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Characterization of breeding sites of *Phlebotomus orientalis* – The vector of visceral leishmaniasis in northwestern Ethiopia

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ABSTRACT

We studied breeding sites of *Phlebotomus orientalis* (Diptera: Psychodidae) the vector of visceral leishmaniasis in northern Ethiopia. Although numbers were rather small, 165 sand flies were captured emerging from vertisol cracks. The most productive breeding sites were cracked vertisols, dry river banks and close to trees. No sand flies were caught emerging from sandy clay loam soils in peri-domestic habitats but a few were captured emerging from gaps in a stone wall. Abiotic parameters in vertisols close to trees and in open field from which *P. orientalis* had emerged, were compared. Soil pH was slightly alkaline and salinity was low. Organic matter contents were similar in both sites. Temperatures and RH remained relatively stable near trees from the end of the rainy season through mid dry season, yet fluctuated markedly at the shallower depth in the open field. During the rainy season, cracks in the soil were sealed resulting in significant lowering of the oxygen concentrations near the tree. Gravimetric water content of soil near trees was lower than open field at shallow depth but similar deeper down. We conclude that ambient conditions suitable for sand fly larvae at shallow depths (45 cm) are restricted to areas close to trees. However, deeper in vertisols (90 cm) suitable conditions are apparently maintained throughout the dry season even in open fallow fields.

1. Introduction

The leishmaniases endanger some 350 million people in 88 countries. Cutaneous leishmaniasis (CL) manifests as a sore at the bite site of the infected sand fly and is usually self healing. Visceral leishmaniasis (VL) also known as Kala Azar, is a life-threatening systemic infection. There are 200–400 thousand new leishmaniasis cases annually, with an estimated 20–40 thousand deaths (Alvar et al., 2012). VL is caused in East Africa and the Indian subcontinent by *Leishmania donovani*, while in Europe, North Africa and Latin America the causative parasite is *L. infantum*. The most important VL foci in Ethiopia are in the Humera/Metema lowlands bordering the endemic areas of Sudan. Most VL cases in the Humera/Metema regions are among migrant agricultural laborers arriving from non-endemic regions to work in the vast sesame fields of the region (Hailu et al., 2007). Recently a VL outbreak was reported from the district of Tahtay Adiyabo (Sheraro), located some 120 km east of Humera where the affected human populations were local villagers (Abbasi et al., 2013).

The vectors of leishmaniasis in the old world are blood-sucking phlebotomine sand flies (Diptera: Psychodidae) belonging to the genus *Phlebotomus* (Maroli et al., 2013). In Sudan and Northern Ethiopia. *Phlebotomus orientalis* has been incriminated as the main vector of VL (Kirk and Lewis, 1955). *P. orientalis*, females are essentially exophagic and rarely enter houses (Gebre-Michael et al., 2010). Dense populations of *P. orientalis* were reported in Acacia-Balanite (*Acacia seyal* and *Balanites aegyptiaca*) woodlands growing on deeply-cracked vertisols (black cotton soil) (Elnaiem, 2011; Hoogstraal and Heyneman, 1969). In recent years, much of the endemic region in the Humera/Metema lowlands has been cleared of trees and converted to agricultural land growing sesame, sorghum and cotton. Despite this, VL remains rampant and migrant

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laborers arriving in the industrialized farms from non-endemic regions in Highland areas of the country, comprise the bulk of the VL cases in this area (Hailu et al., 2007). Sand flies have clearly adapted to the open agricultural fields where they may be captured in large numbers (Gebre-Michael et al., 2010; Moncaz et al., 2013)

Sand flies are holometabolous insects that breed in humid dark habitats where larvae feed on composting organic matter of animal and plant origin. Mature sand flies are small and fragile nocturnal insects that normally fly close to the ground and refrain from flight activity under windy conditions, remaining within several hundred meters of their breeding place during their entire life time (Killick-Kendrick, 1999).

The widest gap in our understanding of sand fly biology remains their larval ecology. In the insectary, optimal rearing conditions for different sand flies are often remarkably uniform. For example, desert-dwelling *P. papatasi* from the Middle East, and Neo-tropical *Lu. longipalpis* from Latin America (as well as other species), are optimally reared under remarkably similar conditions (26 ± 2 °C, 85–95% RH, composting rabbit feces-based larval diet) (Volf and Volfova, 2011). This observation indicates that in nature sand flies must locate and oviposit in specialized ecological niches that offer such conditions for developing larvae. Small numbers of larvae have been recovered from diverse habitats including caves, crevices, animal burrows, termite mounds, cracks in the soil, domestic animal shelters, cracked walls, tree-holes, birds' nests and leaf litter (Feliciangeli, 2004; Singh et al., 2008). There are only two documented examples of more productive sites: In Sardinia 310 *Phlebotomus* spp. larvae were recovered from topsoil inside an abandoned shed (Bettini, 1989). In Panama, 2258 *Lutzomyia* spp. larvae were found in soil samples obtained from forest floors (Hanson, 1961). Additional methods for identifying breeding sites include emergence traps (ETs) specifically designed to capture adults emerging from their breeding sites. Such traps proved useful in characterizing sand fly breeding sites in Brazil and Israel (Casanova et al., 2013; Moncaz et al., 2012).

