Prevalence of asymptomatic visceral leishmaniasis in human and dog, Benishangul Gumuz regional state, Western Ethiopia

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Research

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Abstract

Background: Benishangul-Gumuz region is an important development corridor in Ethiopia. The large-scale projects; the great renaissance dam, mining and agriculture entailed huge environmental modification and settlement pattern changes. Detail epidemiological information of VL in the region is unknown.

Materials and Methods: A cross sectional study to assess the epidemiology and risk factors associated with *Leishmania* infection. Leishmanin skin test (LST) was done for 1342 participants, and for 253 of them rK39 and DAT were done. Thirty-six dogs owned by households with LST positive member(s) were rK39 and DAT tested. A pretested questionnaire was used to capture individual and household characteristics.

Results: Of the 89.2% (1197/1342) who availed themselves for LST reading 6.0% were positive. The rk39 and DAT positivity among the 253 tested were 3.2% and 5.9% respectively. In dogs, positivity rates by rK39 and DAT were 13.9% and 5.6% respectively. Of the household and individual risk factors presence of dog in household (P=0.005), male sex (0.003), residence woreda (0.000) and occupation (0.023) showed a strong positive association with LST positivity. Individuals who lived in household who owned dogs were 2.6 times more likely to be LST positive (AOR = 2.6; 95%CI= 1.54, 4.40). Being female decreased by 0.38 times the probability of being LST positive (AOR = 0.38; 95%CI= 0.20, 0.72). Living in Guba and Kurmuk has 4.7 (AOR=4.74, 95% CI 1.83, 12.31) and 5.9 (AOR=5.85, 95%CI 2.27, 15.09) times more risk of being infected.

Conclusions: We demonstrated presence of active VL transmission in the areas. Thus, we underline the need to establish the responsible vector(s) and reservoir(s) for comprehensive early containment plan to prevent potentially harmful public health and economic consequence.

Background

Visceral leishmaniasis (VL, Kala-azar) is a fatal neglected tropical disease without early diagnosis and proper treatment. VL in east Africa is caused by *L. donovani* species complex. The transmission of *L. donovani* is generally considered anthroponomic. Jambulingam, P., et al., 2017 **[1]** provided a definitive evidence to incriminate dogs as *L. donovani* reservoir in India**,** the status in east Africa remain to be substantiated. Yet, there are reports that associated dogs with *L. donovani* transmission in the Sudan **[2, 3]** and Ethiopia **[4-6]**. Also, there are studies that showed dogs are among the domestic animals that *P. orientalis,* the vector of *L. donovani* in east northern Ethiopia foci, preferentially bites **[7, 8]**.

Visceral leishmaniasis is reemerging with geographic spread and recurrent outbreaks that claimed the life of several hundred of Ethiopians over the past two decades [9]. Among the factors attributed for its spread and outbreaks are individual, household and socio geographic risk factors: age, sex, housing conditions, mass movement of temporary laborers, immunosuppression and ecological modifications [9-12]. The national incidence estimate for Ethiopia based on self-reported cases is up to 4500 new cases per year [13]. Risk model using geographical information system and statistics showed that about 33% of the total landmass, predominantly within the development corridors of significant public health and economic implication, is VL high risk [14].

Benishangul-Gumuz regional state is one of the fastest changing development corridors in western Ethiopia. The mega projects, great Ethiopian Renaissance dam, large scale irrigation and rain feed commercial agricultures and mining activities; have resulted in vast ecological and sociodemographic changes. The rapid assessment by Abera *et al* (2018) [15] reported a 7.3% (20/275) VL asymptomatic infection prevalence in two kebeles (sub districts), in where a VL case reported to have lived [16].

Control strategies for leishmaniasis in Ethiopia rely on case detection and treatment, and vector control. Thus, delineating endemic areas and knowledge about the burden help to attain desired outcome through targeting resources. Also, knowledge on risk factors associated with exposure is important to design behavioral change communication tools to attain active participation and ownership of program by affected communities. Therefore, the objectives of this study were to assess the epidemiology and risk factors associated with explore in humans and dogs in at high risk districts.

Materials And Methods

2.1 Description of study area

Benishangul-Gumuz region, western Ethiopia, is located 34° 10'N and 37° 40'E and 09° 17'N and 12° 06' N. The region is predominantly (75%) lowland. The total population is around 784,345 with an estimated density of 15.91 people per kilometer square (BGRoHB, 2019).The study encompassed six VL high risk districts (Figure 1) as per the environmental factor based risk model by *Tsegaw et al* (2013)[14]; Dangur, Guba and Pawi from Metekel zone, and Banbasi, Kumruk and Sherkole from Assosa Zone. The region is one of the development corridors with large scale agricultural, mining and Dam projects which entailed change in settlement pattern and deforestations, and large influx of peoples for temporary work and/or permanent settlement.

