



Department of Public Health

**Ethiopian Field Epidemiology and Laboratory Training Program
(EFELTP)**

**Assessment of the Determinants of Visceral Leishmaniasis at
Leishmaniasis Treatment Center of Yabello General Hospital,
Borena Zone, Southern Ethiopia: Unmatched Retrospective Case
Control Study**

Investigator: Mustafa Aliyi (BSc, Field Epidemiology Resident)

**A Thesis Submission to the Department of Public Health, St.
Paul's Hospital Millennium Medical College in Partial
Fulfillment of the Requirements for the Degree of Masters of
Public Health in Field Epidemiology**

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October. 2021

Addis Ababa, Ethiopia

**St. Paul's Hospital Millennium Medical College Department of
Public Health
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Case Control Study**

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**October, 2021
Addis Ababa, Ethiopia**

Abstract

Background

Visceral Leishmaniasis (VL) is listed among the 20 neglected tropical diseases by the World Health Organization, and an estimated 50,000 to 90,000 new cases of VL with 20,000 to 40,000 deaths were reported each year. Presence of *Acacia seyal* and *Balanites* trees, staying and sleeping outdoors during night times, live in cracked well house and ownerships of domestic animals are among determinants of VL. There is scarce of literature and records regarding determinants of VL in Borena zone.

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Objective: The aim of this study to assess the determinants of visceral leishmaniasis at leishmaniasis treatment center of Yabello general Hospital, Borena Zone Southern Ethiopia.

Methods

Hospital based unmatched 1:2 case control study design conducted in Yabello General Hospital from March to June, 2021. Case 54 and control 108 enrolled. Case selected from VL registration book and Control from Outpatient department registration book. Systematic random sample technique used. Data was collected by face-to-face interview method using pre-tested structured questionnaire. Data was entered by Epi Info software version 7.2.1.0 and analyzed by using SPSS statistical software ver-23. Data were presented in the form of table and text with description of mean, percent and frequency.

Result

From the total study participants were 162(100%), from this 114 (70.4%) were male and also statistically significant with visceral leishmaniasis (AOR = 3.367; 95% CI: 1.150–9.856), and as Age increase by a year the chance of getting VL disease was decreased (AOR = 0.934; 95% CI: 0.903–0.966). The other determinants of this study were the family with history of VL case (AOR=3.157; 95% CL= 1.239-8.042), Presence of anemia and sleeping under acacia tree were (AOR=4.905; 95% CL= 2.030 -11.856) and (AOR=2.762; 95% CL= 1.119 -6.815) respectively

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Conclusion

The highest number of VL case were male and being male was determinant of VL case. Younger individual more exposed than elders, those living in the house with the history of VL case in the family members, being anemic and sleeping under acacia tree during night times were determinants of VL disease revealed by this study. Health education should be

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strengthen specially for those men who engage in different out rich activity on VL, sand flies with breeding site and on nutrition related awareness.

.
Keywords: - Visceral leishmaniasis, Determinant, Yabello General Hospital, Ethiopia

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First of all, I would like to thank St. Paul's Hospital Millennium Medical College, Department of Public Health for giving me this golden chance to exercise my academic knowledge conducting this research and for their logistic support. Secondly, I would like to thank my Advisors Mr. Hailemichael Bizuneh and Dr. Samrawit Solomon for their great advises and corrective support. I need to forward my appreciation to Mrs. Haimanot Ewnetu, field coordinator and Mrs. Jimmawork Wondimu resident advisor, for their fantastic encouragement and facilitation to this research. I also like to forward my appreciation to those participate on data collection and study participant for their willingness to participate on this research.

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Abbreviation

AIDS	Acquire Immunodeficiency Syndrome
AOR	Adjusted Odds Ratio
GC	Gregorian Calendar
HIV	Human Immune Virus
IRS	Indoor Residual Spray
ITN	Insecticidal Treated Net
OR	Odds Ratio
ORHB	Oromia Regional Health Bureau
PCR	Polymerizes Chain Reaction
PI	Principal Investigator
SNNPR	South Nation Nationality of People's Region
SPHMMC	Saint Paul's Millennium Medical College
SPSS	Statistical Package for Social Science
VL	Visceral Leishmaniasis
YGH	Yabello General Hospital
WHO	World Health Organization

1. Introduction

1.1. Background

Visceral Leishmaniasis (VL) or in other name Kala-azar, is among the 20 neglected tropical diseases by the World Health Organization (1). Visceral Leishmaniasis case is the most serious and is almost always fatal if untreated; and it is one of four main forms of the disease leishmaniasis: Visceral Leishmaniasis, Post-kala-azar Dermal Leishmaniasis (PKDL), Cutaneous Leishmaniasis (CL) and Mucocutaneous leishmaniasis (2).

Visceral Leishmaniasis is severe, often fatal tropical fever caused by a protozoan parasite that enters the body via a sand fly bite. It affects several internal organs such as: spleen, liver, and bone marrow. The illness typically develops within months (sometimes as long as years) of the sand fly bite. Diseased people usually have fever, weight loss, enlargement of the spleen and liver, and low blood counts (a low red blood cell count, a low white blood cell count and a low platelet count) (3).

Visceral leishmaniasis is transmitted by *Leishmania* species by Phlebotomine female sandflies of the genus *Phlebotomus* or *Lutzomyia*. In addition, Human-to-human transmission was reported in addition to anthroponomical or zoonotic, from which: organ transplantation, blood transfusions and needle sharing among intravenous drug addicts are stated among the way of VL transmission can be happen (4).

More than one billion people live in areas endemic for leishmaniasis and are at risk of infection all over the world, in 2017 more than 90% of new cases occurred in only 10 countries: Brazil, Bangladesh, China, Ethiopia, India, Uganda, Kenya, Somalia, South Sudan and Sudan (5).

Indian subcontinent take lion share of VL burden and Eastern Africa is the second-highest-burdened of visceral leishmaniasis (6). In the horn of Africa, Ethiopia is the third most affected country with an estimated 3.2 million people are at risk of VL and an estimated 375,633 square kilometers (33%) of the landmass in northeastern, northwestern, western and southeastern parts of the country is highly suitable for the transmission of VL (7).

Visceral leishmaniasis is concentrated in areas with high density of *Acacia seyal* and *Balanites aegyptica* trees that grow on vertisol soil type. *P. orientalis* prefer to bite mainly people when they are outdoors, especially in the evening time and in the early morning (8). among peoples infected by Veishmaniasis there are those experienced sleep outdoors during night times (9). Those peoples living in area Vector control by insecticide house spraying is

efficient. Sustainability is crucial as interruption of spraying campaigns lead to re-emergence of leishmaniasis (10). Presence of domestic animals dog ownership (11) and owning goats and poor housing conditions, such as cracked house walls. Cracked walls could be made through drying and could be used as ideal breeding and resting sites of sand flies are factors associated with VL (12).

In Borena Zone, little is known about leishmaniasis but not about determinants of VL, conducting this study is important to explore the determinant of VL in Borena Zone to develop specific policy and plan. In considering this, the main objective of this study is to determine determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone Southern Ethiopia.

1.2. Statement of the problem

According to WHO estimation, around 50 000 to 90 000 new cases of VL occur worldwide each year (13). From 2014 up to 2018, a total 3,053 death of VL reported globally with the mean annual death 610. The case fatality rate slightly increases from 2.1% to 3.3% in 2014 and 2018 respectively. In 2018, more than 95% of VL deaths were reported from the countries: Brazil , South Sudan, India and Ethiopia (2).

In America, Visceral Leishmaniasis is endemic in twelve countries with 59,769 new cases reported from 2001-2017 with an average of 3,516 cases per year. Approximately, 57,582 (96%) cases were reported from Brazil, however, southern countries such as Colombia, Argentina, Paraguay and Venezuela are among those with the highest VL case reported [(14)]. Looking south toward the Rift Valley, major VL disease foci can be found along the border between Kenya and Uganda. Visceral leishmaniasis has also been reported as an endemic disease in Turkana, West Pokot and Baringo counties of Kenya. Furthermore, VL epidemics in Kenya have been reported in Rift Valley and eastern regions, specifically Mandera and Marsabit counties (15).

In Ethiopia, the annual incidence ranges from 3,700 to 7,400 cases (7), with the endemicity of VL was recently extended to at least eight administrative regions namely: Amhara, Tigray, Southern Nations Nationalities and Peoples Region, Oromia, Somali, Afar, Gambella, and Benishangul Gumuz Regional States (16). The disease is well established in Metema and Humera plains in Northern Ethiopia, the Omo plains, the Aba Roba focus, and Weyto River Valley in South Nations and Nationalities Peoples region. It was also reported from the Moyale area and Genale river basin in the Oromia regional state, Afder and Liban zones in

Ethiopia's Somali region, and the Awash Valley in the Afar regional state (6). In the southern parts of the country, Districts like Konso, Hamar, Dasenech, and Nyangatom areas in SNNPR; and Teltele in Borena Zone of Oromia region were high risk for VL (17,18).

