Genetic diversity studies of *Plasmodium falciparum* and *Plasmodium vivax* isolates circulating in endemic areas from Panama, Central America

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INTRODUCTION

After more than three decades of apparent control, malaria has reemerged as an important public health problem in Panama, Central America. Since 2001, the incidence of malaria has increased to epidemic proportions, reaching 5,094 new cases during 2004 (4,213; 82.7% *P. vivax* and 881; 17.3% *P. falciparum*), the highest incidence since 1971 (Figure 1). In the last two years, the number of malaria cases showed a negative trend probably due to the change in drug policy to treat *P. falciparum* cases coupled with the intensification of vector control activities.

Malaria genetic diversity studies are important to understand the mechanisms underlying the pathology and epidemiology of the disease in a country. Thus, we conducted a molecular epidemiology study to establish the genetic makeup of *P. falciparum* and *P. vivax*, the two prevalent malaria parasites circulating in endemic regions from Panama (Figure 2).

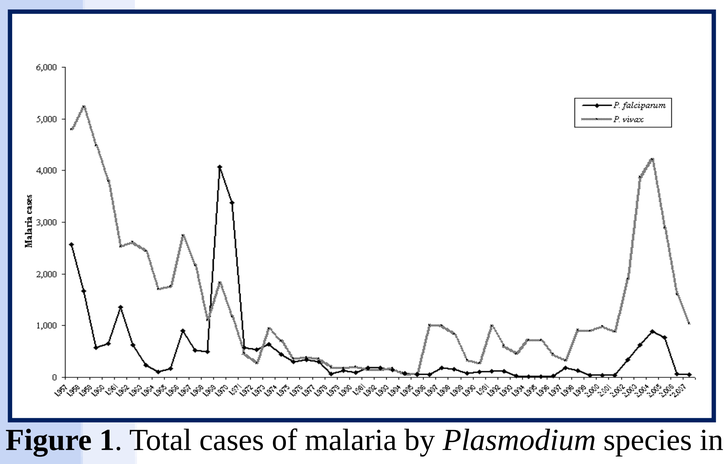

Figure 1. Total cases of malaria by *Plasmodium* species in Panama, 1957 – 2007

METHODS

From 2006 to 2008 an active search for malaria symptomatic patients was performed. Finger-prick blood was collected in filter paper and DNA was extracted by the Chelex method (Figure 3). Malaria positive samples (n = 115; 68 *P. vivax*, 45 *P. falciparum*, 2 mix infections) were diagnosed by a nested PCR (Figure 4). To assess the genetic diversity of malaria parasites circulating in the country, 45 *P. falciparum* and 68 *P. vivax* were selected, representing the main endemic areas in Panama. The following polymorphic markers were analyzed by different molecular methods: *P. falciparum* (MSP-1, MSP-2 and GLURP); *P. vivax* (CSP, MSP-1 and MSP-3)

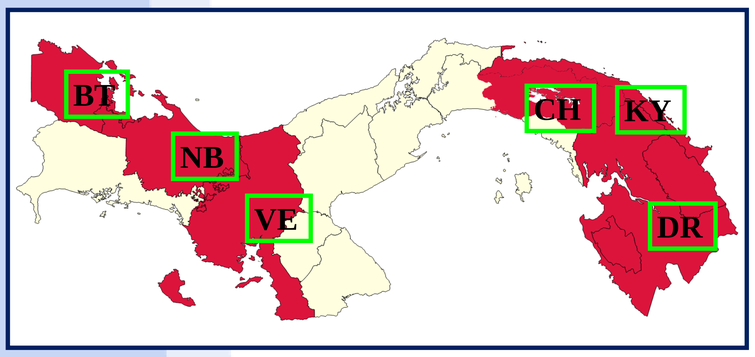

Figure 2. Malaria endemic areas of Panama: BT (Bocas del Toro), NB (Ngobe Bugle), VE (Veraguas) CH (Chepo) KY (Kuna Yala), DR (Darien).

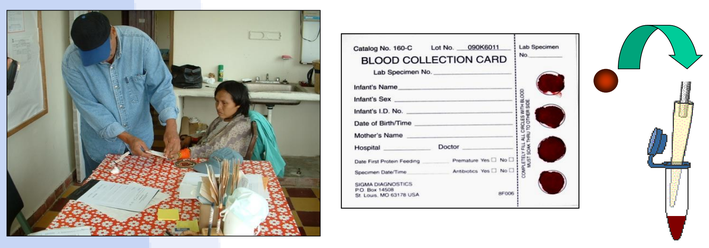

Figure 3. DNA extraction from blood collected in filter paper.