Figure1. Map of the study Woredas, 1=Bambasi, 2=Kurmuk, and 3=Sherkole from Assosa zone, 4=Guba, 5 =Dangur and 6=Pawi from Metekel zone, Benishangul Gumuz regional state, Western Ethiopia.

2.2. Study design and sample size determination

A cross sectional survey was done from 2018 to 2020 to assess the epidemiology, explore if any zoonotic significance of VL and risk factors associated with exposure to *Leishmania* infection. Samples were selected using a multi-staged sampling technique. At primary sampling unit among the three administrative zones of the region, overlaying the environmental factor based risk map [14] two zones namely Assosa and Metekel were selected because they had large areas under VL high risk. Similarly, within the selected zones districts with high risk area were selected. Then an operation map was prepared overlaying the risk map of selected districts and the kebele level shapefile to identify high risk kebeles.

Subsequently, study households were randomly selected from each of the kebeles targeting up to 5% of their total population, with overall sample size of 1342 individuals for LST testing.

Following LST reactive individuals as a focal point dogs were sampled for serological tests, rK39 and DAT. Also, 253 human blood samples, 67 purposively from LST reactive and 185 randomly from non-reactive individuals were tested by DAT and rK39.

After explaining the purpose of the study, a written informed consent was obtained from each participant; parents or guardians for minors. Similarly, informed assent was obtained from dog owners to sample dogs. Blood sample were aseptically collected using 5mL disposable syringe or plain vacutainer tube from cephalic/saphenous vein of both human and dogs. Of the collected blood, 20 µL was used to prepare dried blood spot (DBS) on 3MM Whatman paper (Whatman, Maidstone, UK) allowed to fully air *dry* without exposing to direct sunlight. Sera from both dog and human were used for rK39 ICT and DAT testing.

2.2.1. Leishmanin skin test (LST)

Prior to LST, socio-demographic information was documented from the study participants using pre-tested semi-structured questionnaire. Then, intradermal injection of 0.1 mL leishmanin antigen (Pasteur Institute of Iran, Tehran, prepared from *L. major*) was made at the volar surface of the arm. After 48–72 hours delayed hyper sensitivity reaction was measured with the ballpoint techniques; 5.0 mm and above of the average of the two diameters of an induration was considered positive (Fig 2).

Figure 2: Procedure of leishmanin skin test (LST), photos are from this field work on the same participant: A) Intradermal injection of 0.1mL of LST (Pasteur Institute of Iran, Tehran, Iran) solution after brief shaking, B) Marking of injection point using permanent marker and C) Measuring the induration using the ballpoint pen method after 48 to 72 hours of injection.

2.2.2. rk-39 immunochromatographic test (rK39 ICT)

The rK39 ICT (DiaMed- ITLEISH; Bio-Rad Laboratories, Marnes-la-Coquette, France) was done following the supplier's recommendation. In brief, 20µL serum sample was added to the absorbent pad well with a 150µL (2-3 drop) of chase buffer provided with the kit. Results were read after 10–20 minutes and recorded as follows: positive when both control and test lines appear; negative when only control line appears or invalid when no control line appears (in such cases tests were repeated).**Figure3.** The rK39 immunochromatographic test interpretation: top two positive strips and bottom a negative strip.

2.2.1. Direct Agglutination Test (DAT)

Sera were transported to Benishangul Gumuz Regional laboratory with ice box and stored at −20°C. Then samples were transported to AHRI under cold chain and stored at -20 ^{O}C until processed. Direct agglutination test was performed according to the manufacturer's instructions (Institute of Tropical medicine, Antwerp, Belgium). The presence of antileishmanial antibodies below or at cutoff of 1:3200 titers was used to decide as negative. Both negative and positive controls were run for every batch of kit used. In brief, sera were diluted serially from 1:200 to 1:204800 by transferring 50 µL of diluted serum and discarding the same amount from the last dilution (Fig 4).

Figure 4. A plate showing DAT test results in sera tested with a starting dilution of 1:200 in column

2.2.3. Data analysis

STATA version 13 data software (College Station, Texas, USA) was used for data analysis. Descriptive statistics were employed to summarize in terms of frequencies and percentages. Univariate and multivariate logistic regression were used to determine the association of *Leishmania* infection with the risk factors and expressed as odds ratio and 95% confidence interval. For all analysis a $P < 0.05$ was considered for significant difference.

2.2.4 Ethical consideration

The protocol was approved by AHRI/ALERT ethical review committee (AH01275/0012/18, 19/12/18). Informed consent was obtained from all participants or guardian/parents of minors. For participants between 11 and 18, verbal assent was sought in addition to the parental/guardian consent. Similarly, for dogs informed assent was obtained from the owners.