The increase in Leishmaniasis prevalence can be due to several reasons. Environmental conditions, socio-economic status, demographic, and human behaviors pose significant risks for human Leishmaniasis. Environmental changes as well as population movements, probably alter the dynamics of vectors and reservoirs that may increase human exposure to the diseases (4). An increment of Leishmaniasis /Human Immunodeficiency Virus (HIV) co-infection is an emerging challenge to be addressed as well as an important factor for the spread of the disease. While the global co-infection rate is 2-12% of all VL cases, Ethiopia has reported higher rates of co-infection (16).

Environmental management includes physical modification of the sand-fly habitat, relocation of human settlements away from known vector habitats, and the implementation of environmental sanitation programs to eliminate actual or potential breeding sites. Long-Lasting Insecticide Treated Nets (LLITNs) distributed for malaria control and prevention, however, it is also used for sand-fly to controlling in door transmission. Insecticide spraying is only effective in indoor or per-domestic transmission and not in sylvatic or outdoor situations and others like utilization repellent and vector control are among the intervention implemented in Ethiopia so far (16). According to Borena Zone Health Department report (unpublished) from July up to October 2019, there was Leishmaniasis outbreak in Borena Zone with 153 suspected VL case and nine suspected VL death. Case fatality rate was estimated 1.4 per 100,000 population (Borena Zone 2020 report).

Even if there are many studies conducted regarding VL and its associated factors in the Northern and Northwestern part Ethiopia (9,11,12,19–21), different study design conclude their finding in different way which cannot represent all parts of the country. Furthermore, there are different risk factors in different areas and also the life style, cultural and behavior of the community is different. In the Southern part, especially in the Borena Zone, there is no literature and record on the determinant factor of visceral leishmaniasis as far as I know in this study area. The local determinants of VL will be identified which is important for local public health intervention and provide the data to planner in order to incorporate with local policy.

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1.3. Significant of the study

In the Borena Zone, visceral leishmaniasis is continued as community and governmental concern. Because of VL cases are still reporting, even the outbreak happened in 2019. However, Visceral Leishmaniasis related literature especially on its determinants, not published as far as I know in Borena Zone and nearby area. Moreover, the identified associated factors in Northern part of the country may not work for Southern part. This study will reveal determinant of VL and provide the evidence data, this data will change to information. Using this information to filling gap related to VL and use for: (1) to conduct public health intervention and specific measurement on VL on time, because VL is neglected tropical fever and fatal if untreated. So, prevention and control of Leishmaniasis is depend on knowing different factors of Visceral Leishmaniasis. (2), this study will be used for improvement of public health practice on specified activity. (3), to develop Policy, this will be conducted after recommendation forwarded to develop local specific policy, strategic and technical plan on VL control and prevention for Borena Zonal administration. But not only for Borena zone, but also for those on the same setup and nearby community, we will be address to different audience through publication on relevant peer reviewed journals.

2. Literature Review

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2.1. Literature overview

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In Uganda at Amudat Hospital, there were 4,428 admissions for VL investigation between 2000 and 2006. In Kenya Kacheliba Health center, there were 2,301 cases of visceral leishmaniasis tested between 2006 and 2010(15). A total of 1128 VL cases were admitted at Kahsay Abera Hospital from July 2011 to August 2013(12).

The most frequently reported symptoms of VL cases; In Kacheliba of Kenya, fever were (100%), weight loss (87.8%), loss of appetite (86.8%), spleen enlargement (77.1%), nose bleeding (21.9%), vomiting (16.5%), headache (14.6%), abdominal pain (13.0%), abdominal swelling (11.4%), and diarrhea (8.5%) (15). In Ethiopia, 96.2% fever, 12.5% spontaneous bleeding, 94.2% weight loss and 6.7% jaundice were reported (22).

The diagnoses were based on the patients history, clinical examinations, parasitological investigations, and/or rk39 dipstick test results and Hematological examinations were among diagnosis conducted in Ethiopia (12).

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2.1.1. Socio-demographic factors

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According to the VL outbreak investigation between September and November 2009 in Nepal, case control study design conducted on the total sample size of 563, among those living in the Large households size (>5 person) 3.6 times more likely to develop VL case than those living in the small number of households (AOR: 3.55, 95%CI: 1.84 – 6.83 with P value >0.0001) (23).

The outbreak investigation conducted in Mersabet County, Northern Kenya, from May to October 2014, from the total 136 cases Males were more affected than females 105 (77%). Although all age categories were affected but majority of the cases were older male between 15 and 44 years 61 (42%) (24).

According to case control study conducted on kahsay abera hospital catchments population, setit Humera district, Tigray region, Northwest Ethiopia, among 283 (case 94 and control 189) participant enrolled in the study from January to July 2013, The odds for visceral leishmaniasis consistently increased as the size of the family increased in the household (OR1.3; 95% CI: 1.026-1.799) (12).

In other study conducted in North Gondar zone, northwest Ethiopia, from September 1-30, 2013, unmatched case control study conducted among 545 (case =109 and control 436), Malnourished individuals were about 3 times more likely to be infected with VL than Normal (9).

2.1.2. Environmental factors

According to the study conducted in Worldwide risk factors in leishmaniasis, Household design and construction material including number of floors, number of rooms, dirt floor, damp earthen floors and houses with cracked mud or thatched plastered house walls are the common risk factors of leishmaniasis(4).

According to Cohort study conducted in India and Nepal on 20 clusters (14 in India and 6 in Nepal) from November 2006 to May 2009, Presence of other VL cases in the house where high risk than those no any case in the household, OR: 3.03, 95%C; 1.68- 5.48 with P-value 0.001 (25). In The other hand, VL outbreak investigation between September and November 2009 in Nepal, case control study design conducted on the total sample size 563, those Proximity to other VL cases (within 50 m) 3.97 fold at risk than those far away from VL cases (23).

According to unmatched case control study conducted in the catchment area of the MSF Tabarak Allah Hospital, Sudan, among 1,080 (270 cases and 810 controls) study unit from September 2012 to July 2013, Presence of dog in the yard 2.43 times more likely to have visceral leishmaniasis than those in counterpart (26).

According to the study conducted on Visceral Leishmaniasis in Ethiopia, an Evolving Disease, 2014 : Changes in land coverage such as deforestation and continually replace by irrigation and farmlands has an increasing risk for leishmaniasis. As the population of the reservoir hosts such as dogs, wild canids and rodent increases, the prevalence and incidence rate of VL has increased and the sand fly vectors have become peri-domestic(18).

According to the study conducted in Ethiopia on Identification of environmental parameters and risk mapping of visceral leishmaniasis in Ethiopia by using geographical information systems and a statistical approach, 2012, the annual average temperature shows a high leverage (33%) on VL occurrence, the second most important determinant for VL case was soil type 27% which is Vertisol, the black-cotton soil type. The other determinant for VL occurrence was Attitude a relative percentage of influence was 20% and rainfall also determinant for VL occurrences its percentage of influence was 12% (17).

According to case control study conducted on kahsay abera hospital catchments population, setit Humera district, Tigray region, Northwest Ethiopia, among 283 (case 94 and control 189) participant enrolled in the study from January to July 2013, Those People who are living in the house of cracked mud wall were 6.4 times more risk than those not living in cracked mud wall, and those who owned a goat had 6.4 times increase odds of a being VL case compared with those who did not own goats (12).

According to the study conducted Kefta Humera, Tigray northwest Ethiopia, two parallel case-control studied from 2009 to 2011 and total individual included were 308 (151 resident cases and 157 migrant), an individual sleeping under an acacia tree during the day or at night time conferred 4- to 7-fold increased risk of Visceral leishmaniasis and sleeping near to dog 6.79 times higher risk of getting visceral leishmaniasis infection than those slept far apart from dogs (19).

According to survey conducted in Humera, Addis zemen and sheraro from October to November 2010, among 546 tested for exposure to *Leishmania* spp and sand flies in domestic animals in northwestern Ethiopia, the most suspected animal reservoirs for *L. donovani* are dogs, which are known to play a key role as reservoir hosts in the transmission cycle of the closely related *L. infantum*. And also many other domestic animals have been seropositive for *Leishmania* species, indicating these domestic animals (donkeys, goats, and sheep) as putative host species in local VL foci. Nevertheless, it is crucial to mention that neither PCR-positivity nor seropositivity indicates that an animal is able to maintain the parasite for a long period of time(21).

In other study conducted in North Gondar zone, northwest Ethiopia, from September 1-30, 2013, unmatched case control study conducted among 545 (case =109 and control 436) Living in a house made from mud wall increases the odds of getting visceral leishmaniasis infection by 2.5 times, and those their home around acacia tree were 3.19 times more likely to have Visceral leishmaniasis than those far apart (9).

2.1.3. Behavioral factors

In The other hand, VL outbreak investigation between September and November 2009 in Nepal, case control study design conducted on the total sample size 563, those Proximity to other VL cases (within 50 m) 3.97 fold at risk than those far away from VL cases, and Sleeping on a bed (Vs on the floor) AOR: 0.25 95%CI: 0.08 – 0.78 P value 0.016 (23).

According to unmatched case control study conducted in the catchment area of the MSF Tabarak Allah Hospital, Sudan, among 1,080 (270 cases and 810 controls) study unit from September 2012 to July 2013, those people sleeping outside door 3.91 times risky than those not sleeping outside. In the same study and smoking from Acacia seyal as indoor repellent were 1.57 time more likely to have visceral leishmaniasis than those not smoking acacia seyal as indoor repellent (26).

