

Full Length Research Paper

# A preliminary survey of domestic animal visceral leishmaniasis and risk factors in North West Ethiopia

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**Screening of domestic animals was carried out from 2008 - 2009 to detect antibodies against *L. donovani* and to see the involvement of animal reservoirs. A total of 203 domestic animals were included in the study. Serum and biopsy samples were collected. Screening of serum samples by Modified Direct Agglutination Test (DAT) for canine reservoirs was used to screen serum samples at  $\geq 1:320$  cut off titer. Data were also collected using pre-tested questioners to evaluate the involvement of some potential risk factors. Antibody against *L. donovani* in domestic animals was detected and the overall proportion of occurrence was 30.5%. No *Leishmania* parasite was isolated from spleen, liver, skin snip and exudates, bone marrow and lymph node of dogs.**

**Key words:** *L. donovani*, anti-leishmanial antibodies, Kala azar treatment risk factor, domestic animal, Libokemkem, Northwest Ethiopia.

## INTRODUCTION

Leishmaniasis is caused by a diphasic protozoan parasite of the genus *Leishmania*. *Leishmania donovani* is first recognized by Leishman and Donovanin in 1903; almost simultaneously (Herwaldt, 1999).

In East Africa, VL is endemic in parts of Sudan, Ethiopia, Somalia and Kenya and, causes at least 4000 deaths annually and a loss of approximately 385000 DALYs (Reithinger *et al.*, 2007). The overlapping geographical distribution of VL and AIDS in this region significantly increased the mortality rates (Horst *et al.*, 2008), and the highest co-infection rate reported in the world (Alvar *et al.*, 2008).

In Ethiopia, long-recognized VL-endemic foci lie in the lowlands (<1500 meter above sea level); these are situated in Metema and Humera Northwest of Ethiopia bordering east Sudan (Mengesha and Abuhay, 1978),

which accounts for approximately 60% of VL case of the country and reflecting the first ecologic pattern. The second ecological patterns are regions of Lake Abaya, Omo River, and the lower Omo, Segen and Woyto valleys in the south and southwest (Fuller *et al.*, 1979; Ayele and Ali, 1984; Ali and Ashford, 1994). Sporadic epidemiological surveys showed the presence of VL in more than 40 isolated localities in Ethiopia. Recent outbreak of VL in the highlands of NW Ethiopia, Libo district; south Gondar has claimed the lives of hundreds (Alvar *et al.*, 2007) before medical interventions were put in place.

In east Africa (Sudan, Ethiopia, Kenya and Somalia), *L. donovani sensu lato* is the cause of visceral leishmaniasis (VL) and anthroponotic transmission seems predominates (Soulsby, 1982) and Post Kala azar dermal leishmaniasis (PKDL) patients also play an important role in VL transmission (Sundar and Rai, 2002). Some wild animals are incriminated as a reservoir host in Sudan like grass rat (*Arvicanthis niloticus*, Rodentia: Muri

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dae), spinus mouse (*Acomys albigena*, Rodentia: Muridae), serval (*Felis serval*, Carnivora: Felidae) and genet (*Genetta genetta*, Carnivora: Viverridae) (Hoogstraal and Heymann, 1969), jackal (*Canis* spp) (sixl *et al.*, 1987) and Egyptian mongoose (Elnaiem *et al.*, 2000). Natural leishmaniasis by *L. infantum* is reported in horses (Ramos-Vera *et al.*, 1996), cats (Salano-Gallego *et al.*, 2000) and goats (Williams *et al.*, 1991) but their role in vector infectivity is not studied.

Dogs are incriminated as a reservoir host in east Sudan for *L. donovani* (Dereure *et al.*, 2000; 2003) and in Mediterranean region for *L. infantum* (El Harith *et al.*, 1989).

In the Horn of Africa, there are two distinct ecologic settings of visceral leishmaniasis: the semi-arid regions where *Phlebotomus orientalis* breeds in cracks in the black cotton clay soil (Hoogstraal and Heymann, 1969) and the savannah and forest areas in the south where the vectors *P. martini* and *P. celiae* are found in association with *Macrotermes* termite mounds (Gebre-Michael and Lane, 1996).

The main vectors of VL in Ethiopia are *P. martini* and *P. celiae* in south (Gebre-Michael and Lane, 1996) and *P. orientalis* in the southwest (Hailu *et al.*, 1995). Studies implicate *P. orientalis* as the most probable vector of VL in Humera and Metema endemic areas of NW Ethiopia and highly suspected in the recent outbreak areas of Libo district (Gebre-Mitchael *et al.*, 2010) as well as in Bellessa high land valley in the north (Ashford *et al.*, 1973).