Result

3.1. Prevalence of asymptomatic visceral leishmaniasis

Of the total 1342 participants LST tested; 89.2% [801 males and 396 females] availed for result reading. The LST based prevalence was 6.0% (72/1197). The seroprevalence was 3.2% (8/253) and 5.9% (15/253) respectively by rk39 and DAT. Three of the 8 rk39 and 7 of the 15 DAT reactive individuals were LST positive, while 5 of the rk39 and 8 of the DAT reactive were LST negative (Table 1).

Table 1: The prevalence of asymptomatic visceral leishmaniasis in Benishangul Gumuz, western Ethiopia, by age and sex as measured by LST (n = tested positive, N = 1197), DAT (n = tested positive, N = 253) and rK39 (n = tested positive, N = 253), 2018-2020.

Variable	LST positive %(n/N)	rK39 positive %(n/N)	DAT positive %(n/N)
Sex			
Male	7.37(59/801)	3.49(6/172)	5.81(10/172)
Female	3.28(13/396)	2.47(2/81)	6.17(5/81)
Age (in years) group			
<5	0(0/2)	0(0/2)	0(0/2)
5-12	9.09(3/33)	0(0/33)	3.03(1/33)
13-18	30.06 (49/163)	4.29(7/163)	6.75(11/163)
>18	27.27 (15/55)	1.82(1/55)	5.45(3/55)

Variation in asymptomatic infection was observed among sites: higher prevalence was detected in kebeles from Guba (16.1 %, 32/199) and Kurumuk (14.0 %, 27/191) districts respectively. The highest LST positivity was recorded at Abulhorse kebele (28.3 %, 17/60) from Guba district (Table 2).

Table 2. The kebele level asymptomatic visceral leishmaniasis as measured by leishmanin skin test (LST), Benishangul Gumuz, western Ethiopia. Where n = number of LST positive and N = number of LST test per kebele, 2018 - 2020.

Study locations			LST Reactive % (n/N)	
Zone	Woreda	Kebeles	Woreda*	Kebele**
Metekel			2.73(6/220)	
	Pawi	Mender-104		0 (0/71)
		Mender-24		8.22(6/73)
		Hidase		0 (0/76)
	Guba	Almahal	16.08(32/199)	12.66(10/79)
		Abulta		8.33(5/60)
		Abulhorse		28.33(17/60)
	Dangur	Gubulak	3.66(7/191)	0(0/71)
		Qota		11.67(7/60)
		Bawulana dilate		0 (0/60)
Assosa				
	Banbasi	Dabus	0	0 (0/60)
		Keshmando		0 (0/70)
		Woneba		0 (0/60)
	Kurumuk	Kurumuk	14.14(27/191)	1.64(1/61)
		Kutaworke		25.00(15/60)
		Dulshitalu		15.71(11/70)
	Sherkole	Mekazen	0	0 (0/67)
		Fadursefabegu		0 (0/68)
		Awelbegu		0 (0/71)

*Pr = 0.000 **Pr= 0.000

The seroprevalence of asymptomatic visceral leishmaniasis in dogs

Of the 36 dogs owned by households which had LST reactive member(s) 5 (13.9%) and 2 (5.6 %) were reactive by rk39 and DAT respectively (Table 3). The trend in distribution of the seroprevalence in dogs paralleled that observed in human; more positive dogs were found in sites where there were more LST positive humans (Tables 2 and 3).

Table 3: Seropositivity of dogs (N=36) owned by households with LST positive member(s), Benishangul Gumuz, Western Ethiopia, 2018 – 2020.

Kebele	By rK39		By DAT		DAT and/or rK39
	Tested	Positive	Tested	Positive	Positive n (%)
Mender -24	3	0	3	0	0
Almahal	4	1	4	0	0
Abulhorse	10	3	10	1	3
Qota	4	0	4	0	0
Kurumuk	1	0	1	0	1
Kutaworke	8	0	8	0	0
Dulshitalu	6	1	6	1	1
Total	36	5 (13.89)	36	2 (5.56)	5 (13.89)

3.2. Factors associated with asymptomatic *Leishmania* infection

The exposure to *Leishmania* infection showed significant gender difference. Females were about 0.4 times less likely to be affected compared to Males (AOR = 0.38; 95% CI= 0.20, 0.72). Yet age showed no significant association with VL exposure. Presence of dog in a household was found to increase by 2.6 times the likelihood of being LST positive (AOR = 2.60; 95% CI= 1.54, 4.40). Living in Kurmuk district (AOR 5.85, 95%CI 2.27, 15.09) had the highest risk followed by Guba districts (AOR 4.74, 95%CI 1.83, 12.31) (Table 4).

Table 4: Risk factors associated with asymptomatic visceral leishmaniasis as measured by leishmanin skin test, Benishangul-Gumuz, western Ethiopia, 2018 - 2020.