According to the study conducted Kefta Humera, Tigray northwest Ethiopia, two parallel case-control studied from 2009 to 2011 and total individual included were 308 (151 resident cases and 157 migrant), an individual s those slept on the ground 2.96 time more likely to have visceral leishmaniasis than those not sleep on the ground (19).

In other study conducted in North Gondar zone, northwest Ethiopia, from September 1-30, 2013, unmatched case control study conducted among 545 (case =109 and control 436), Subjects who had experience of outdoor sleeping were 6.3 times more likely of getting visceral leishmaniasis infection than those who never sleep outside (9).

2.2. Conceptual framework

- This conceptual framework prepared by following disease causal, agent and environmental triangle model. Which means, the disease is Visceral leishmaniasis, the agent is sand fly and the environmental factors are those facilitate the transmission like: Socio-demographical, environmental factors, Behavioral factors and health service related factors. this conceptual framework is adopted from (4,9,12,17 19,21,23,24,26)

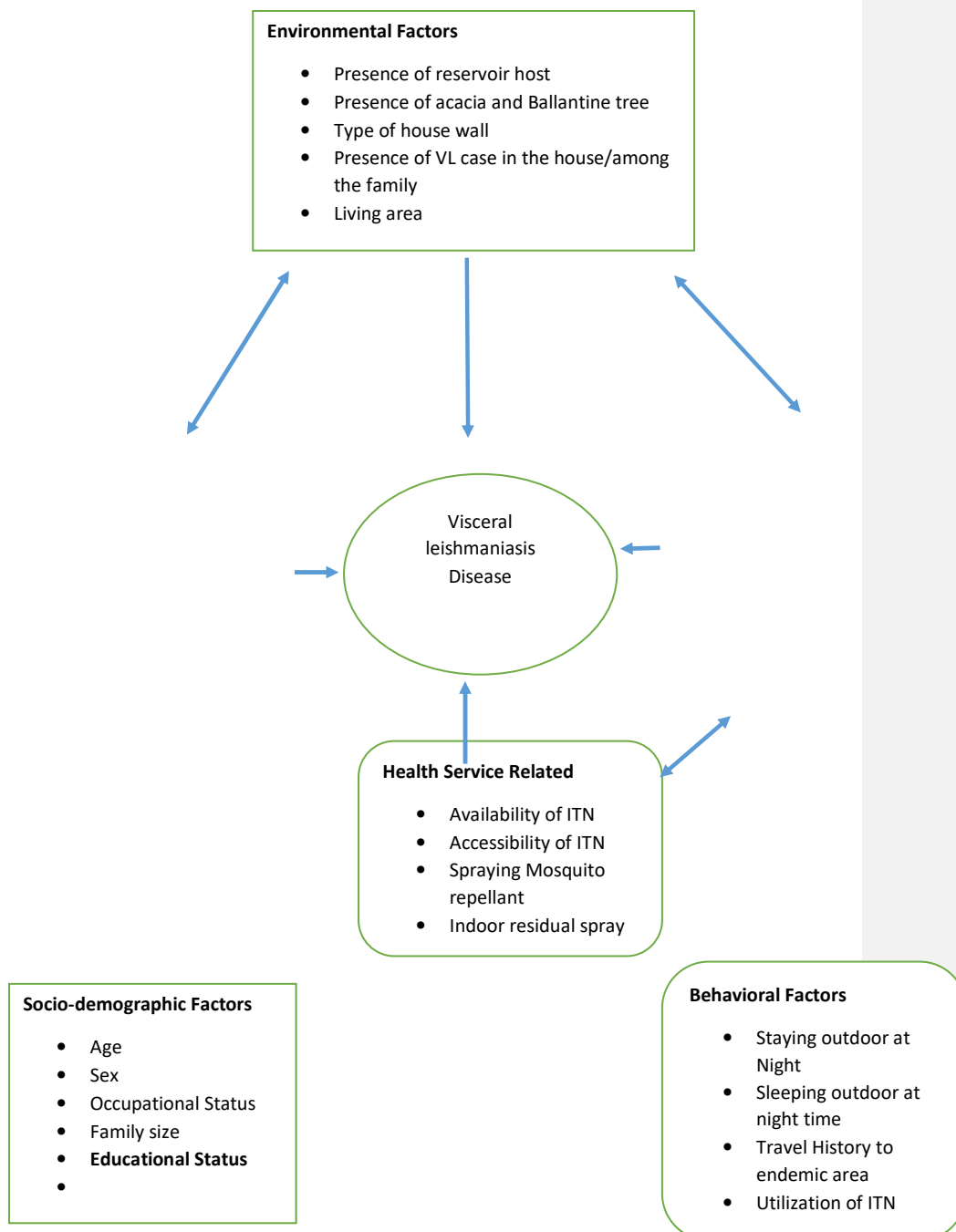


Figure 1 Description of conceptual framework of research on Visceral Leishmaniasis, Ethiopia 2021.

3. Objective

3.1. General Objective

The purpose of this study is to assess the determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone, Southern Ethiopia March to June, 2021

3.2. Specific Objectives

- To determine socio-demographic determinant of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone.
- To identify environmental determinant of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone.
- To assess behavioral determinant of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone.

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4. Methods and Materials

4.1. Study setting and period

The study was conducted in Borena Zone which is one of 21 Zones in Oromia Region, Ethiopia. Borena is bordered on the south by Mersabet County of Kenya, on the west by the Southern Nations, Nationalities, and Peoples' Region (SPNNR), on the north by West Guji and Guji, and on the east by Ethiopian Somali Region. Yabello Town is serving as zonal administration town. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the projected total population of the Zone is about 516,423 with 120,112 households, of which 259,125 were female. Most areas of Borena Zone lies below 1,500 meters above mean sea level; vast area of the Zone is warm and semi-arid lowland. The annual rainfall of the Zone ranges between 592 and 984 ml and the warmest period in the year is from January to March while the lowest annual temperatures occur between the months of June and August. The mean annual temperature ranging from 27° C to 29° C (27)(28).

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Total Governmental health facility in the Borena zone; three hospital (Moyale, Mega and Yabello hospital), 44 health centers and 149 health posts. There are 7 private medium clinics and 45 lower clinics. Except in the Yabello General Hospital, there is no services on diagnosis and treatment for Visceral Leishmaniasis, but they are giving health education services for their community.

Yabello General Hospital had been established since 2009 GC. The hospital is serving its catchment community and the only health facility giving health care services for Visceral Leishmaniasis (Kala-azar) including diagnosis and serves as treatment center to Zonal population and its neighbors Zone including Konso, Burji, and Segan Zones of SNNPR, West Guji Zone of Oromia region, and Mersabet county of Kenya. More than 200 clients attended the outpatient department of the Yabello General Hospital daily. More than 5 suspected case sent to laboratory department for VL test (rk39 rapid test) per day, in average 5-6 VL case admitted to Leishmaniasis treatment ward to stay at least one-month duration on treatment in the ward. The study conducted from March to June, 2021.

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4.2. Study design

A hospital-based unmatched retrospective Case Control (1:2 ratio) study design was used to determine determinants of VL at Yabello General Hospital.

4.3. Population

4.3.1. Source population

All clients who visited the Yabello general hospital during study period were the source population.

4.3.2. Study population

Randomly selected cases and controls at Yabello General Hospital were the study population

- Case: those who developed the sign and symptoms of V. Leishmaniasis, who also tested positive for rk39 serologic tests, and those on treatment and completed the treatment during the study period were included in the study.
- Control: those clients who registered for any case other than VL case and from selected participants, who tested negative for rk39 serologic tests were included as controls.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

For both (Case and Control) we were include: - Those above 18 years old, mother/father or caregiver for <18 year old of participants were included, those has been live in the current resident area for at least one year.

4.4.2. Exclusion criteria

- Case: Those with relapse cases were excluded, because of the factors for relapse cases may be different from active cases.
- Control: those has a history of VL case.

4.5. Sample size determination and sampling procedures

4.5.1. Sample size determination

The sample size is determined by Kelsey formula using the Epi Info version 7.2.1.0 computer software by considering one variable assumed to bring a difference in two groups. So that sample size calculation was based on the following assumptions: Two-sided 95% Confidence level (CI), 80% Power, 1:2 ratio of cases to controls and from a similar study design conducted in North Gondar (9), the percent of controls proportion of sleeping on the ground is 42% with adjusted odds ratio (AOR) of 2.92. Thus, the total sample size by considering a

10% non-response rate were 162 (54 cases and 108 controls). The predictor variables considered during sample size calculations are presented in Table 1.

Table 1. The proportions of determinants of visceral leishmaniasis variables considered for sample size calculation using Epi Info Version 7.2.1.0 computer software.

Variables	% of controls exposed Proportion	AOR	Case + 10%	Control + 10%	Total	Reference
Sleeping on the Ground	42	2.92	54	108	162	(9) (selected)
Presence of acacia tree near to home	38.3	3.01	51	100	151	(9)
Family Size	27.9	3.55	40	79	118	(25)
Live in cracked wall	44.4	6.37	23	46	69	(12)

4.5.2. Sampling technique and procedures

Yabello General Hospital is the only hospital giving diagnosis and treatment with VL (Kala-azar) admission ward in Borena Zone, and also serving as referral site of Zonal health facility. This hospital selected purposively, to get VL case in the Borena Zone and nearby administration area and admitted at Kala-azar ward.