The purpose of the present study was to detect the presence of antibody for visceral leishmaniasis in selected domestic animals and to identify some potential risk factors in the cycle of the disease

## MATERIALS AND METHODS

### Study Area

Libo Kemkem wereda is located in the Amhara Regional state, Northwestern Ethiopia at an altitude of 2,000 meters above sea level. The district is made up of 30 kebeles (the administrative) with an estimated population of 196,813 in 2004. Addis Zemen (the district capital, population of 19,755) is located between Bahir Dar and Gondar on the major road connecting Addis Ababa to dry port Galabat, Sudan; crossing known foci of intense VL transmission in Metema, Ethiopia, and Gedaref, East Sudan. The district has one health center and 10 health posts.

Two kebeles (Bura sand Egziarab) of Libo Kemkem wereda, were the first VL outbreak of 2004 had occurred and claimed the life's of hundreds. These villages were selected purposively to detect the rate of infection and antibody development in domestic animals. The Amhara Regional State although reported the outbreak as Quartum resistant malaria, later epidemiological investigation by Médécain Sans Frontières Greece (MSF-

G) proved that it was VL of up to 7% cumulative incidence (Alvar *et al.*, 2007). The village comprises 1200 households (families living together) and the agriculture is mainly rain fed and very little irrigation is practiced in the winter season.

### Study Design

The domestic animals, included in the study were dogs, cats, cattle, donkeys, sheep and goats and the number of animals sampled from each group in that order were 90, 3, 43, 15, 42 and 10. Among domestic animals from previous studies dog was found be affected by the *Leishmania* and in this study an attempt was made to increase dogs number compared to the domestic animals. The sample selection of animals (sampling strategy) was based on owner's willingness, and convince.

### Ethical Approval

This study was approved by the ethical committee of animal welfare of the Research and publication office of Gondar University.

### Sample Collection

Serum samples were collected aseptically from jugular vein of cattle, sheep, donkey and goats; from cephalic veins of dogs and cats, and allowed to clot at room temperature. The serum was separated by centrifuging at 2000 rev/min for 15 min and collected into a sterile vial and stored at -20°C.

Biopsy from animals was taken after obtaining informed consent from the owners, and Fine needle percutaneous aspiration was done to get spleen, lymph node, liver and bone marrow aspirates from seven dogs and immediately smeared on a sterile glass slide and fixed with methanol for giemsa stain, and cultured in Novy macNeal Nicolae (NNN) media in the field and later incubated in laboratory for isolating the parasite. The standard surgical procedures and biopsy materials were applied to get fine needle aspirates from inguinal lymph node, liver, spleen and bone marrow aspiration from trochantric fosa of the femur bone by applying 2% lignocaine hydrochloride local infiltration. Serological tests were carried out in Leishmania research and training center, Gondar.

### Diagnostic Methods

In this study for the diagnosis of *Leishmania* in domestic animal sampled (dogs, cattle, donkeys, goats and sheep) Direct Agglutination Test (DAT) and parasitology (Giemsa stain and culture methods) were used. So far no gold

standard test is suggested both for human and animal Leishmaniasis.

### Serological Method

DAT is a highly sensitive (92-100%) and specific (72-100%) test to monitor low level of antibody in canine reservoirs for *Leishmania donovani* (Mohebbi *et al.*, 2006). DAT was performed according to El Harith *et al.*, (1989) modified version for the canine and human reservoir and 1:320 titer was taken as the cut off. The test was conducted using V-shaped microtiter plate. Both negative control (from diluent and antigen) and positive control (from confirmed human VL patient) was applied. Serum samples were diluted serially starting at 1:20 to 1:20480 by transferring 50 $\mu$ l of diluted serum and this same amount was discarded from the last dilution. The diluent used was a solution containing 0.15M NaCl, 0.2M 2-Mercaptoethanol and 1% (vol/vol) fetal calf serum (SIGMA). After serial dilution, 50 $\mu$ l DAT antigen (KIT, Amsterdam) was added to the sera in the micro-well plate containing 50 $\mu$ l of diluted serum and the plates were shaken clockwise and anticlockwise by preventing splashing off. After 18 hours of incubation at room temperature, the DAT reading was made over white board.

The agglutination activity was detected visually and by comparing with the negative and positive human sera was used as a control.

### Parasitology

#### Giemsa Stain

Smears prepared from biopsy of spleen, lymph node, skin snip and bone marrow were fixed with absolute methanol and stained with Giemsa and examined carefully by light microscope at high magnification (1000x oil immersion) for the presence of Donovan bodies (amastigote stages).