Risk factors	COR (95% CI)	P value	AOR (95% CI)	P value
Own Dog				
Yes	3.37(2.08, 5.46)	0.000	2.6(1.54, 4.40)	0.000
No	1 (Ref.)	-	1 (Ref.)	-
Sex				
Male	1 (Ref.)	-	1 (Ref.)	-
Female	0.43 (0.23, 0.79)	0.006	0.38(0.19,0.72)	0.003
Age				
<5	1 (Ref.)	-	1 (Ref.)	-
5 - 12	0.22 (0.08, 0.61)	0.004	0.40 (0.13, 1.24)	0.112
13 - 18	0.65(0.36, 1.18)	0.156	1.23(0.59, 2.58)	0.586
>18	-	-	-	-
Woreda				
Pawi	1 (Ref.)	-	1 (Ref.)	-
Guba	6.83(2.79, 16.73)	0.000	4.74(1.83, 12.31)	0.001
Dangur	1.36(0.45, 4.11)	0.589	0.91(0.29, 2.86)	0.874
Bambasi	1	-	-	-
Kurmuk	5.87(2.37, 14.55)	0.000	5.85(2.27, 15.09)	0.000
Sherkole	1	-	-	-
Occupation				
Farmer	1 (Ref.)	-	1 (Ref.)	-
Trader	0.69(0.08, 5.86)	0.737	0.28(0.03, 2.67)	0.270
Civil servant	0.14(0.02, 1.06)	0.056	0.09(0.01, 0.72)	0.023
Laborer	1	-	-	-
Driver	-	-	-	-
Students	0.35(0.19, 0 .64)	0.001	0.52(0.24, 1.13)	0.102

Discussion

Benishangul-Gumuz is one of the crucial development corridors in western Ethiopia. Accompanying the large-scale projects there is a huge sociodemographic and ecological changes. Large areas in region were predicted to be a high-risk for VL based on the environmental factor based geographical information and statics risk mapping [14]. Yet, data hardly existed on the epidemiology of VL in the region [17]. This epidemiological survey is the first that assessed asymptomatic *Leishmania* infection covering wider at high risk districts in the region in humans and dogs.

The prevalence of *Leishmania* infection was 6.0% based on LST positivity. The seroprevalence in human *Leishmania* infection was 3.2% (8/252) by rk39 and 5.9% (15/252) by DAT. The LST positivity rate in our study is in agreement with previously reported *Leishmania* infection prevalence, Hailu *et al*. (2009) from Aba Roba, southern Ethiopia and *Bsrat et al.*(2018) from Welkait, northern Ethiopia, of 5.4%**,** 5.6% and 5.88%, respectively [4, 18, 19]. Also is lower than results reported by Ali *et al.*(2004) from lower Awash valley, eastern Ethiopia and Tadese *et al.*(2019) from Raya Azebo, North eastern Ethiopia, who reported reactive rates of 38.3%, and 9.08%, respectively [20, 21]. The seroprevalence in Benishangul-Gumuz by rK39 (3.2%) was lower than the report by Alebie et *al.* (2019*)* from Gode and Adale districts of Shebele zone (12.7%), south eastern Ethiopia [22]. The DAT positivity rate was in agreement with Hailu *et al.* (2009) who found 5.4% [19], and was higher than that of Tadese *et al*. (2019) who reported 0.8% [20]. The difference in prevalence is expected as the risk factors or level of risk factor for exposure to sand fly bite differ in different at-risk communities.

Understanding the determinants of VL exposure in an area is important information for designing infection prevention methods. Thus, we examined personal and household factors implicated with *L. donovani* infections. The significant differences in exposure between males and females observed in the present study was supported by Ali *et al*., (2002), Hailu *et al*., (2009) and Bantie *et al*., (2014) [4, 19, 21]. This could be due to the reason that males are mostly engaged in outdoor activities and stay out door that might have increased their chance to sand fly bites.

In the current study participants who owned dog had 2.6 (95% CI 1.54, 4.40) times higher chance of being LST positive, a finding that paralleled the report by Bsrat *et al*. (2018) [4], yet, the seroprevalence in dogs was higher in the current study. This difference might be due we purposely sampled dogs owned by households with LST positive members and also the small sample size, as our aim was not to determine prevalence in dogs but generate a lead data if dogs are implicated in the transmission.

The relatively lower *Leishmania* infection prevalence detected in this study could indicate most probability that VL is a recent (re) emergence in/introduction to Benishangul Gumuz region. The lack of significant exposure risk difference between age groups corroborates our argument of VL being a recent phenomenon to Benishangul Gumuz Region. Further, the relatively higher prevalence in districts like Guba, were high socioecological modifications happened within Benishangul Gumuz and Metekel area that shares borders with the high VL burdened foci in Amhara, thus the recent move to areas uninhabited hitherto and/or influx of people from all corners, mostly project employees (civil servants) to the region might have precipitated the transmission in the community. Lack of sand fly data, limited and purposive sampling of dogs hampered a conclusion as to their contribution to the transmission. The risk factors captured in the study were not exhaustive enough due to lack of experience what is going on the ground which also limited to dissect in and make recommendations.