Case: Seventy eight cases admitted VL cases to YGH from March to June, 2021. Out of which one case ruled out due to He was from Kenya, fifty four sample selected out of 77 VL cases admitted to VL ward included to study. By using systematic random sampling technique, we selected consecutive two cases and jumping one case till sample size reached.

Control: control were selected from patients registered at outpatient department for any other illnesses than Leishmaniasis and those tested negative for VL with a serological test (rK39) from selected participant. Participants were selected by using systematic random sampling technique, taking cumulative number from last year DHIS 2 report on the same months, then dividing to number of control participant, the k^{th} value was determined. The first control was selected using lottery method.

According to DHIS 2 reported last years from March to June, 2020 total 3,843 client attended outpatient department at YGH.

$N/n = k^{th}$, which is $3892/108 = 36^{th}$, every 36 clients, one client was selected for control

Where: N = total VL cases admitted to sampling frame

n = number of sample size

4.6. Variables

Dependent Variable

- The Visceral Leishmaniasis case (Positive and Negative).

Independent variables

- **Socio-demographic status variables:** Age, Sex, Residency, Occupation status, and Educational Status.
- **Environmental factors** like: Type of house and its wall, type of ground floor, presence of reservoir host
- **Behavioral factors:** Staying outdoor at night, sleeping outside the door at day and night, and knowing of VL, transmission, and prevention mechanism.
- **Health service related:** like IRS, ITN and repellents

Measurements

In the Yabello General Hospital rk39 test is the test conducted for visceral leishmaniasis diagnosis in addition of Patient history, clinical examination and hematological investigations. Its Immunodiagnosis rapid diagnosis and antibody test for VL. It is a recombinant antigen, rK39 has been shown to be specific for antibodies in patients with VL caused by members of the *L. donovani* complex. This test was performed by medical laboratory professionals, this was after the finger is pricked, half a drop of blood is smeared at the tip of the strip, and the lower end of the strip is allowed to soak in 4 to 5 drops of phosphate-buffered saline, placed on a clean glass slide or tube. If the antibody is present, it will react with the conjugate (protein A colloidal gold) that is pre-dried on the assay strip. The mixture moves along the strip by capillary action and reacts with rK39 antigen on the strip (29), yielding a pink band the procedure and test interpretation attached in annex IV .

A meta-analysis estimated rK39 and direct agglutination test sensitivities of 79% and 93% in East African study with specificities of 85% and 96% respectively (30). A recent article from the study hospital showed rK39 rapid test and DAT sensitivities of 84% and 94%, respectively(31). It is the only available for diagnosis of VL with acceptable sensitivity and

specificity levels which is also inexpensive and simple and no need of special setup to perform the test and in field area(29).

Hemoglobin test performed for all participants by using HemoCue-301 hemoglobin analyzer to determine the presence of anemia. We measured the weight and height of our respondents to assess nutritional status. Tuberculosis and malaria screening conducted and GeneXpert tested for those TB suspected and blood film test conducted for malaria suspected participants.

4.7. Data collection method, tool, and procedures

The data was collected from March to June, 2021. A face-to-face interviewing used by using structured questionnaires. The structured questionnaire was adapted from similar previous literatures (9,12,13,19). The questionnaire was first written in English, and translated into local languages (Afaan Oromo) and translated back into English by language expertise.

Two trained health professions (one BSc Nurse and one Laboratory professional) were used for data collection. Socio-demographic, behavioral and environmental factors of patient data recorded by BSc Nurse, and the other data like laboratory related test and record were admitted by Laboratory professional. Principal Investigator of this study supervised the overall data collection process.

4.8. Data quality control

Two days on-site training on the contents of the questionnaire, on the steps of data collection, quality assurance techniques and ethical related issue were provided for data collectors. The questionnaire was pre-tested at Negele Borena General Hospital on 10% (5 case and 10 Controls) of the total sample size to avoid data contamination. Feedback, suggestions, and comments obtained from the pre-test were incorporated in the final version of the questionnaire.

The principal investigator had made daily checks on the data collection process to ensure the completeness and consistency of the gathered information and errors found during the process had been corrected. Data was also controlled during data entry, using double data entry and Check code data entry software (Epi-Info). Quality control procedure had been

followed during conducting rk39 test for control participant according to manufactures instruction.

4.9. Data processing and analysis

Data entered into Epi Info version 7.2.1.0 and exported to SPSS version 23 for statistical analysis. Descriptive statistics was performed to summarize and present the information in the form of frequencies, mean and standard deviation for quantitative data and percentages and frequencies for categorical data s. A bivariable logistic regression analysis used to examine the determinants of VL cases and a p-value below 0.2 were entered into the multivariable logistic regression model. Multivariable logistic regression analysis with backward LR procedure was used to identify the determinants of VL case. A p-value of less than 0.05 in multivariable logistic regression analysis was considered as statistical significance. Both crude and adjusted odds ratios with a 95% confidence interval (CI) was presented. Hosmer and Lemeshow goodness-of-fit greater than 0.05 was used to test for model fitness. Graphs and tables were used to present the finding of the determinants.

4.10. Ethical considerations

Ethical approval obtained from the Institutional Review Board (IRB) of Saint Paul s Hospital Millennium Medical College (SPHMMC) (Ref. no. pm 23/533) on the date 08 March, 2021 and delivered with Supportive latter to Oromia Regional Health Bureau (ORHB). Support letter written from Oromia Regional Health Bureau (ORHB) (Ref. No BEFO/HBTfH/46/1076) on the date of 13 march, 2021 and submitted to Yabello General Hospital. A written informed consents obtained from each study participant but from those less than eighteen years old participants, we obtain from their care givers. Furthermore, all information that obtained from the respondents had been kept confidential and there was no name of participant written.

4.11. Plan for dissemination of the study results

Study findings will be submitted to SPHMMC, Department of Public Health. The findings will be also submitted to Oromia Regional Health Bureau, Borena Zone Health Department, and Yabello General Hospital. Additionally, this finding will be presented on different scientific/research conferences and meetings. At the end, attempt will be made to publish the findings on the relevant peer-reviewed open access journal.

4.12. Operational definitions

Case: Clinical signs and symptoms associated with VL include fever for more than two weeks, fatigue, weakness, loss of appetite and weight, malaise, cough, diarrhea, epistaxis, cachexia, abdominal pain, anemia, pancytopenia, and associated with parasitic invasion of blood and reticulo-endothelial system, enlargement of lymph nodes, spleen, and liver (16).

Control: Control selected from patients registered at outpatient department for any other illnesses than Leishmaniasis and negative for serological test (rK39).

Stay outside overnight: staying outside the house before going to sleep

Travel history to VL endemic Area: who has a history of travel to Visceral Leishmaniasis endemic area for at least one day and above from him / her regular residency area?

Wearing of full cover clothe: wearing of long sliver clothe and covering of head during staying outside, especially during evening and early morning time.

History of Visceral leishmaniasis in the family: - indicate that presence of VL diseased individual at least before two years in the family.

5. Result

5.1. Socio-demographic status

The study participant rate of were 162 (100%), the mean age of this study was 32 years old and SD were 14. Male were 114 (70.4%), participant related to residency 63 (38.9%) were form urban, the rest from the Rural. From total participants, 81 (50.0%) were categorized under Occupation status of livestock or pastoralist and 68 (42%) were those following Islamic religion show table 2.

From the total VL case participants included in the study, one case with malaria and three cases with Tuberculosis **Conc-omitant OI**

From the total study participates **49 (94.2%)** present with weakness, 47(90.4%) with fever more than two weeks and **23 (44.2%)** were those state with fatigue sign and symptoms fig 2.

Commented [U14]: Need revision on language. Advised to improve the sentences construction so that readers can understand.

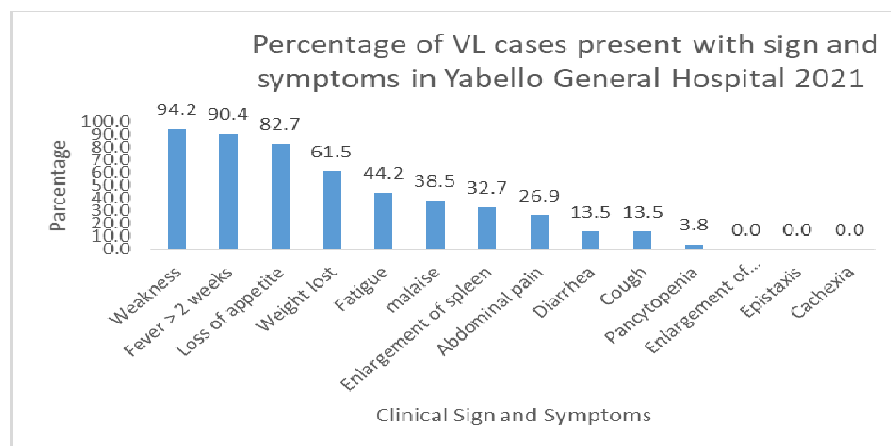


Figure 2 clinical presentation of VL diseased participants in Leishmaniasis treatment center of YGH,

SN	Variable	Category	Case n	Control n	COR	95% C.I.	P-value
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Commented [U16]: No need to report the P value-as far as you reported CI

Borena zone, Southern Ethiopia 2021.

