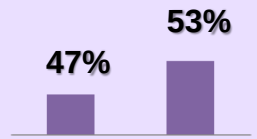
RESULTS

Country of origin



- Sub-Saharan Africa → Equatorial Guinea 47.1%
- Asia
- South America
- Others / no afilliation

Study Population



Mean Age

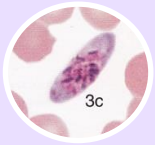
38.4 ± 13.6

3.1% children <14

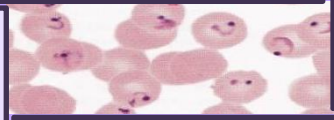
10% Positive cases

2 *P. malariae*

2 *P. vivax*



85 *P. falciparum*



qPCR as Gold Standard

	Microscopy	RDT
Sensitivity	75.4 %	86.4 %
Specificity	100 %	98.4%*

*12 false positive results

Submicroscopic malaria

34.8% Negative Microscopy but Positive qPCR results
80.6% with altered analitical determinations: anaemia and thrombocytoapaenia, and higher levels of LDH and transaminases

REFERENCES

[1]Rougemont M, et al. 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. 2004;42(12):5636-43.
[2]Shokoples SE, et al. Multiplexed real-time PCR assay for discrimination of Plasmodium species with improved sensitivity for mixed infections. J Clin Microbiol. 2009; 47(4):975-80.