Conclusions

It is noteworthy that our approach of risk modeling to targeted surveillance in areas hitherto not known to be VL endemic is proved to be useful. We showed the presence of active VL transmission in key developmental corridor, Benishangul-Gumuz. We recommend the regional health bureau and responsible stakeholders to be vigilant, and plan early containment measures to avoid possible public health and economic consequences due to VL.

Declarations

Ethics approval and consent to participate

This research was approved by the AHRI/ALERT ethics committee (AH01275/0012/18, 19-12-18). Specimen collection was done with the informed consent of the household head for dogs. Informed consent of participant, Parent/or guardian for minors and verbal assent for 11 to 18 years in addition to parental/legal guardian was sought for humans.

Consent for publication

Not applicable

Availability of data

The data supporting the conclusions of this article are included within the article and anonymized data could be shared upon request to the corresponding author as per data sharing policy of the Armauer Hansen Research Institute.

Competing interests

Dr Nigus Manaye is the employee of the funding institute.

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Author Contributions

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Reference

1. Jambulingam P, Pradeep Kumar N, Nandakumar S, Paily KP, Srinivasan R: **Domestic dogs as reservoir hosts for Leishmania donovani in the southernmost Western Ghats in India**. *Acta Trop* 2017, **171**:64-67.
2. Hassan MM, Osman OF, El-Raba'a FM, Schallig HD, Elnaiem DE: **Role of the domestic dog as a reservoir host of Leishmania donovani in eastern Sudan**. *Parasit Vectors* 2009, **2**(1):26.
3. Dereure J, El-Safi SH, Bucheton B, Boni M, Kheir MM, Davoust B, Pratlong F, Feugier E, Lambert M, Dessein A *et al*: **Visceral leishmaniasis in eastern Sudan: parasite identification in humans and dogs; host-parasite relationships**. *Microbes Infect* 2003, **5**(12):1103-1108.
4. Bsrat A, Berhe M, Gadissa E, Taddele H, Tekle Y, Hagos Y, Abera A, M GM, Alemayhu T, Gugsa G *et al*: **Serological investigation of visceral Leishmania infection in human and its associated risk factors in Welkait District, Western Tigray, Ethiopia**. *Parasite Epidemiol Control* 2018, **3**(1):13-20.
5. Bashaye S, Nombela N, Argaw D, Mulugeta A, Herrero M, Nieto J, Chicharro C, Canavate C, Aparicio P, Velez ID *et al*: **Risk factors for visceral leishmaniasis in a new epidemic site in Amhara Region, Ethiopia**. *The American journal of tropical medicine and hygiene* 2009, **81**(1):34-39.
6. Kalayou S, Tadelle H, Bsrat A, Abebe N, Haileselassie M, Schallig HD: **Serological evidence of Leishmania donovani infection in apparently healthy dogs using direct agglutination test (DAT) and rk39 dipstick tests in Kafta Humera, north-west Ethiopia**. *Transboundary and emerging diseases* 2011, **58**(3):255-262.
7. Gebresilassie A, Abbasi I, Aklilu E, Yared S, Kirstein OD, Moncaz A, Tekie H, Balkew M, Warburg A, Hailu A *et al*: **Host-feeding preference of Phlebotomus orientalis (Diptera: Psychodidae) in an endemic focus of visceral leishmaniasis in northern Ethiopia**. *Parasit Vectors* 2015, **8**:270.
8. Yared S, Gebresilassie A, Abbasi I, Aklilu E, Kirstein OD, Balkew M, Brown AS, Clouse RM, Warburg A, Hailu A *et al*: **A molecular analysis of sand fly blood meals in a visceral leishmaniasis endemic region of northwestern Ethiopia reveals a complex host-vector system**. *Heliyon* 2019, **5**(7):e02132.
9. Alvar J, Bashaye S, Argaw D, Cruz I, Aparicio P, Kassa A, Orfanos G, Parreno F, Babaniyi O, Gudeta N *et al*: **Kala-azar outbreak in Libo Kemkem, Ethiopia: epidemiologic and parasitologic assessment**. *The*

American journal of tropical medicine and hygiene 2007, **77**(2):275-282.