The aim of the current study was to identify and characterize putative *P. orientalis* breeding sites in vertisol cracks and peridomestic habitats by capturing adults emerging from suspect habitats. In addition we measured several abiotic parameters in the soil to assess whether ambient conditions may be suitable for sand fly larvae.

2. Materials and methods

2.1. Study sites

Kafta Humera Woreda (Western Tigray) – 14.17′19″ N 36.37′18″ E 600 m′ AMSL. The town of Humera is surrounded by vast fields of sesame, sorghum and cotton. The Humera and Metema districts comprise the largest and most important focus of VL in Ethiopia affecting mostly migrant agricultural laborers (Deribe et al., 2012; Hailu et al., 2007). Sand flies were collected during late November 2010 – early dry season, shortly after the sesame and sorghum harvests.

Shiraro [=Sheraro]–Tehatay Adiabo Woreda (North Western Tigray) – 14.17′19″ N 36.37′18″ E 1000 m′ AMSL. Rural area with a stable population, considered an emerging focus of Kala Azar. Sand flies were collected during different periods between October 2011 and March 2013.

2.2. Putative breeding sites

Preliminary surveys (Fig. 1) identified several putative sand fly breeding sites in vertisols: (1) Trees (fissures in bark, holes in trunks and root systems of common woody species); (2) Cracked vertisols in sesame and sorghum fields; (3) Termite mounds; (4) Dry riverbeds and river banks; (5) Rodent burrows; (6) peridomestic environments in clay loam soil, included areas under trees and bushes, stone walls and stone piles resulting from the collapse of residential hut (Tukul). Sand fly trapping was restricted to the dry season because adult sand flies were very scarce during the rainy season and dirt roads became impassable.

2.3. Trapping methods

Emergence traps (ETs) were constructed as described previously (Moncaz et al., 2012). Briefly, large horizontal sticky traps (LHSTs) were placed over or next to the exits of suspected breeding sites and covered with a sand fly-proof net (186 holes per square centimeter). The LHSTs were made of white polypropylene boards, measuring 60 cm × 60 cm, placed horizontally on square metal frames approximately 15 cm above ground. The top sides of the boards were smeared with sesame oil. Individual or multiple LHSTs were covered by sand fly-proof netting to enclose smaller or larger areas. ETs with one LHST covered an area of approximately 4 m^2.

ETs were placed over cracked vertisols (Fig. 1A and B), in dry riverbeds (Fig. 1C), over tree root systems (Fig. 1D), tree trunks and buttresses (Fig. 1E), loose stone walls surrounding farm yards (Fig. 1F) and over a stone pile produced by a collapsing hut (=tukul). ETs for larger areas comprised several LHSTs enclosed under a common net (hereafter tunnel-trap). Tunnel ETs covered areas greater than 10 m^2 (Fig. 2A). ETs for vertical soil river banks comprised multiple LHSTs supported by pegs driven into the soil bank and covered in netting (Fig. 2B). In some cases, prior to placement of the LHSTs and the net cover, approximately 50 cm of the soil river bank were excavated to expose the more humid subterranean milieu (Fig. 2C).

2.4. Identification of sand fly species, gender, and age

Sand flies were removed from the sticky straps using fine watchmakers' forceps and placed in 70% ethanol. They were mounted in Hoyer's medium with their heads separate from thoraces. Flies were identified to species based upon cibarial and pharyngeal armature as well as spermatheca of females and external genitalia of males (Lewis, 1982). Age-grading of wild-caught male sand flies was performed based on the orientation of their external genitalia (Fig. 2D–E). The external genitalia of male sand flies rotate through 180° around the longitudinal body axis during the initial 16–24 h of adult life (Moncaz et al., 2012).

2.5. Microclimatic and physicochemical soil parameters

Several abiotic soil parameters were monitored in putative breeding sites to determine their potential suitability as breeding sites. The volumetric water content (VWC) of the soil was measured continuously under trees as well as in the open fields at different depths from the end of the rainy season (Sept. 2012) until mid dry season (March 2013). Oxygen concentration in subterranean air spaces was sampled periodically. Soil samples were taken for laboratory analyses of physicochemical parameters during mid-dry season.

2.5.1. Monitoring of the soil microclimate

Temperature and relative air humidity (RH) sensors as well as volumetric water content (VWC) sensors (Onset© S-THB-M002) were buried 1 m from the trunk of a *B. aegyptiaca* tree within its root system in partial shade of its canopy. A second set of sensors was buried in an open field 10 m away from the tree. Two sensors each were buried at depths of 45 and 90 cm in both of these habitats. The wirings of the sensors were inserted into PVC tubes and