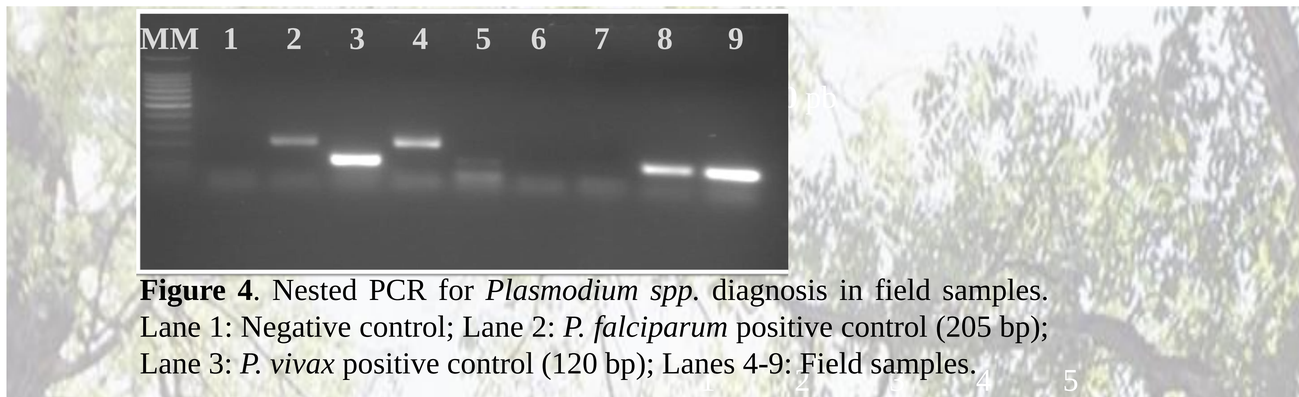

Figure 4. Nested PCR for *Plasmodium spp.* diagnosis in field samples. Lane 1: Negative control; Lane 2: *P. falciparum* positive control (205 bp); Lane 3: *P. vivax* positive control (120 bp); Lanes 4-9: Field samples.

RESULTS

Plasmodium falciparum

GLURP: Three genotypes were found. Genotype A (~ 1,200 bp) was found in 20 samples (16 from Chepo, 3 from Darien and 1 from Eastern Panama). Genotype B (~ 700 bp) was observed in 25 samples from Kuna Yala and Genotype C (~ 800 bp) in one imported case (Figure 5)

MSP-2: Two genotypes were observed. In Kuna Yala, 23 samples belong to the 3D7 and 1 to FC27 allelic family. In Chepo and Darien all 19 samples belong to the 3D7 family. In two imported cases one sample was FC27 and the other 3D7 (Figure 6).

MSP-1: Only one genotype was detected in all the field isolates.

Plasmodium vivax

CSP: Two different CSP sub-types were observed in isolates bearing the VK210 repeats (Figure 7).

MSP-1: A single genotype was observed in all the samples

MSP-3: Three different genotypes were detected. In Bocas del Toro and Veraguas only Genotype I was observed, while in Darien (Genotypes I, II and III) were circulating. (Figure 8)

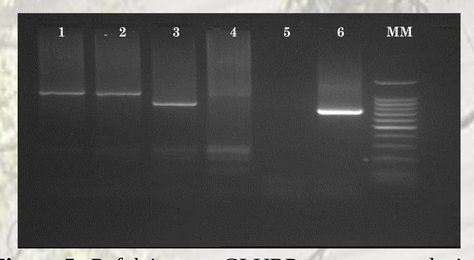

Figure 5. *P. falciparum* GLURP genotype analysis by nested PCR. Lanes 1, 2 and 4:1,200 bp; Lane 3: 800 bp; Lane 5: Negative control; lane 6: 700 bp.

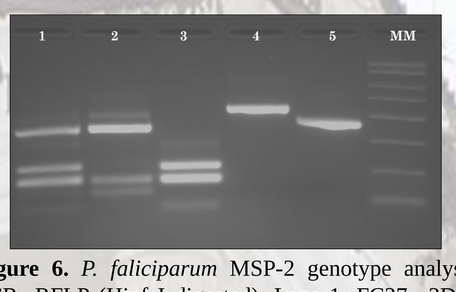

Figure 6. *P. faliciparum* MSP-2 genotype analysis by PCR- RFLP (Hinf I digested). Lane 1: FC27– 3D7 mix infection; Lane 2: 3D7; Lane 3: FC27; Lanes 4 and 5: Non-digested products.

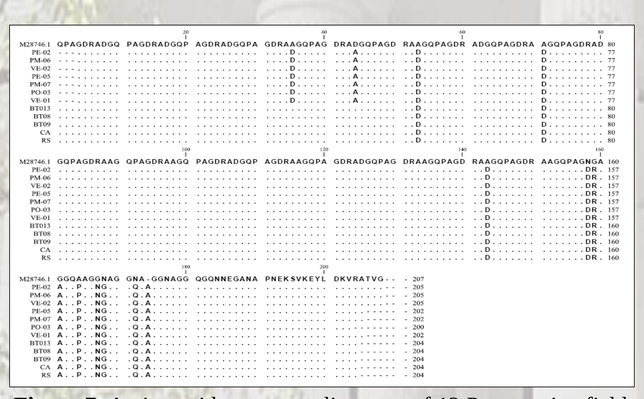

Figure 7. Amino acid sequence alignment of 12 Panamanian field isolates representing *P. vivax* from the main Panamanian endemic areas and bearing the Vk10 repeat type.

Figure 8. *P. vivax* MSP-3 genotype analysis by PCR-RFLP (Alu I digested). Lanes 1 and 2: Allelic pattern I; Lanes 3, 4 and 6: Allelic pattern II: Lane 5: Allelic pattern III; Lane 7: Non digested PCR product

CONCLUSIONS

- Genetic analysis from both malaria species demonstrate that parasites circulating in Panama present a limited genetic diversity, a result that is in agreement with the low transmission intensity observed in the country.
- *Plasmodium* parasites isolated from Darien Province, near Colombian border, presented the higher diversity (at least three different genotypes). This may be the consequence of the high migration between both countries.
- Since genotype analysis may serve to establish association with epidemiological parameters it is important to establish a regional surveillance network for monitoring the propagation of pathogenic genotypes.

Acknowledgement: This study was partially funded by the Secretaria Nacional de Ciencia, Tecnología e Innovación (SENACYT, FID06-173).