#### Culture

Biopsy of inguinal lymph node, liver, skin snip and exudates, bone marrow and spleen of dog was cultured on Novy macNeal Nicolae (NNN) media. And then incubated at room temperature, passage and examined weekly over a 6- week period at different time.

Growth of the parasite was detected by using inverted microscope and Giemsa staining. NNN media was prepared from nutrient agar containing 10% whole rabbit blood over laid with liver infusion tryptose broth containing 200,000IU/ml penicillin G and 1Ug/ml streptomycine.

### Data Collection

Data was collected using pre-tested questioners to evaluate the involvement of some potential risk factors including Kalazar history of owners (treated for the disease or not), living near the dump and sharing the same house with domestic animals during night time. While physical examination of dog was carried out at the time of taking biopsy aspirates.

### Data Analysis

The data was analyzed by logistic regression with Intercooled Stata version 11. A statistically significant association between variables was said to exist if the computed p-value is less than 0.05.

## RESULTS

The overall proportion of domestic animals showing the existence of *Leishmania* antibody in DAT was high as 62 of 203 animals (30.54%) found to be positive (table 1). Highest seropositivity of 41.9% and 40 was found in cattle and dogs, respectively. Sera collected from dogs had agglutination reaction (n=92), 25.6% had a titration of 1:320 and 15.6% had >320.

Sera collected from cattle showed a high rate of agglutination reaction activity against *L. donovani* coomassie blue stained antigen of the 43 tested sera 41.9% had  $\geq$ 320. Sera collected from donkeys had a medium rate of agglutination activity (n=15) and 33.3% had a titer of  $\geq$ 1:320. Sera collected from sheep showed a very low agglutination activity (n=42) and 4.7% had a titer of  $\geq$ 1:320. No parasite was observed from cultured samples and stained slides.

Dogs owned by kala azar treated owners have showed high proportion of DAT positivity and high risk of antibody development (OR 8.9375, p = 0.016) (see table 3); and sharing the same house with the owners have also showed high risk of exposure (OR 5.464, p < 0.001) (see table 3).

Cattle sharing the same house have high risk of exposure to the parasite (OR 4.667, p < 0.001) but there is no relationship between those cattle owned by treated owners and non treated owners (OR 0.8839, p = 0.927) (see table 3).

Sheep have showed weak relation among kala azar treated owners and non treated owners and sharing the same house and separate house (OR 0.625 p = 0.945 and OR 1.2777, p = 0.725) (see Table 3).

A total of 6 dogs which have positive DAT titer were examined for the presence of amastigotes in a total of 26 tissue aspirates (Lymph node, liver, skin snip, spleen and bone marrow) were cultured in NNN media. None of them were positive.

**Table 1.** antibody titer against *L. donovani* in domestic animals (seropositivity) using Direct Agglutination Test (DAT).

Description	dogs	cats	cattle	donkey	sheep	goats	Total
No animals examined	90	3	43	15	42	10	203
No. of positive( $\geq 1:320$ )	36	0	18	5	2	1	62
Proportion (%)	40	0	41.9	33.3	4.8	10	30.5

Dogs owned by Kala azar treated owners were seropositive in a very high proportion than their counter parts (Table 2) and most of dogs are clinically normal (see Table 3) but serologically positive ( $p < 0.01$ ).

**Table 2.** Proportion of animals owned by owners Treated for Kala azar and not treated .

Description	Dogs		Cattle		Sheep		Total
	KRx	KN	KRx	KN	KRx	KN	
No animals examined	38	52	25	18	19	23	175
No. of animal $\geq 320$ positive( $\geq 1:320$ )	22	8	9	7	1	1	48
Proportion (%)	57.89	15.38	36	38.89	5.26	4.35	27.43

Where: KRx mean owners treated for Kalazar  
KN means owners not treated for Kalazar

## DISCUSSION

In 2004, there was an active transmission of VL in the study area (Bura and Egziarab) and up to 7% cumulative incidence of human cases and two dog cases (out of 40 examined)(5%) was reported due to *L. donovani* complex (Alvar *et al.*, 2007). In our study, November 2008 to April 2009, a higher occurrence was observed in dogs, where 36 were sero-positive out of the 92 (39.13%) and this difference could be as a result of the time gap between the human outbreak and canine outbreak time difference. The high prevalence seen in our data may reflect we sampled before high mortality among infected dogs after the epidemic (2004/5). In Brazil, high canine seroprevalence preceded the human epidemic by several years (Werneck *et al.*, 2007).

