Leishmania species identification using PCR-RFLP, DNA sequence, HRM methods

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Introduction

Leishmania parasite is specific; it has its own specific sand fly species and specific reservoir host.

Leishmania detection and identification is important in order to understand disease transmission.

Different methods can be used for *leishmania* parasite detection and identification.

Hypothesis:

We hypothesize that the DNA sequencing is the most reliable method of leishmania parasite detection

Objectives:

1. To detect and identify leishmania parasite using ITS PCR-RFLP.
2. Conduct DNA sequencing to identify leishmania parasite.
3. Using PCR- HRM method to detect and identify leishmania parasite.

Method	HRM	DNA sequence	RFLP
Advantage	1. Cost effective 2. Fast / simple 3. More accurate 4. Low reagent/sample consumption	1. More accurate 2. Fast	1. Cost effective 2. Simple
Disadvantage	1. Overestimation (Cybergreen dye) 2. Close melting point	1. Expensive 2. Reference genome required	1. Slow 2. Long process 3. Require large sample size 4. Less widely use

Results:

Sample	HRM	DNA sequence	RFLP
1	Positive	*L. tropica*	No result
2	Positive	*L. infantum*	No result
3	Positive	*L. tropica*	No result

Discussion:

- DNA sequencing is the most reliable method to detect *Leishmania* parasite.
- In HRM the melting point of *L. infantum*, *L. donovani*, and *L. tropica* are almost the same.
- In the RFLP experiment, the results did not appear. ???

Thank you

Questions??

- Problem solving of primer cross-reactivity.
- The possibility to develop a quantitative blood meal analysis that can differentiate between old and fresh blood meals.
- Drug testing – how can you adapt for your parasite