Table 2 Socio-demographic Characteristics and bi-variable analysis of VL in leishmaniasis treatment of YGH Borena Zone, Southern Ethiopia 2021.

Commented [U15]: Suggested to have text description for variable in Table 2

			(%)	(%)		Low	High	
	Age	Mean= 32 SD = 14			0.953	0.926	0.980	0.001
2	Gender	Male	45 (39.5)	69 (60.5)	2.83	1.249	6.393	0.013
		Female	9 (18.8)	39 (81.2)	1			
3	Residency	Urban	8 (12.7)	55 (87.3)	1			
		Rural	46 (46.5)	53 (53.5)	5.967	2.575	13.825	0.000
4	Marital status	Married	27 (24.1)	85 (75.9)	1			
		Unmarried	27 (54.0)	23(46.0)	3.696	1.826	7.478	0.000
5	Educational status CAT	Unable to write and read	18 (32.1)	38 (67.9)	1			
		Primary	28 (38.4)	45 (61.6)	1.314	0.631	2.734	0.466
		Secondary and above	8 (36.4)	14 (63.6)	1.206	0.255	1.789	0.722
6	Religion	Muslim	19 (27.9)	49 (62.1)	1			
		Orthodox	8 (23.5)	26 (76.5)	0.794	0.306	2.058	0.634
		Protestant	9 (40.9)	13 (59.1)	1.785	0.656	4.860	0.257
		Wakefeta	18 (47.4)	20 (52.6)	2.321	1.014	5.314	0.046
7	Family Size	≤4	18 (36.0)	32 (64.0)	1			
		≥5	36 (32.1)	76 (67.9)	0.842	0.418	1.697	0.613

Commented [U17]: What this value indicates? In the text you described the mean value but here there is no mean report? It is also good to report the mean age for cases and control participants.

P value= less than 0.05, COR= crude odd ratio, C.I= Confidence interval and "1" in COR indicate the reference category.

5.2 Environmental related

Characteristics and Bi-variable analysis of Environmental determinants described in the below table, all variables eligible for Multivariable logistic regression except Presence of termite hill around the home which P-value is above 0.2 show table 3

Table 3 Characteristics and Bi-variable analysis of Environmental factors of VL in leishmaniasis Treatment center of YGH, Borena Zone, Southern Ethiopia 2021.

S.N	Variables	Category	Case n (%)	Control n (%)	COR	95% C.I		P-
						Low	High	
1	Type Of House floor	Mud	45(39.1)	70(60.9)	2.714	1.199	6.147	0.017*
		Cement	9(17.0)	38 (83)	1			
2	Type Of House Wall	Thatched	4 (28.6)	10 (71.6)	1.600	0.406	6.299	0.501
		Mud or with mud plastered	41 (39.8)	62 (60.2)	2.645	1.153	6.067	0.022*
		Brick with cement plastered	9 (20.0)	36 (80.0)	1			
3	Type Of House Roof	Thatched	34 (44.7)	42 (55.3)	2.671	1.361	5.243	0.004*
		Non-thatched	20 (23.3)	66 (76.7)	1			
4	IRS Status	Yes	18 (66.7)	9 (33.3)	0.182	0.075	0.441	0.000*
		No	36 (26.7)	99 (73.3)	1			
5	Presence of Acacia Tree around the resident area	Yes	44 (40.0)	66 (60.0)	2.800	1.273	6.158	0.010*
		No	10 (19.2)	42 (80.8)	1			
6	Stay Under Acacia day time	Yes	31 (43.1)	41 (56.9)	2.203	1.133	4.282	0.020*
		No	23 (25.6)	67 (74.4)	1			
7	Sleep Under Acacia night time	Yes	25 (56.8)	19 (43.2)	4.038	1.948	8.371	0.000*
		No	29 (24.6)	89 (74.5)	1			
8	Presence of Ballantine tree	Yes	14 (24.7)	45 (76.3)	0.490	0.239	1.006	0.052

		No	40 (38.8)	63 (61.2)	1			
9	Wearing full cover clothe at evening and night time	Yes	22 (43.1)	29 (56.9)	1			
		No	32 (28.2)	79 (71.2)	0.534	0.268	1.064	0.075
10	Presence of termite hill around the home	Yes	37 (36.3)	65 (63.1)	1.440	0.721	2.875	0.302
		No	17 (28.3)	43 (71.7)	1			

5.3 Behavioral factors

Bi-variable logistics regression analysis of the behavioral factors: - those has behavior of staying outside the door during night time were 74 (45.7%), sleeping outside the door 32 (19.75%), travel history 36 (22.2%) and knowing of visceral leishmaniasis with sign and symptoms as a protective 81 (50%) were eligible for Multivariable Binary logistic regression analysis show table 4.

Table 4 Characteristics and Bi-variable analysis of behavioral factors of VL in leishmaniasis Treatment center of YGH, Borena Zone, Southern Ethiopia 2021.

SN	Variables	Category	Case n (%)	Control n (%)	COR	95% C.I		P-value
						Low	High	
1	Stay Outside overnight	Yes	32 (43.2)	42 (56.8)	2.286	1.174	4.452	0.015*
		No	22 (25)	66 (75)	1			
2	Sleep outside overnight	Yes	14 (43.8)	18 (56.2)	1.750	0.793	3.862	0.166
		No	40 (30.8)	90 (69.2)	1			
3	Travel history	Yes	17 (47.2)	19(52.8)	2.152	1.008	4.595	0.048*
		No	37 (29.4)	89 (70.6)	1			
4	History of VL case in the family	Yes	23 (59.0)	16(41.0)	4.266	2.001	9.093	0.000*
		No	31 (25.2)	92(74.8)	1			
5	Possessing of functional ITN	Daily	10 (41.7)	14(58.3)	1			
		Sometimes	23 (46.0)	27 (54.0)	1.193	0.446	3.190	0.726
		Not At all	21 (23.9)	67 (76.1)	0.439	0.439	0.170	0.089
6	Utilization of ITN	Yes	32 (44.4)	40 (55.6)	1			

Commented [U18]: What about other variables? I think there are also additional variables who are eligible for multivariable analysis which indicated in the Table.

1. History of VL case in the family
2. Utilization of ITN
3. Knowing of VL
4. Knowing of VL prevention
5. Anemia Status

Commented [U19]: Eligible for Multivariable analysis?

Commented [U20]: Eligible for Multivariable analysis?

		No	22 (24.4)	68 (75.6)	0.404	0.207	0.789	0.008*
7	Utilization of repellent	Yes	4 (19.0)	17 (81.0)	1			
		No	50 (35.5)	91 (64.5)	2.335	.745	7.320	0.164
8	Knowing of VL	Yes	46 (38.0)	75 (62.0)	1			
		No	8 (19.5)	33 (80.5)	0.395	0.168	0.930	0.033*
9	Knowing of Sign and symptoms	Yes	35 (43.2)	46 (56.8)	1			
		No	19 (23.5)	62 (76.5)	0.403	0.205	0.792	0.008*
10	Knowing of VL prevention	Yes	31 (43.1)	41 (56.9)	1			
		No	23 (25.6)	67 (74.4)	0.454	0.234	0.883	0.020
11	Anemia Status	Presence of Anemia	31 (46.3)	36 (53.7)	2.696	1.377	5.276	0.004
		Absence of Anemia	23 (24.2)	72 (75.8)	1			

Commented [U21]: Eligible for Multivariable analysis?

Commented [U22]: Eligible for Multivariable analysis?

Commented [U23]: Eligible for Multivariable analysis?

Commented [U24]: How anemia status is considered as behavioral related factor?

P value= less than 0.05, COR= crude odd ratio, C.I= Confidence interval and 1 in COR indicate the reference category.

Multivariable Binary Logistic Regression Analysis conducted, those eligible for multivariable analysis. From those statistically significant variables: - Age, Gender, History of VL case in the Family member and presence of Anemia and sleeping under acacia tree during night times.

Among those statistically significant variables, being Male were 3.367 times more likelihood of getting Visceral Leishmaniasis than its counterpart. AOR = 3.367; 95% CI: 1.150 - 9.85. Those being anemic were 4.9 times higher chances of getting VL cases than those not become anemic, AOR = 4.905; 95% CI: 2.030- 11.85.

Those experienced sleeping under acacia during night times were 2.76 times more chance of getting Visceral leishmaniasis cases than those not sleep under acacia tree, AOR = 2.762 ; 95% CI: 1.119- 6.815 and the other significant variables show on Table 5.

Table 5 Multivariable Binary Logistic Regression Analysis of VL in Leishmaniasis Treatment center of YGH, Borena Zone, Southern Ethiopia, 2021.