Another study in Sudan (Mukhtar *et al.*, 2000) using DAT showed 68.7 % (66/96), 21.4 % (9/42) and 8.5% (5/59) in donkeys, cows and goats, respectively using DAT for human patients with cutoff value  $\geq 1:3200$  cut off (El Harith *et al.*, 1988). They have also tested six dogs and 25 sheep sera and none were reactive. Compared to our result of 33.3 % (5/15), 41.9% (18/43) and 10(1/10) in donkeys, cattle and goats, respectively. Mukhtar *et al.* (2000) report was high only in donkeys but lower in cattle and goats and were used the old version of DAT to screen human VL patients while in our study we use the modified version of DAT to detect asymptomatic carrier in canine and vulpine reservoirs (El Harith *et al.*, 1989).

Another study conducted on cattle in Bangladesh using ELISA and DAT (9.4%,  $n=138$  and 3%,  $n=138$ ) were seroconvert positive, respectively. But the parasite DNA is not detected using Ln PCR and (Loop-mediated isothermal amplification) LAMP (Alam *et al.*, 2011). The occurrences of antibody for *L. donovani* differ among different species in the present and previous studies (Mukhtar *et al.*, 2000). In our study the highest seropositivity of 41.9% was found in cattle while other similar study Bhattaria *et al.*, (2010) in Nepal found goats have high seropositivity 16% (23/144) followed by cattle and buffalo 5% (1/20) and 4% (1/24), respectively.

In the Indian subcontinent *P. argentipes* is the principal vector and these flies 5x more attracted to cattle than person and feed more on animals than on persons. Study showed that much of the blood meal of sandy flies is satisfied from cattle followed by goats (Bern *et al.*, 2010). Cattle sharing the same house have strong correlation of seroconvert positive (OR 4.6667,  $p < 0.001$ ). ELISA-based blood meal analysis of 273 fresh fed *P. orientalis* females collected from Metema revealed a remarkably high bovine blood feeds (92%) with only 2.2% of human blood feeds (Gebre-Michael *et al.*, 2010). Feeding of sandy fly on equines has been also previously reported (WHO, 1991). Cattle may provide zooprphylaxis and the application of insecticide to cattle could cause sand flies to increase their feeding on human (Kolaczinski *et al.*, 2008). Due to the hairy nature of sheep these fragile sand flies are attracted seldom.

**Table 3.** Logistic regression models of risk factors for visceral leishmaniasis among domestic animals in Libo kemkem, Ethiopia.

Species	Risk factors	Odds ratio	Standard Error	P-value	95% CI
<b>Dog</b>					
	Sharing the same house	5.464	0.4937	<0.001	2.0478-14.58
	Kala azar treatment history	8.9375	0.5021	0.016	3.713 -12.304
<b>Cattle</b>					
	Sharing the same house	4.6667	0.8591	<0.001	0.5059-11.018
	Kala azar treatment history	0.8839	0.6382	0.927	0.462 - 8.053
<b>Sheep</b>					
	Sharing the same house	0.625	1.452	0.945	-0.5437-0.280
	Kala azar treatment history	1.2777	1.4488	0.725	0.3975-0.9660

Dogs owned by households with history of Kala azar treatment and sharing the same house with human beings found to be more affected by VL and this difference was statistically significant (OR 8.9375,  $p = 0.016$ ).

In our house hold survey, we also got two PKDL and one ocular (uvitiis) form of post Kala azar leishmaniasis cases in addition to their history and this figure has almost similar to the past findings of Alvar *et al.*, (2007) where three PKDL cases were recorded. And 50% of treated VL cases develop PKDL in Sudan (Zijilstra *et al.*, 1995). Subclinical cases can have a detectable circulating DNA in their blood, such cases can be reservoir and formerly treated VL patients can remain a reservoir for a long term (Scafer *et al.*, 1995). Domestic dogs may act as infection reservoirs, but large outbreaks are usually thought to involve anthroponotic transmission (Dereure *et al.*, 2003). The failure of isolation of *L. donovani* from dog skin indicates the negligible role played by these animals in the transmission cycle in the study area.

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## Author Contributions

Review Literature: Ambaye Kenubih, Tamerat Abebe, Prof Asrat Hailu, Shimelis D and Gizat Almaw. Collect Sample: Ambaye Kenubih, Tamerate Abebe, Wossen Seged Lemma and Prof Asrat Hailu. Data Analysed: Ambaye Kenubih Gizat Almaw and Shimelis D. Diagnose Lab samples Ambaye Kenubih, Yegnasew Takele, Prof Asrat Hailu and Tamerate Abebe.

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