814 - Ecology and Transmission Dynamics of Visceral Leishmaniasis in Ethiopia: results of a prospective study to determine human infection rates in an endemic area of north Ethiopia

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Ecology and transmission dynamics of Visceral Leishmaniasis in Ethiopia
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Introduction

Globally, visceral leishmaniasis (VL), a systemic protozoan infection caused by *Leishmania donovani* spp. is estimated to afflict some 500,000 persons annually. In Africa, the worst affected regions are southern Sudan (15,000-20,000 cases/yr) and Ethiopia (4,000-7,000 cases/yr). VL is considered an emerging disease in north Ethiopia where it is associated with seasonal migration of agricultural laborers to endemic areas and exposure to HIV infection. A prospective cohort study was designed to elucidate the transmission dynamics of *Leishmania donovani* infections in endemic communities in northern Ethiopia.

Objectives

The overall aim of the project is decipher the transmission dynamics of visceral leishmaniasis in northern Ethiopia and, thereby, facilitate the design of effective control strategies for VL in sub-Saharan Africa.

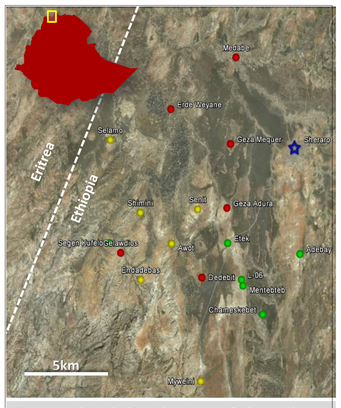
Specific aims

1) To determine the prevalence of symptomatic and asymptomatic infections in humans using serological and molecular techniques.
2) To determine parasite-carrier rates in persons with asymptomatic/sub-clinical *L. donovani* infections using quantitative real time PCR.
3) To provide quantitative estimates of parameters needed for mathematical modeling of transmission.
4) To determine infectivity of asymptomatic human carriers to sand flies by xenodiagnosis.

Materials & Methods

Sheraro, an endemic locality in northern Ethiopia, was selected for in-depth prospective (repeated cross-sectional) epidemiological studies of VL transmission. A base-line survey was carried out in March 2011 involving 4,883 individuals living in 18 villages (clusters). Participating households (n=1,386) were numbered and their coordinates were recorded. Demographic and socio-economic data were collected. Screening for VL by physical and laboratory examination was performed. Exposure to *Leishmania* was assessed by Leishmanin Skin Test (LST). Infection was assessed in blood samples by Direct Agglutination Test (DAT) in sera and RT-PCR in dried blood spots. RT-PCR, targeting the *Leishmania* kDNA was optimized beforehand.

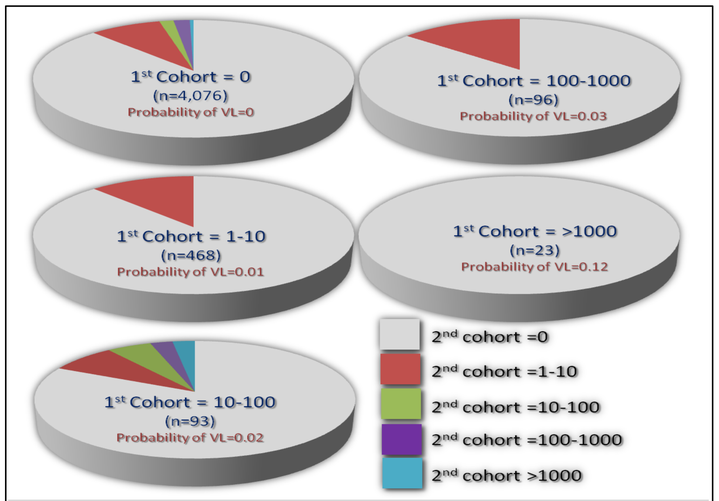
In February 2012, 2,675 individuals living in 13 villages (777 households) were selected for a second screening one year after base line in March 2011. It was aimed to select villages at higher risk of infection as deduced from base-line data. The selected villages were planned to undergo complete annual assessments, employing similar techniques (i.e., LST, DAT and RT-PCR coupled with clinical and parasitological assessments).



