1st Cohort sampling in the district of Sheraro (March 12-April 10, 2011)

Team Members:

From AAU Faculty of Medicine - Prof Asrat Hailu (PI), Aysheshm Kassahun (coordinator and research assistant), Dr Workagegnehu Hailu (Physician), Asrat Bizuneh, (Research Assistant / lab technician), Welelta Shiferaw (Research Assistant / lab technician), Abiot Tadesse (statistician), Michael Berhanu (driver and project assistant), Shewaye Belay (PhD student-Mekele University)

From AAU Aklilu-lemma institute of Pathodiology - Prof Teshome Gebre-Michael (co-PI, entomologist), Araya Gebreselassie (PhD Student, entomology)

Field Station Personnel - Kedir Ali (Lab tech, Humera), Siltan Gebreselassie (Nurse, Humera), Hagos Teka (lab tech, Sheraro), Hadas Gebreyesus (nurse, Sheraro), Hadush Demoz (field assistant, Sheraro), Abel Haile (field assistant, Humera), Kibrom Tafere (driver & field assistant, Sheraro), Haile Gebremariam (translator & lab assistant, Sheraro), Gebremichael Teklemariam (translator, Sheraro)

Introduction:

Following the discussions held during several meetings, it was decided to conduct a large-scale longitudinal cohort study in the villages surrounding Sheraro. The villages are spread out with separate compounds occupying distant hilltops, several hundred meters to 3 km from each other. Thus, the study localities represent an extensive geographical region.

A preliminary census of the entire population of 3 Farmers' Associations (FAs); namely, Lemlem, Metebteb and Ademiti was conducted during January and February 2011. This included enumeration of the residents (total ~11,000 in the 3 FAs), marking of houses, obtaining GPS readings and recording relevant epidemiological/sociological data. Following the census, entire families of VL affected villages were selected for the cohort study - totaling approximately 5,000 persons.

The team from Addis Ababa University arrived in March 11 and the sampling plan was reviewed with the other team members. Each person was assigned roles for the field activity. Prior to the team's arrival for sampling in a particular village, a

team member with a translator was dispatched to coordinate with the local chief(s), identify a suitable site for the makeshift clinic and to let all the villagers know the activities of the next day during which the kala-azar team would be working in their village.

Medabe-Lemlem (22/5/2011):

On the morning of the 22nd of March, 2011 the team arrived at the village of Medabe-Lemlem at 7:30 am (1:30 Ethiopian time) and immediately began preparing the two class rooms of the newly built school house for the day's work. Within 30 minutes the clinic was ready and a long line of villagers had formed along the northern wall of the school-house .

Each villager would go through several steps to ensure that all data were properly recorded and all tests were performed and properly performed.

The early-bird villagers gathering outside the school waiting for Abiot to assign them numbers - while Siltan, Kedir and Hagos finish setting up the blood-sampling station inside

1. Informed consent was sought and obtained from adults or parents of, minors.
2. Preliminary registration – volunteers were allocated sequential numbers in the queue and the team filled in a form with personal details such as age, ethnic affiliation, address, travel history, existing conditions and diseases (including history of Kala Azar) and assigned identifying codes. The volunteers' weight and height were recorded.

Gebre-Michael obtains consent for participation samples after providing information to the adults in their own language, Tigrigna. Aysheshm and Welelta fill in the forms, assign cohort IDs and hand out filter papers for collection of blood samples for PCR.

Kedir draws venous blood sample from a young fellow while his elder sister keeps watch. Asrat performs Leishmanin skin test by injecting 0.1 ml of the antigen solution in the skin. The injection site was marked and reactions were assessed 48-72 hours later (bottom right)

3. Venous blood was drawn by medical technicians for serological tests Leishmanin skin tests were performed injecting Leishmanin antigens to determine exposure

and immune statusPhysical exam and discussion with a physician and reports are recorded on Clinical recording form (CRF)

4. Suspect clinical cases underwent a thorough physical examination and tested on the spot using rK39-based kala azar kits
5. VL patients were provided with financial aid and transport to Humera hospital for treatment

A boy with enlarged abdomen was examined by Dr Workagegnehu Hailu. A rapid rK39-based kala azar test proved strongly positive. The boy and his father were sent off to Humera Hospital for confirmation of diagnosis and treatment. The visible scars on the abdomen were a result of burns inflicted by a traditional village healer in attempt to cure the boy.

Summary:

Working hours were from daybreak till dusk and sampling continued throughout. Between 150-300 villagers were sampled each day during 27 days. Total population sampled was ~5,000. Four cases of visceral leishmaniasis were diagnosed and treated. Positive anti-Leishmanin skin test reactions were recorded. Dot agglutination tests (DATs) and ELISAs will be performed using *Leishmania donovani* promastigote antigens. kDNA and/or 7SL PCR diagnosis will be performed using high resolution melt (HRM) analysis by real time PCR. Sera from PCR-positive samples will also be tested for reactivity against *Phlebotomus orientalis* saliva. Subsequent data analysis will facilitate our decisions regarding continued sampling and xenodiagnostic studies.

In the evenings, after all the sampling of the day were done, the team gathers back in the station to centrifuge blood samples, catalogue data and mark samples. Top Left: Asrat Bizuneh, Kedir, Wlelta & Aysheshm preparing the sera. Top Right: Asrat Hailu, Abiot & Workagegnehu checking the clinical report forms (CRFs) for quality and recoding name, age and sex on separate forms for recording LST results 48-72 hours later.