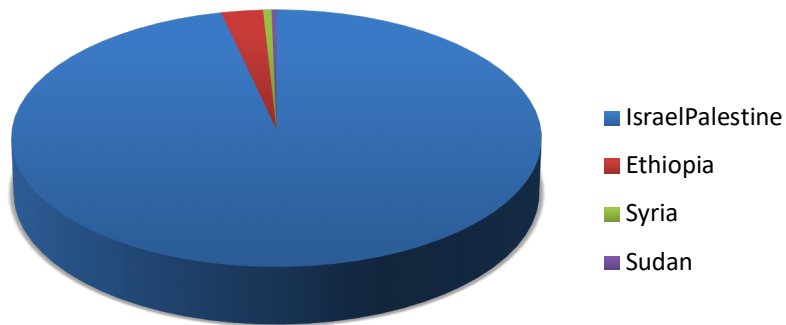


# **Next Generation Sequencing-ITS1**

MeBOP 2018

# NGS-ITS1

## Total Reads per country

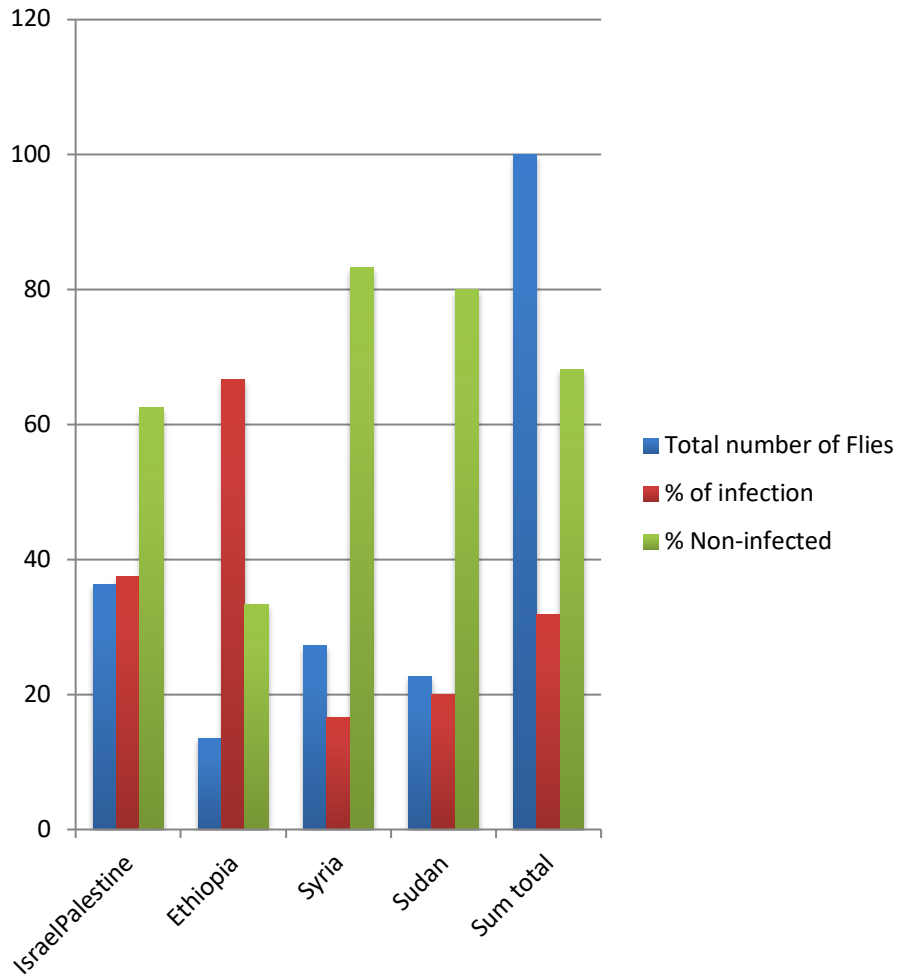


Country	total
Israel-Palestine	4749
Ethiopia	134
Syria	27
Sudan	16

The highest readout from Israel-Palestine followed by Ethiopia

# ITS1-NGS

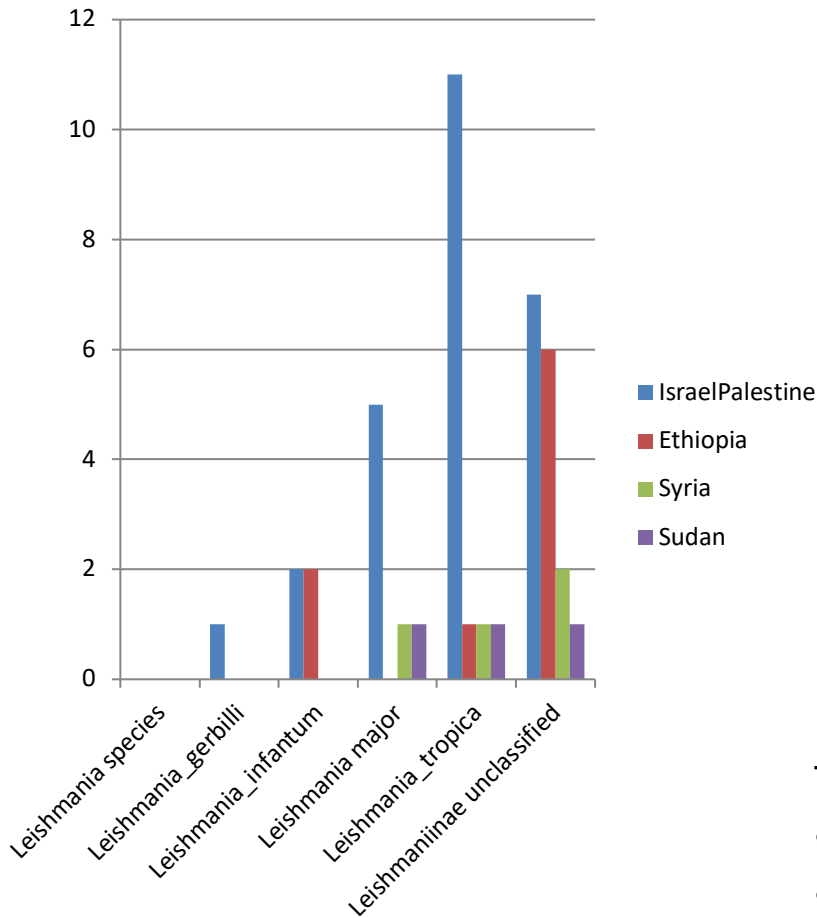
## Infected flies per country



	Total number SF	Infected
Israel-Palestine	32 (36,4%)	12(37,5%)
Ethiopia	12 (13,6%)	8 (66,7%)
Syria	24 (27,3%)	4 (16,7%)
Sudan	20 (22,7%)	4 (22,7%)
Total	88(100%)	28 (31,8%)

# ITS1-NGS

## Identified Leishmania species per country



	country					
	IsraelPalestine	Ethiopia	Syria	Sudan	Sum total	%
L. gerbilli	1	0	0	0	1	2,4
L. infantum	2	2	0	0	4	9,5
L. major	5	0	1	1	7	16,7
L. tropica	11	1	1	1	14	33,3
Leishmaniinae unclassified	7	6	2	1	16	38,1
					42	100,0

The dominant leishmania species were

- L. tropica
- L. major
- L. Infantum

Mixed infection per sand fly

Israel-Palestine

taxlevel	rankID	taxon											
taxonomy	total	S77	S79	S80	S81	S82	S83	S84	S85	S86	S87	S88	S96
		1	14	188	3189	8	7	1301	3	6	28	3	1
Root	4749												
L. gerbilli;	50	0	0	5	0	0	0	0	0	0	0	0	0
L. nfantum;	2910	0	0	0	0	0	4	287	0	0	0	0	0
		0	0	23	3093	3	1	842	0	0	0	0	0
L. major;	3962												
L. tropica;	2081	0	0	145	19	5	1	1	3	4	25	3	1
		0	14	15	77	0	1	171	0	2	3	0	0
L. unclassified;	283												

Syria

taxlevel	rankID	taxon				
taxonomy	total	S21	S23	S24	S36	
Root	27	22	3	1	1	
L. major	1	0	0	0	1	
L. tropica;	3	0	3	0	0	
L. unclassified	23	22	0	1	0	