Commented [U25]: Before this it is good indicate the sub section for example. Risk factors for visceral leishmaniasis

Commented [U26]: What about other variables for example

Sociodemographic

- 1.residence
2. marital status

Environmental factors

- 1.Type Of House floor
- 2.Type Of House Roof
- 3.IRS Status
- 4.Presence of Acacia Tree around the resident area
- 5.Stay Under Acacia day time
- 6.Sleep Under Acacia night time

Behavioral factors:

1. Stay Outside overnight
2. Travel history
3. Utilization of ITN
4. Knowing of VL
5. Knowing of Sign and symptoms
6. Knowing of VL prevention

AND

No findings (descriptive and inferential) about health service related factors at all

Variables	Category	COR	AOR	95 % C.I.	P value
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				Lower	Upper	
Age		0.953	0.934	0.903	0.966	0.000
Gender	Male	2.826	3.367	1.150	9.856	0.027
	Female	1	1			
History of VL case in the family members	Yes	4.266	3.157	1.239	8.042	0.016
	No	1	1			
Anemia Status	Presence of Anemia	2.696	4.905	2.030	11.856	0.000
	Absence of Anemia	1	1			
Do you sleep under acacia during night time	Yes	4.038	2.762	1.119	6.815	0.027
	No	1	1			

P value= less than 0.05, COR= crude odd ratio, AOR = Adjusted Odd ratio, C.I= Confidence interval and 1 in COR and AOR indicate the reference category.

6. Discussion

From the total study participants male were 114 (70.4%) and also statistically significant with Visceral leishmaniasis when compared with Females, as age increases the chance of getting VL disease decreased, those living in the house with an individual has history of VL cases were more like to get leishmaniasis than those no living with history VL case in the family member. Anemia also one of VL pre-determinant of VL (13), we had also reveal that being anemic was among determinants of visceral leishmaniasis and those sleep under acacia tree at night times were statistically significant variables.

In endemic parts of the county, VL is a disease of children as older people will have gained immunity but in area has recently been introduced, all age groups are susceptible and most cases occur in groups that have regular contact with sand flies habit (18,32). According to this study finding, as age increase by one year the chance of getting Visceral leishmaniasis were decreased by 0.934. Similar with this study conducted in north Gondar under fifteen years were more at risk than those greater than fifteen (9). The study conducted in Northern Ethiopia on asymptomatic under fifteen children, as age increase by a year the probability of being asymptomatic for VL infection were AOR = 1.15 (33). The study conducted in the

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- Explain the meaning and importance of the findings
- Relate the findings to those of similar studies
- Consider alternative explanations of the findings.

Amudet Hospital Uganda, for those age group less than or equal to nine years old almost two times more like to get VL disease than those above nine years old (34). the study conducted in Nepal, those age group between 14_ 24 were 2.22 times more like of getting VL infection (25). This may be due to pastoralist community engage young male on searching and feeding of their cattle s.

In Ethiopia around 90% of Visceral leishmaniasis cases were male (13). In this study finding, being male was 3.367 more likelihood of getting Visceral leishmaniasis disease than its counterparts. This finding in line with the study conducted in Northern Ethiopia in Ayder referral hospital with 97.7% study participants were Male (35), and the other case control study conducted in Gedaref state Sudan with AOR= 6.4 (26) and the study conducted in India and Nepal revealing that, the risk of Male getting VL infection 1.27 time higher than Female(36). This finding perhaps show that, Male was engage in different outdoor activity, like keeping cattle, farm and others activity, since our study area where in the pastoralist community, their life style were move from place to place due to seeking cattle foods locally known as Godaansa which is mostly performed by male.

This study demonstrated that an individual live in the family member having History of Visceral leishmaniasis diseased were 3.157 felt of getting VL disease than those don't has VL case in the family. This result in line with the study conducted in northern high land of Ethiopia, from the total study participants, serologically positive VL case were those living with an individual has history of VL case in the family member with AOR = 4.67 (33),The study conducted in Nepal, the AOR =3.03 more times like to get VL disease (23), the other study with the same area India and Nepal (25) and the other study in line with this finding was from Saran district of Bihar state, India (37) and the other systematic review of visceral leishmaniasis in South Asian (38). In contrast to this finding, the study conducted in North Gondar, Ethiopia not show statistical significant (9) and the study in Sudan (39). This may be indicate that sand flies can bite in the house including at daytime if the room darkened, the other probable reason of this finding may be immune response of individual to agent and may be similar source of infection but due to development of disease is vary from weeks up to 1 or 2 years.

The other determinant revealed by this study were those became anemic were 4.905 times more chance to get VL disease than those do not anemic. Similar with the study conducted in Gedaref state, Sudan with AOR= 4.7 an individual person who become anemic more like to get VL disease (39), and other reported from previous study conducted in Sudan, that Anemic

individual more at risk than those not anemic (32) and this is due to those exposed to insufficient nutrition intake have poor: protein-energy, iron, vitamin A and zinc and other nutritional status which is affect Immunity defense mechanism (40), and also may be due the pastoralist community moving from place to place to feed their cattle s which limited access to nutritional variety.

Those experienced sleeping under acacia during night times were 2.76 times more chance of getting Visceral leishmaniasis cases than those not sleep under acacia tree. This study supported by the study conducted in Kafta-Humera, Tigray, with AOR = 5.22 (19). The study conducted in Amhara region, with AOR = 2.65 and 3.1 (9,41). The other study in line with this study conducted in Gedaref state, Sudan with AOR = 1.72 (26). This may be due to acacia tree or locally known as 'Waacuu Dimaa' is common breeding and resting site of sand flies.

Even if Yabello general hospital is the only health facility giving VL related service and giving referral service for Borena zone and nearby community, VL diseased participant were those come to Hospital for seeking of health service, therefore, its affect generalization of study finding to Borena zone because of its performed in the hospital which is not include all VL patients in Borena zone due to many VL diseased people not attend hospital. Control participants also selected from hospital registration book. From those already diseased persons may lack exposing to infection due to many people stay at home when they are sick. This may be slightly affect the determinant of VL. The other limitation of this study may be recall bias for both case and control participants. In Yabello general hospital, the test has been performed for VL diagnoses was rk39 rapid antibody test with history of patient, clinically and hematological tests support, there was no confirmatory test conducted like spleen aspiration, bone marrow aspiration and molecular test.

7. Conclusion and Recommendation

As much as our knowledge, this the first study regarding VL determinants in the leishmaniasis treatment center of Yabello General Hospital and Borena zone. In this study finding, the highest number of VL case were male and being male was determinant of VL case. Younger individual more exposed than elders, those living in the house with the history of VL case in the family members were determinant of visceral leishmaniasis. Being anemic and sleeping under acacia tree during night times were also determinants of VL disease revealed by this study.

Health education should be strengthen specially for those men who engage in different out rich activity recommended to WoHO

Screening and measurement also recommended for those living with an individual has history of VL case recommended to YGH, WoHO and BZHD.

Finally, farther study related to anemia and its associated factors on pastoralist community is what we recommen ORHB and YGH.

Strength of our study was conducting research on disease categorized under neglected tropical disease, revealing almost the determinants of visceral leishmaniasis in Yabello General Hospital and in Borena zone.

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9. Annexes

Annex 1 Dummy Table

Variables	Category	Case N (%)	Control N (%)
Sex	Male		
	Female		
Age	0-14		
	15-29		
	30-44		
	45-59		
	>=60		
Occupation	Farmers		
	Student		
	Livestock		
	Employment		
	Others		
Residency	Urban		
	Rural		
Family size	<4		
	>=4		
Type of floor	Cement		
	Mud		
Presence of acacia tree near to home	Yes		
	No		
Stay Outdoor at night time	Yes		
	No		
Sleep outside at night	Yes		

	No		
Possession of ITN	Yes		
	No		
Use of ITN	daily		
	sometimes		
	Never used		
Travel history	Yes		
	No		
Use of repellent	Yes		
	No		
Know about VL	Yes		
	No		
VL sign and symptoms	Yes		
	No		
Know about VL prevention	Yes		
	No		
Do you believe in modern treatment	Yes		
	No		

Annex 2 General information, Consent and data collection tool (English version)

Greeting; good morning/afternoon!

Title of the research proposal: Assessment of the Determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone, Southern Ethiopia: Unmatched Retrospective Case Control Study

Name of the principal investigator: Mustefa Aliyi

Name of the organization: Saint Paul's Hospital Millennium Medical College (SPHMMC).

Sponsor: SPHMMC

Introduction: This information sheet is prepared for Yabello General Hospital. The aim of this form is to make the above-concerned offices clear about the purpose of the research work, data collection procedure and get permission to undertake research.

Purpose: The main purpose of this study is, to assess the determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone Southern Ethiopia: Unmatched Retrospective Case Control Study, 2021

Procedures: In order to achieve the above objective, all clients who visit the Yabello general hospital during study period were the source population and randomly selected cases and controls included in this study.

Risk of the study: Participating in this study will not have any risk or harm except Blood sample collected for investigation so, there is minimal risks.

Benefit of the study: there is no direct benefit to the participants but accordingly the result was provide important information on addressing the determinant factors associated with visceral Leishmaniasis to the catchment population.

For control participant the test will be performed free of cost and if asymptotically positive for rk39 VL test, he or she advice to get treatment at leishmaniasis treatment center of Yabello general hospital.

Rights of Participants: You have full right to participate or not in this study. You may respond to all the questions or you may not answer to questions you do not want to and you may end the interview at any time you need. You can ask any question that is not clear for you.

