LB-5219
Poster Session B

Sequence Analysis of *Plasmodium falciparum* histidine-rich protein 2 and 3 genes from returning travelers to Africa

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Background:

- WHO reported 219 million new cases of malaria with half million deaths in 2017
- Sub-Saharan Africa and Children under 5 accounted for majority of cases and deaths.
- 5 human species – *Plasmodium falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*
- *Plasmodium falciparum* accounts for 99% of Malaria deaths
- Malaria is not endemic in Canada but we have competent vectors for transmission
- Travellers from endemic areas account for 500 cases/year
- Current diagnosis of Malaria in Canada include microscopy, rapid diagnostic test by antigen detection of histidine-rich proteins 2 and 3, and PCR
- We aimed to study the sequence heterogeneity of histidine-rich protein 2 and 3 genes in *Plasmodium falciparum* cases which failed detection by HRP2 specific rapid diagnostic test

Materials and Methods:

- *Plasmodium falciparum* whole blood samples from cases which failed BinaxNow rapid diagnostic test were identified
- DNA extraction was performed and species were confirmed by real time PCR
- Plasmodium histidine-rich protein 2 (*pfhrp2*) and 3 (*pfhrp3*) genes were PCR amplified and Sanger Sequenced
- Sequence alignment and analysis were performed by MEGA 6.06 software

Figure 1. Real time PCR to confirm malaria species

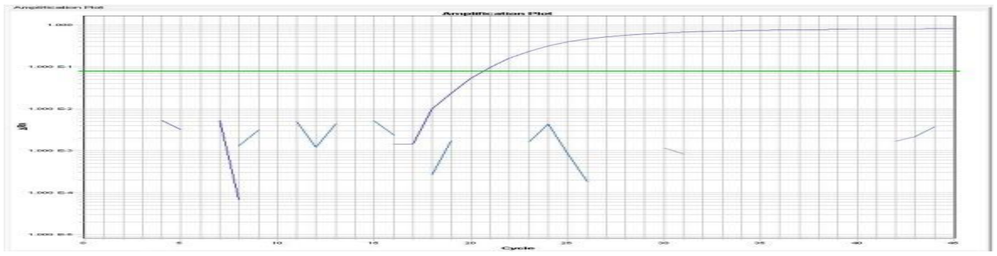

Figure 2. DNA sequence generated by Sanger Sequencing

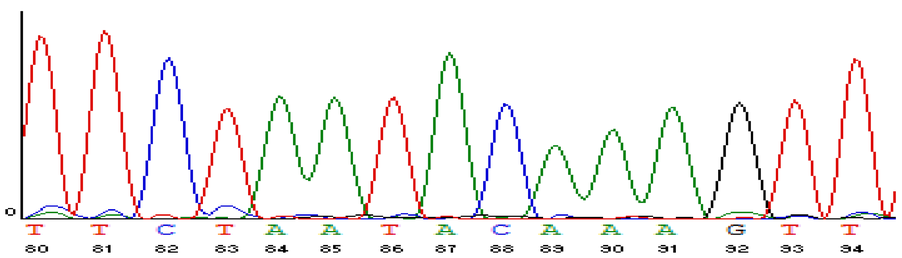

Table 1. Deletion of *pfhrp2* and *pfhrp3* genes described in the literature

Gene	basepair	Gene Structure	Expression	Mutation/Deletion
Pfhrp2	1064	2 exons, 1 intron	HRP2	Deletion (Brazil, Peru) Partial Deletion (Bangladesh) Partial and full deletion (Kenya)
Pfhrp3	983	2 exons, 1 intron	Epitopes homologous to HRP2	Deletion (Brazil, Peru) Mutation (Bangladesh)

Results:

- Four *Plasmodium falciparum* cases that failed BinanxNow rapid diagnostic test were identified
- Real time PCR confirmed three were mono-*Plasmodium falciparum* infections whereas one was a mixed infection

Table 2. *Plasmodium falciparum* cases that failed BinaxNow rapid diagnostic test
TBD – To be determined

Case	Travel (Country of Acquisition)	Year	Age	Sex	Microscopy	Parasitaemia	BinaxNow T1 (PfHRP2)	BinaxNow T2 (Pan-Aldolase)	*Pfhrp2*	*Pfhrp3*
1	Nigeria	2016	39	M	*Pf*, Rings	<0.1%	-	-	No mutation compared to control (400bp of exon2)	No mutation compared to control (400bp of exon 2)
2	Nigeria	2017	42	M	*Pf*, Rings	<0.1%	-	+	No mutation compared to control (700bp of exon 2)	TBD
3	Tanzania	2017	13	M	Neg but *Pf* PCR Pos, low level infection	N/A	-	-	TBD	TBD
4	Ivory Coast	2018	50	M	*Pf* and *Pm* mixed infection. Rings, Trophozoites, Schizonts	0.2%	-	-	TBD	TBD

Conclusions:

- No mutation was found in exon 2 region of pfhrp2 and pfhrp3 DNA sequence analysis for the cases where primary material was available for sequencing
- Detection failure may be due to low expression of PfHRP2 -> detection failure by BinaxNow RDT
- In non-endemic lab setting with waning microscopy expertise
  Ambiguous microscopy smear + PfHRP2 RDT Neg may lead to:
  -> Misdiagnosis as non-*falciparum* Malaria or Neg for Malaria
  -> Inappropriate treatment with Chloroquine for presumed *Plasmodium vivax* or other non *falciparum* malaria
  -> Outcome can be detrimental (Death)!

Future Analysis:

- Further DNA analysis of exon 1 and full exon 2 of both genes
  -> Insight on any sequence heterogeneity that may affect binding to the RDT
- Analysis of remaining PfHRP2 RDT neg Plasmodium falciparum cases
  -> Etiology of detection failure
- Continual Surveillance
  -> Inform lab diagnostics and physicians of potential pitfalls
  -> Possible modification in diagnostic algorithm

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