Fogging of hyrax dens by Pyrethroid derivatives: an attempt to control sandfly density in rural areas in Palestine-TDR 18-7

SECTION A. GENERAL INFORMATION

- PI name: Dr. Amer Al-Jawabreh
- Reporting Period: October-December 2019
- Objectives of the study:
    - General: To test the efficacy of pyrethroid thermal-fogging method in reducing the incidence rate of human CL by reducing the sandfly density in rock hyrax dens
    - Specific:

1. Investigating the effective of thermal fogging of Hyrax dens by sandfly density and species diversity in the study hyrax dens before and after spraying.
2. Comparing the species on the study dens with control ones where no fogging occurs.
3. Determining the prevalence of and type of Leishmania parasite in the collected sand fly species.

SECTION B. TECHNICAL REPORT:

INTRODUCTION:

Cutaneous leishmaniasis (CL) is endemic in Palestine and considered a public health problem. Incidence rate ranges from 8 to 12 per 100,000 (Ministry of Health, 2010-2016). Tubas district was the most affected district, about 816 cases were reported during 1990 to 2018, where the disease is considered to be zoonotic, caused by *L. tropica,* vectored by *Phlebotomus sergenti,* and rock hyraxes, *Procavia capensis* serve as the reservoir animal.

Prevention measures of CL in Palestine are almost limited to the control of adult sand flies using residual insecticide in dwellings and its surrounding sprayed at the beginning and end of the biting season. In case of zoontic cutaneous Leishmaniasis (ZCL), control of adult sandflies in habitats of coexistence with the reservoir animal could be promising effective control measure. This controlled intervention research aims to

study the impact of utilizing thermal fogging of insecticide using Permithrin, in and outside hyax's dens, on sandfly density and reducing the number of sandflies infected with Leishmania parasites.

METHODOLOGY:

Study design:

A pilot intervention study design is used to assess efficacy of thermal fogging on sandfly density in Tubas district in Palestine.

Sandflies Classification and Identification:

Collected sandflies were removed from traps, washed in detergent solution, labelled and stored in 70% alcohol, for each trap separately. Then sandflies were dissected and mounted in Berlese's medium, identified based on the taxonomic keys (Lewis 1982, Büttiker & Lewis 1983, Lane 1986). Gravid and engorged female sandflies were dissected, head and the terminal abdominal segments mounted in Berlese's medium for identification and the remaining parts kept in 70% ethanol for DNA extraction and PCR to check for the presence and species of leishmania parasite.

Genomic DNA Extraction from Sandflies:

Genomic DNA from sandflies was extracted using manual phenol-chloroform method modified after Casaril *et al.*, 2017. Briefly, Individual sandly was picked from the 70% ethanol-filled 1.5 ml tube and transferred into new 1.5 ml tube. Two hundred µl of DNA lysis buffer were added. The tube was beaten by bead beater for 5 min at 1850 rpm with glass bead to crush and homogenize the sandfly. Ten µl of proteinase K (10 mg/mL) were added and incubated overnight at 56 °C. Two hundred and fifty µl of Phenol solution (PH:8) were added to samples and vortex for 1 min then centrifuged for 3 minutes at 14000 rpm, and the aqueous layer which contain the DNA was collected in new 1.5 ml . One tenth µl of 0.2 M sodium acetate and 700 µL of ice-cold 100% ethanol were added and samples incubated overnight at -20°C to precipitate DNA. The samples were then centrifuged for 30 minutes at 14000 rpm. The supernatant was discarded, and the pellet was washed with 0.5 ml of 70% ethanol,

followed by centrifugation for 5 minutes at 14000 rpm. Ethanol was evaporated using thermal block (Eppendorf) at 60°C. The samples were re-suspended in 50 µl of 1× TE and kept -20 C until further use.

Data collection:

Data was collected using computerized excel sheets into which codes of sandflies were fed. The code consisted of number and type of traps, and geographical location. The form was filled by the entomologist performing the classification and the laboratory technician conducted the DNA extraction.

Ethical considerations:

The study does not need an approval from a research ethics committee because it involves solely sandflies outside the towns and villages and has no involvement of human subjects of any kind.

Activity implementation:

Time period	Activities
March-April	CDC traps, sticky traps, tubes, ethanol, markers, racks, and other consumables were prepared.
Recruit staff	Field workers including an entomologist and lab technician were recruited
Prepare staff	26.6.2019: A field visit was conducted to select the work sites and familiarize the team with the work.
Pre-Intervention Sandfly trapping	3 trapping sessions in the second half of June to select study sites based on sandfly density
Sandfly trapping	13 trapping-collection sessions from July to September.
Thermal fogging	One thermal fogging session was conducted, mid-August.
Dissection and classification	6000 sandflies were classified
DNA extract	150 out of 300 engorged sandflies were DNA-extracted.
PCR	Pending, waiting for materials to be delivered.
Data entry	Pending
Data analysis	Pending
Final report	Pending

Preliminary Results:

The total number of sandflies trapped from the 9 sites in 13 session were 10,000. 6000 sandlies were mounted on slides using Berles's mounting media. Slides will be stored for one month for mounting media to start clearing sandflies and make them ready for classification. Around 60% of the sandflies were *Sergentomyia spp* and 40% *Phlebotomus spp*.

Of the 6000 dissected sandflies, the midgut of the 250 engorged (blood-filled) were kept in 1.5 ml tube filled with 70% ethanol and stored until use. The genomic DNA was extracted from 150 engorged sandflies manually using phenol-chloroform method according to Casaril *et al.*, 2017.

References:

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