Confidentiality: Any information forwarded confidentially and names were not be written or specified. As to the information given ahead, Participating in this research will be not exposed to any risk. Your name will not be written on this form and the information you give would never share to others. Now I would like to tell you that you selected randomly to participant of the Study. Your genuine response to the interviews was very important for the purpose of the study. At the same time we would like to appreciate your voluntarily participation in the survey after a thorough understanding of the information given to you.

Person to contact: This research proposal will be reviewed and approved by the institutional review board (IRB) of SPHMMC. If any case you want to know more information about the research and its undertakings, you can contact the committee through the address of the advisor and /or principal investigator.

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3. Mustefa Aliyi (EFETP resident) principal investigator,
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Information Sheet for study participants (English version)

A. Information sheet:

Greeting

Hello, how are you dear respondents?

My name is ----- . I am a member of the research team conducted by Mustefa Aliyi (Field Epidemiology Resident) in the Saint Paul s Hospital millennium medical college (SPHMMC) department of public health conducting on the assessment of the determinants of visceral Leishmaniasis at leishmaniasis treatment center of Yabello General Hospital, Borena Zone Southern Ethiopia. I am here to gather information about determinants of visceral Leishmaniasis at leishmaniasis treatment center of Yabello General Hospital. This study will help to improve public health intervention, measurement, and practice and also used for development of policy. Therefore, I want to ask you some questions to take parts in this research. Your name will not be included in the information. I promise to keep the confidentiality of your response. The interview will take about 20 to 30 minutes.

Are you willing to participate in the study? Yes_____ No_____

B. Consent form:

I have been requested to participate in the study of assessment of the determinants of visceral Leishmaniasis . I clearly understood the objective. Since it doesn't affect my personal life, I don't need any remedy. Consequently, I here approve my consent to take part in the study as an Interviewee with my signature. It is therefore with full understanding of this situations that I agreed to give the informed consent voluntarily to the researcher to give an information.

I----- after being fully informed about the detail of this study here by give my consent to participate in this study.

Signature _____ date_____

Name of data collectors _____ signature----- date_____

Name of Principal investigator _____ signature----- date_____

Saint Paul s Hospital Millennium Medical College, Department of Public Health

Questionnaires to assess the determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone Southern Ethiopia: Unmatched Retrospective Case Control Study, 2021

This questionnaire is prepared for collecting data about determinants of Visceral Leishmaniasis at Leishmaniasis

Name of data collector _____ Signature _____ Date _____

Name of supervisor _____ Signature _____ Date _____

SN	ID _____	Participant status	A. Case	B. Control
1	Socio-demographic status			
Q101	Address _____	Zone _____	Woreda _____	kebele _____ village _____
Q102	Age _____			
Q103	Gender : -	A. Male	B. Female	
Q104	Residency	A. Urban	B. Rural	
Q105	Marital Status	A. Married	B. Single	
		C. Divorce	D. Widowed	
Q106	Education	A. Can't read or write	B. Primary	
		C. secondary	D. Diploma and above	
Q107	Occupation	A. Civil servant	B. Livestock	
		C. Farmer	D. House wife	
		E. Student	F. Merchant	
		G. Other (specify) _____		
Q108	Religion	A. Muslim	B. Orthodox	
		C. protestant	D. Catholic	
		E. wakefeta		
Q109	Number of family _____			
1.1	1.1. Care giver/ Family socio-demographic status for under 18 participants			
Q110	Q110. Marital Status	A. Married	B. Single	
		C. Divorce	D. Widowed	
Q111	Q111. Education	A. Unable to read and write	B. Primary	
		C. secondary	D. Diploma and above	
Q112	Occupation	A. Civil servant	B. Livestock	
		C. Farmer	D. House wife	
		E. Student	F. Merchant	
		G. Other (specify) _____		

Q113	Religion	A. Muslim		B. Orthodox	
		C. protestant		D. Catholic	
		E. wakefeta		F. Other _____	
Q114	Number of family _____				
2	Environmental factors				
Q201	Type of floor	A. Cemented		B. Mud	
Q202	Type of wall	A) Brick with cement plastered		B. Brick	
		C. Thatched		D. Mud and brick with mud plastered	
Q203	Type of roof	A) Cemented and asbestos		B. corrugated iron sheet	C. Thatched
Q204	Dwelling status	A. No cattle shed	B. Separate dwelling		C. mixed dwelling/attached cattle shed
Q205	Do you sleep near domestic animals?		A) Yes		B) No
Q206	If yes for Q305, specify them _____				
Q207	IRS-status		A) Yes		B) No
Q208	If yes for Q207 when IRS sprayed				
	A) Before 1 year	B) before 2 years		C) Before 3 years	D) Not sprayed
Q209	Presence of acacia tree around home		A) Yes		B) No
Q210	Do you stay under acacia during day time?		A) Yes		B) No
Q211	Do you sleep under acacia during night time?		A) Yes		B) No
Q212	Presence of Ballantine tree around home		A) Yes		B) No
Q213	Do you sleep under Ballantine tree during night time?		A) Yes		B) No
Q214	Do you wear long silver protective clothe during night time		A) Yes		B) No
Q215	Types of soil around residency area _____, _____				
Q216	Presence of termite hills around home		A) Yes		B) No
Q217	Nutritional status	A. Normal		B. Moderate Malnourished	C. sever malnourished

Q218	Anemia status:	Height _____m	Weight _____kg	MUAC _____for Children s
Q219	TB Status	Negative	Positive	Unknown
Q220	Malaria Status	Negative	Positive	Unknown
Q221	Do you have History of blood transfusion	Yes	No	I Don t remember
3	Behavioral factors			
Q301	Do you stay outside overnight?	A) Yes		B) No
Q302	Do you sleep outside overnight?	A) Yes		B) No
Q303	Travel history to VL endemic Area	A) Yes		B) No
Q304	If yes for Q303, Where:	Woreda _____	Kebele _____	Village _____
Q305	History of VL case in family member	A) Yes		B) No
Q306	Has functional bed net	A) Yes		B) No
Q307	How often you used:	A) daily	B) sometimes	C) never used
Q308	Did you use repellent	A) Yes		B) No
Q309	Do you know about leishmaniasis?	A) Yes		B) No
Q310	If yes for Q309 do you know the factor of VL?	A) sand fly		B) Mosquito
		C) From God		D) I don t know
		E, other (specify) _____		
Q311	Do you know the sign and symptoms of VL case	A) Yes		B) No
Q312	If yes for Q312 how, please mention it _____, _____, _____			
Q313	Do you think that VL is curable?	A) Yes		B) No
Q314	Do you think that VL is preventable?	A) Yes		B) No
Q315	If yes for Q313 how, please mention some of them _____			
Q316	How do you manage yourself/family members when you suspect VL illness?	A visit health facility		
		B, go to traditional hill		

		C, go to holly water	
		D, buy drug from pharmacy	
		E, other (specify) _____	
Q317	Did you believe in VL modern medicine?	A) Yes	B) No
Q318	Any comment on VL? _____		
5	Laboratory Result		
Q501	Rk39 test Result	A. Positive	B. Negative
		C. Invalid	
Q502	Hemoglobin/ hematocrit	_____	
6	Clinical presentation (for case only)		
Q601.	Date onset of disease _____		
Q602	Sign and symptoms	A) Fever for more than two weeks	B) fatigue
		C) weakness	D) Loss of appetite
		E) Weight,	F) malaise,
		G) cough,	H) Diarrhea,
		I) epistaxis,	J) Cachexia,
		K) abdominal pain,	L) Pancytopenia
		M) Enlargement of lymph nodes	N) Enlargement of spleen,

This is all what I want to ask you. Thank you for spending your time and Valuable information you gave me. Do you have any question that I can address for you? _____

Annex 3 Oddeeffannoo waligalaa, waligalteefi Unkaa oddeeffannoo ittiin guurramu (Afan Oromo version)

Mata-duree qorannoo kanaa: Assessment of the Determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone, Southern Ethiopia: Unmatched Retrospective Case Control Study

Qindeessaan qorannoo kanaa: Obbo Musxafaa Aliyyii

Dhaabbata: Hospitaala fi koolleejjii fayyaa milineemii St. Phawuloos (SPHMMC).

Sponsorri: SPHMMC

Ibsa qorannichaa: Odeeffannoon kun kan qopha e Hospitaala woliigalaa

Ya aball oottif. Kaayyoon isaa qaama olitti ibsame kanaaf waa ee qorannichaa kanaa hubannoo fi ibsa kennuudhaaf. dabalataanis akkaataa oddeeffannoon itti guuramuufi qorannoo kanaaf hayyama arkachuuf.

Kaayyoo Qo annoo:- bara 2021 naannoo oromiyaa godina booranaa hoospitaala yaaballootti rakkoollee dhibee tiittee gurraattii waliin wal qabatan gargar baasuufi

Akka ittiin hojjatamu: kayyoo qorannoo kanaa galmaan ga uuf, maamiltootni yeroo qorannoon kun addemsifamu hospitaala ya aball ootti yaalaman hundi madda qorannoo kanaattii dablataaniis maammiltootni dhukkuba qabaniifi kan hin qabne carraadhaaf filaman ni hirmaatu

Rakkoo qo annoon fidu danda u: Rakkoo hamaan hin jiru garuu yeroo qorannoof dhiiga fuudhan xiqqoo ishee nama dhukkubuu danda a

Faayidaa Qo annichaa:- qorannichi faayidaa kallattii hirmaataadhaaf hin qabu, garuu bu aan qo annoo kanaa rakkoollee naannoo kanatti dhibee kanaa waliin wal qabatan gargar baasuuf fayyada.