10. Gebremichael Tedla D, Bariagabr FH, Abreha HH: **Incidence and Trends of Leishmaniasis and Its Risk Factors in Humera, Western Tigray**. *J Parasitol Res* 2018, **2018**:8463097.
11. Gadisa E, Tsegaw T, Abera A, Elnaiem DE, den Boer M, Aseffa A, Jorge A: **Eco-epidemiology of visceral leishmaniasis in Ethiopia**. *Parasites & vectors* 2015, **8**:381.
12. Custodio E, Gadisa E, Sordo L, Cruz I, Moreno J, Nieto J, Chicharro C, Aseffa A, Abraham Z, Hailu T: **Factors associated with Leishmania asymptomatic infection: results from a cross-sectional survey in highland northern Ethiopia**. *PLoS neglected tropical diseases* 2012, **6**(9):e1813.
13. FMoH: **Guideline for diagnosis, treatment and prevention of leishmaniasis in Ethiopia.** Edited by Unit N, second edn. Addis Ababa: Ethiopian Federal Ministry of Health; 2013.
14. Tsegaw T, Gadisa E, Seid A, Abera A, Teshome A, Mulugeta A, Herrero M, Argaw D, Jorge A, Aseffa A: **Identification of environmental parameters and risk mapping of visceral leishmaniasis in Ethiopia by using geographical information systems and a statistical approach**. *Geospatial health* 2013, **7**(2):299-308.
15. Abera A, Tasew G, Tsegaw T, Kejella A, Mulugeta A, Worku D, Aseffa A, Gadisa E: **Visceral Leishmaniasis in Benishangul-Gumuz Regional State, Western Ethiopia: Reemerging or Emerging?** *The American journal of tropical medicine and hygiene* 2016.
16. Abera A, Tasew G, Degu A, Almneh M, Mulugeta A, Aseffa A, Gadisa E: **VISCERAL LEISHMANIASIS FROM AN AREA PREVIOUSLY NOT KNOWN TO BE ENDEMIC; DANGUR, BENSHANGUL-GUMUZ, REGIONAL STATE, NORTHWEST ETHIOPIA: A CASE REPORT**. *Ethiopian medical journal* 2016, **54**(1):33-36.
17. Abera A, Tasew G, Tsegaw T, Kejella A, Mulugeta A, Worku D, Aseffa A, Gadisa E: **Visceral Leishmaniasis in Benishangul-Gumuz Regional State, Western Ethiopia: Reemerging or Emerging?** *The American journal of tropical medicine and hygiene* 2016, **95**(1):104-108.
18. Gadisa E, Custodio E, Cañavate C, Sordo L, Abebe Z, Nieto J, Chicharro C, Aseffa A, Yamuah L, Engers H: **Usefulness of the rK39-immunochromatographic test, direct agglutination test, and leishmanin skin test for detecting asymptomatic Leishmania infection in children in a new visceral leishmaniasis focus in Amhara State, Ethiopia**. *The American journal of tropical medicine and hygiene* 2012, **86**(5):792-798.
19. Hailu A, Gramiccia M, Kager PA: **Visceral leishmaniasis in Aba-Roba, south-western Ethiopia: prevalence and incidence of active and subclinical infections**. *Annals of tropical medicine and parasitology* 2009, **103**(8):659-670.
20. Tadese D, Hailu A, Bekele F, Belay S: **An epidemiological study of visceral leishmaniasis in North East Ethiopia using serological and leishmanin skin tests**. *PloS one* 2019, **14**(12):e0225083.
21. Ali A: **Leishmaniases survey in the Awash Valley: leishmanin skin test profile in the Upper Awash and surrounding areas**. *Ethiopian medical journal* 1997, **35**(4):225-233.
22. Alebie G, Worku A, Yohannes S, Urga B, Hailu A, Tadesse D: **Epidemiology of visceral leishmaniasis in Shebelle Zone of Somali Region, eastern Ethiopia**. *Parasites & vectors* 2019, **12**(1):209.