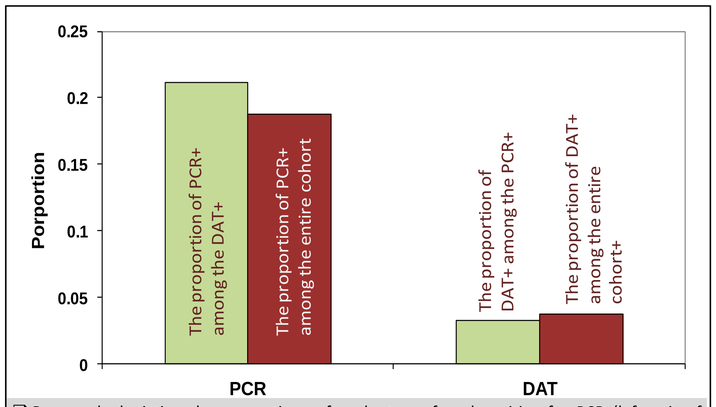
Geographical distribution of the villages sampled during the 1st cohort. The different colors denote percentage of kDNA PCR positive persons in the population.
● >15% PCR+ ● 5-15% PCR+ ● <5% PCR+

1	2	3	4
Category Parasites /ml	Retested by kDNA RT-PCR	kDNA RT-PCR+ (on retesting)	Level of uniformity
0	107	9	91.6%
1-10	108	64	59.3%
11-100	48	41	85.4%
101-1000	24	23	95.8%
Above 1000	19	19	100%

Validation of qRT-kDNA PCR results from the 1st cohort. Representative samples (Column 1) were reexamined using the same protocols (Column 2). The samples that were positive upon re-examination are depicted in Column 3. Levels of uniformity (Column 4) show the percentage of samples that gave the same result in both tests. Negative and high positive samples were consistent. However, low positive samples were undependable.



Pie charts depicting the likelihood of 1st cohort volunteers (n=4,756) converting to a different (or remaining in the same) PCR category upon re-examination during the 2nd cohort (~12 months later, n=2,327) .
- Each pie chart represents an entire PCR infection-category from the 1st cohort
- Colors represent 2nd cohort PCR categories (*L. donovani* parasites per mL blood)
- Volunteers from any 1st cohort category were most likely to be PCR negative.
- Volunteers who were PCR negative had a significant probability of becoming PCR positive.
- Volunteers with moderate parasitemias (10-100 & 100-1000 parasites/ml) were most likely to remain PCR positive.
- Volunteers from the highest PCR category (>1000) became PCR negative or VL patients.
- The probability of becoming a VL patient (n=30) was directly correlated with the intensity of parasitemia.



- Bar graph depicting the proportions of volunteers found positive for PCR (left pair of columns) or DAT (right pair of columns).
- Red columns depict the proportion out of the entire 1st cohort (n=3,376)
- Green columns depict the proportion out of the complementary category (i.e. PCR+ out of DAT+ [left green column], DAT+ out of the PCR+ [right green column])
- Results indicate a lack of correlation between PCR and DAT positivity among the cohort volunteers.

Results

At base-line, the LST rate among 4,554 individuals was 10.1% and remained surprisingly low (35%) among 126 previously treated VL cases. Serological screening (DAT) of 4,788 individuals with no prior history of VL, identified 3.9% positives. Of 4,757 dried-blood samples tested by quantitative RT-PCR, 680 samples (14.3%) were found positive for *Leishmania* k-DNA. Of those, 119 (17.5%) harbored over 100 parasites per ml of blood. Sequencing of ITS1-PCR products of PCR positive cases (n=21) showed that 90% (19 of 21) were *L. donovani.* During February 2012, 2,098, 2,359 and 2,361 individuals were screened by LST, DAT and RT-PCR, respectively. The number (and percent) positive were 257 (12.2%), 132 (5.6%) and 329 (13.9%) respectively. The rates were 11.4%, 3.3% and 14.6% respectively, when previously treated cases of VL were excluded from the analysis. The incidence of new infections measured by LST, DAT and RT-PCR conversions (from base line negative to follow-up positive) after one year (March 2011 – February 2012) was 6.8%, 3.1% and 14.6% respectively. From March 2011 to February 2012, a total of 34 new cases of VL (22 males, 12 females) were found amongst the study population. Thus, the annual incidence of active VL in the study localities is at least 7.0 per 1,000 persons. The mean age of these patients was 16.8 (±12.5). Of these 34 cases, 38.2% were DAT positive in March 2011. Similarly, 15.6% were positive by LST, and 30% were positive by k-DNA RT-PCR. These data show that the incidence of infection (measured by LST conversions) is at least 10 fold to the incidence of active VL. When incidence of infection is measured by DAT or RT-PCR conversions respectively, the ratio is 1:4 (DAT) and 1:20 (RT-PCR).

Summary

The study is ongoing, more data will accrue and the results of in-depth analysis will be reported in due course. Preparations are now underway to conduct xenodiagnostic procedures in VL patients and in those individuals with incident/pre-existing evidence of infection (LST, DAT & RT-PCR). The data is expected to give us information on infectiousness of asymptomatically infected individuals in comparison with active VL patients, and to highlight their role in transmission.



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