# Mixed infection per sand fly

## Ethiopia

taxlevel	rankID	taxon							
taxonomy	total	S48	S49	S50	S54	S56	S58	S59	S61
Root	13428	1	2	20	57	4	20	2	
Leishmania_infantum;	640	0	0	16	48	0	0	0	
Leishmania_tropica;	40	0	0	0	0	4	0	0	
Leishmaniinae_unclassified	28	1	2	4	9	0	20	2	
;	66								

## Sudan

taxlevel	rankID	taxon	daughterlev els	total
taxonomy	total	S10	S2	S9
Root	16	10	5	1
Leishmania_major;	1	0	0	1
Leishmania_tropica;	10	10	0	0
Leishmaniinae_unclassified;	5	0	5	0

Q2:

- Comparison between ITS-1 and cytochrome b PCRs in terms of:
  - Sensitivity and specificity. What factors can affect PCR sensitivity?
  - Possibility of post amplification analysis that could lead for species identification.
-

## **Comparision between ITS-1 and Cytochrome b PCR in terms of sensivity and specificity.**

- ITS-1 is more sensitive but less specific
- Cytochrome b is specific but less sensitive
- Sensitivity means that: how much the test can detect the positive cases
- Specificity means that: how much the test can detect the negative cases



# What factors can affect PCR sensitivity?

- Amount of DNA sample
- Denaturation, melting temperature
- Cycle number
- Primer length and primer design
- Elongation time

# **Possibility of post amplification analysis that could lead for species identification?**

- DNA sequencing
- HRM analysis

Q4:

- qPCR-kDNA system followed by HRM
  - Sensitivity compared to other systems.
  - If other used PCR systems are suitable for HRM analysis (what type of experiments are needed).
- Answer
  - QPCR-HRM is more sensitive. Because it detects a single nucleotide change
  - Sequencing of a gene is done first then HRM follows.

Q5:

- ITS1 and Leishmania cytochrome b PCR sequence analysis their suitability for creating phylogenetic tree.
  - We have ITS1 and cytochrome b DNA sequence for the Old World *Leishmania* species, can these genes be used for New world *Leishmania* species?
  - What do we need to check?
  - **Answer:**
    - **Occurrence:**
      - **Old world Leishmanias:** Eastern hemisphere-endemic in Asia, Africa and Southern Europe
      - **New world Leishmaniasis:** Endemic in western hemisphere extending from South central Texas to central and South America (except Chile and Uruguay).
      - **Not present in:** Australia, Antarctica or Pacific islands
    - **Diagnosis**
      - ITS1-PCR-Restriction Enzyme Analysis-Old World Leish
      - Leish-Cytochrome PCR: both old world and New world PCR

**Table 2 The detection limit of seven PCR methods for the detection of *L. siamensis***

Target gene	PCR results by											
	Number of parasites/μl*						DNA concentration (pg/μl)					
	500	50	5	0.5	0.05	0.005	23000	2300	230	23	2.3	0.23
ITS1	+	+	+	+	+	-	+	+	+	+	+	-
<i>cpb</i>	+	+	+	-	-	-	+	+	+	-	-	-
<i>cyt b</i>	+	+	+	-	-	-	+	+	+	-	-	-
<i>hsp70</i>	+	+	+	+	-	-	+	+	+	+	-	-
mini-exon	+	+	+	+	-	-	+	+	+	+	-	-
SSU-rRNA	+	+	+	+	+	-	+	+	+	+	+	-
<i>tim</i>	+	+	+	+	-	-	+	+	+	+	-	-

Hitakarun et al. Parasites & Vectors 2014, 7:458:

the small subunit ribosomal RNA region (SSU-rRNA), the internal transcribed spacer 1 region (ITS1), cysteine protease B (*cpb*), cytochrome b (*cyt b*), heat shock protein 70 (*hsp70*), the spliced leader mini-exon, and the triose-phosphate isomerase (*tim*) genes were compared.

### ITS1-More sensitive than Cytochrome B

- Using the ITS1 region showed a high sensitivity because approximately 20–400 copies per *Leishmania* genome exist
  - Specificity ?=>positive PCR products have to be confirmed by sequence analysis
- *Cyt b*=> low sensitivity but high specificity (100%)

*Thank You very much*