Figures

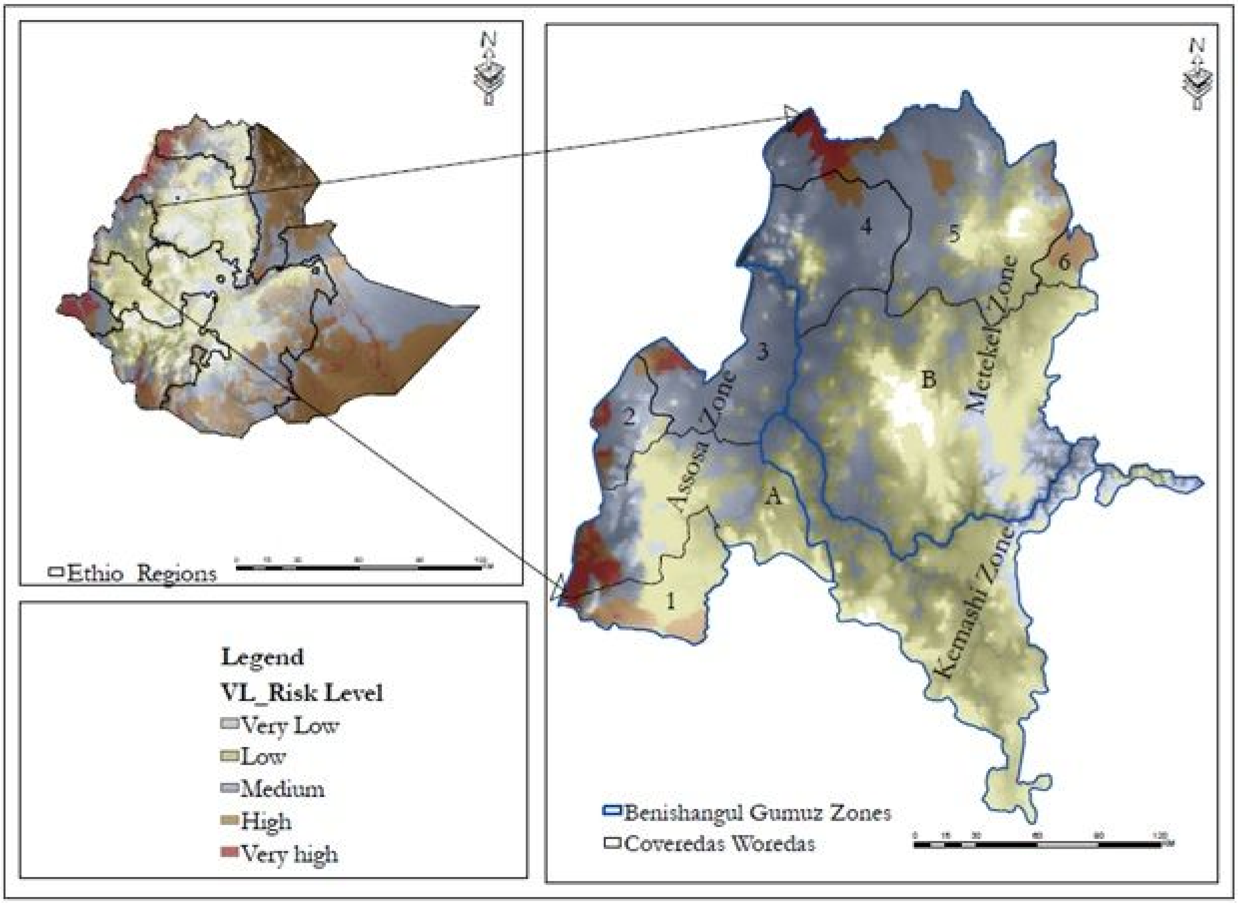

Figure 1

Study area, Shibabaw et al.

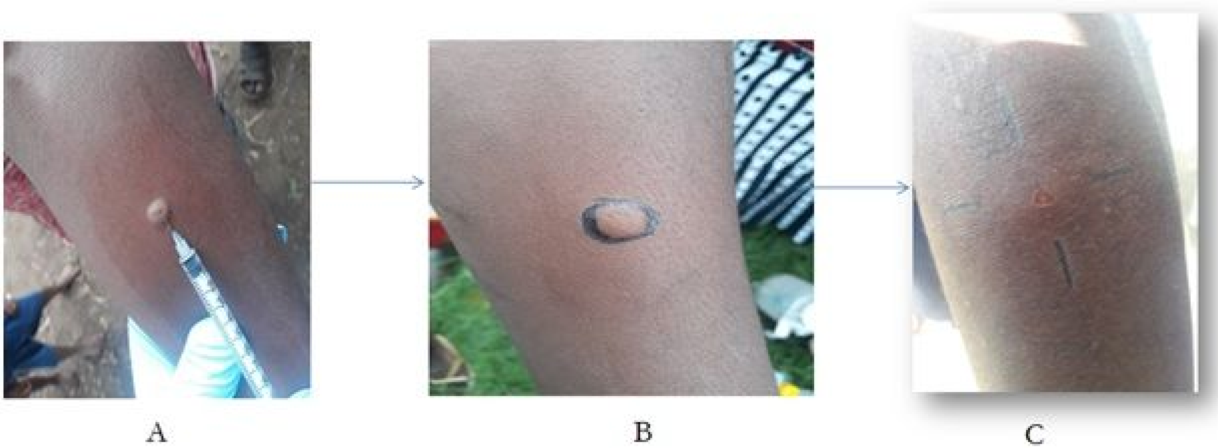

Figure 2

LST procedure, Shibabaw et al

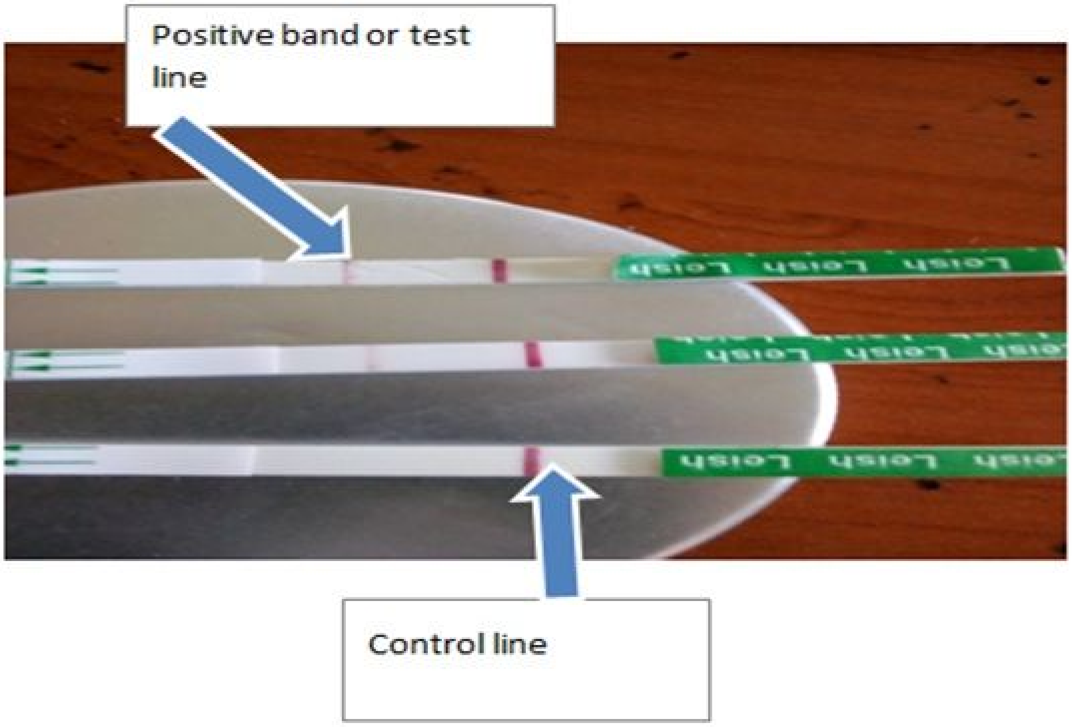

Figure 3

rK39 Procedure, Shibabaw et al

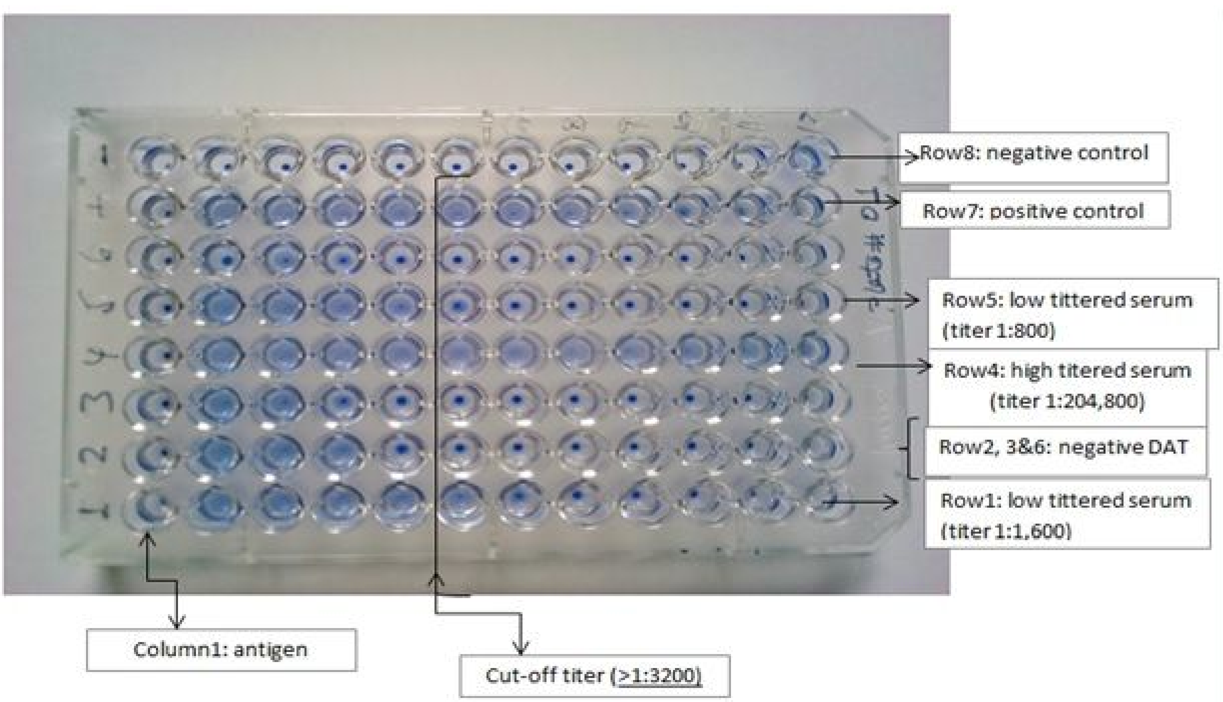

Figure 4

DAT procedure, Shibabaw et al

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- Shibabawetal.GraphicAbstract.tif