Hirmaattonni dhibee irraa bilisa tahan bilisaan qoratamaniit yoo mallattoolee tokko malee kan bu aan qorannoo rk39 jiraachuu dhibee mirkaneessa tahe hoospitaala yaaballootti akka yaala argatu ni taasifama.

Mirga hirmaattotaa: hirmaataan tokko hirmaachuu fi hirmaachuu dhiisuuf mirga guutuu qaba. Gaafii hundumaa deebisuuf tahee deebisuu dhiisuuf/kan barbaade qofa deebisuuf mirga qaba. Gaaficha yeroo barbaadetti addaan kutuuf mirga qaba. Gaafii ifa hin tahin gaafachuufis mirga qaba.

Iccitii hirmaataa eeguu: Gaaffii fi deebiin godhamu maqaan waan irratti hin barreeffamneef iccitiin hirmaataa ni eegama.

Akkuma olitti ibsamaa ture, qo annoo kanarratti hirmaachuun dhiibbaa fidu hin qabu. Maqaan keessan uunkaa kanarratti hin barraa u, deebiin keessan nama tokkooyyuutti hin dabarfamu. Amma kaniin isinitti himuu barbaadu, isin carraadhaan qo annoo irratti hirmaachuuf filatamtan. Deebiin isin naaf deebistan fi yaanni isin naaf kennitan kaayyoo

qo annichaatiif murteessaa dha. Haaluma kanaan fedhiidhaan qo annoo keenya irratti hirmaachuuf hayyamamoo tahuu keessaniif isin galateeffanna.

Nuqunnamuuf: Piroposaaliin Kun institutional review board (IRB) Hospitaalaa fi kollejii fayyaa St. Phawloosii tiin kan mirkaneessame ta'e, yoo barbaachisaa taanaanifi gaafi dablataa yoo qabaattan, nuti isin keessummessuuf qophiidha, toora armaan gadii kanarratti nu arkachuu ni dandeessu.

4. Mr. Hailemichael Bizuneh (BSC, MPH), Advisor
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Waliigaltee hayyamaa

A. Odeeffannoo

Nagaan oltee/nagaan bullee/nageenyi isiniif haa ta'u!!!

Maqaan kiyya _____ ani garee Obbo Musxafaa Aliyyii (Reezidantii Field Epidemiology) Hospitaalaa fi Koollejjii Fayyaa Milineemii St. Phaawloositti kan qorannoo kana hojjachaa jiraniif odeeffannoo sassaabuu dha. Akka qorannootti ani wantoota dhibee tiittee gurraattiif sababa tahan Hoospitaala Yaaballootti qorachuufi. Kanaaf isin immoo qorannichaaf namoota csraadhaan filataman keessaa tokko waan taataniif gaaffiilee

qophaa an akka nuuf guuttaniif isin barbaanna. Odeeffannoon ani isinirraa argadhu rakkicha gargar baasuuf, tarkaanfii fudhachuuf akkasumas furmaata rakkoo Sanaa barbaaduufi imaammata fayyaa fooyyessuuf nu gargaara. Isinis namoota gaaficha akka naaf deebisaniif carraan filataman keeysaa tokko tahuu keeysaniin daqiiqaa 20-30 fayyadamtanii akka naaf deebistan kabajaan isin gaafanna. Maqaan keessan waan hin barreefamneef wantii isiinirratti dhaqabu goonkuma hin jiru, kanaaf iccitiin isaa kan egameedha.

Kanaafu hayyamamoodhaa? A) Eyyeen B) Iyyootti

B. Hayyamaa

Qorannoo sababa dhibee Tiittee gurraattii ta annirratti godhamu kanaaf akkaan odeeffannoo keennuu carraadhaan afferameetiin jira, ibsa isaan naaf godhanilleen hubadheettiin jira, rakkoon tokkolleen akka narra hin dhaqabnee beekeettiin jira. kanaafu tola kiyaan hayyamamaa ta uu kiya mallattoo kiyaaniin mirkaneessa.

Ani ergaan gadi fageenyaan hubadhe booda _____ ta uu kiya ibuun barbad.

Mallattooo hirmaataa _____ Guyyaa _____

Maqaa nama odeeffannoo guruu _____ Mallattooo ----- Guyyaa _____

Maqaa nama odeeffannoo qindeessuu _____ Mallattooo ----- Guyyaa _____

Saint Paul's Hospital Millennium Medical College, Department of Public Health

Mata-duree:- Questionnaires to assess the determinants of Visceral Leishmaniasis at Leishmaniasis Treatment Center of Yabello General Hospital, Borena Zone Southern Ethiopia: Unmatched Retrospective Case Control Study, 2021

Unkaa Odeeffannoo ittiin guuran

Maqaa nama odeeffannoo guruu _____ Mallattooo ----- Guyyaa _____

Maqaa nama odeeffannoo qindeessuu _____ Mallattooo ----- Guyyaa _____

SN	ID ____	Hirmaataan	A) Dhukkubsataa	B) Fayyaa
1	Socio-demographic status			
Q101	Address	Godina _____	Aannaa _____	Ganda _____
Q102	Umrii/Ganna _____			
Q103	Saala : -	A. Dhira	B. Dhalaa	
Q104	Qubsumtii	A. Magaalaa	B. Baadiyyaa	
Q105	Haala Gaa ilaa	A. Fuudhe	B. Hinfuune	
		C. Gargar Baane	D. Du aan Gargar Baane	
Q106	Haala Barnootaa	A. Barreesuu fi Dubbusuu Hin danda'u	B.marsaa jalqabaa	
		C. Marsaa Lammaffaa	D. Dipiloomaafi sani ol	
Q107	Haala Hojiii	A. Hojjataa Mottummaa	B. Horsiisee Bulaa	
		C. Qonnaan Bulaa	D. Haadha manaa	
		E. Barataa	F. Daldalaa	
		G. Kan Biroo (Ibsi) _____		
Q108	Waa ee Amantaa	A. Muslima	B. Ortodooksii	
		C. Kaatolikii	D. Protestaantii	
		E. Waaqeffataa	kan biroo	
Q109	Baayina Maatii _____			
1.1	1.1. Care giver/ Family socio-demographic status for under 18 participants			
Q110	Haala Gaa ilaa	A. Fuudhe	B. Hinfuune	
		C. Gargar Baane	D. Du aan Gargar Baane	
Q111	Haala Barnootaa	A. Barreesuu fi Dubbusuu Hin danda'u	B.marsaa jalqabaa	
		C. Marsaa Lammaffaa	D. Dipiloomaafi sani ol	
Q112	Haala Hojiii	A. Hojjataa Mottummaa	B. Horsiisee Bulaa	
		C. Qonnaan Bulaa	D. Haadha manaa	
		E. Barataa	F. Daldalaa	
		G. Kan Biroo (Ibsi) _____		
Q113	Waa ee Amantaa	A. Muslima	B. Ortodooksii	
		C. Kaatolikii	D. Protestaantii	
		E. Waaqeffataa	kan biroo	
Q114	Baayina Maatii _____			

2	Wantootaa dhibee tiittee gurraattiittiif nama saaxilan			
Q201	Gosa Lafa Manaa	A. Simintoo	B. Biyyee	
Q202	Gosa Dhaaba Manaa	A) Dhakaa	B. Bulukeetti	
		C. Citaa ykn Chibraa	D. Biyyee	
Q203	Gosa Gombisa Manaa	A) Simintoo B) Biyyee	C) Qorqorroo	D) Citaa
Q204	Haala mooraa loonii	A. moraa looni hin qabu	B. mana jireenya maatiitiin gargari	C. mana jireenya maatiitiin wal-keessa/walitti dhiyoodha
Q205	Beeyilada manaa cinaa ni raftanii?		A) Eyyeen	B) Iyyootti
Q206	Yoo eyyeen ta e gaaffiin lakk.Q205, Kam faadha Adda baasi_____			
Q207	Keemikaalli Farri ilbisaa mana keessaa bifamee	A) Eyyeen	B Iyyootti	
Q208	Yoo eyyeen ta ee gaaffiin Lakk. Q207, Yoom			
	A) woggaa tokkoo dura	B)woggaa lamma dura	C) woggaa sadii dura	D) hin bifamne
Q209	Dhaddachii (Waacuu Dimaa) naannoo jireenya keessanii ni jiraa	A) Eyyeen	B) Iyyootti	
Q210	Guyyaa dhaddacha(Waacuu Dimaa) jalatti hara ni galfattuu?	A) Eyyeen	B) Iyyootti	
Q211	Halkan halkan (Waacuu Dimaa) jala ni Raftuu?	A) Eyyeen	B) Iyyootti	
Q212	Hindheessi naannoo mana jireenya keessanii ni jiraa	A) Eyyeen	B) Iyyootti	
Q213	Guyyaa hindheessa jalatti hara ni galfattuu?	A) Eyyeen	B) Iyyootti	
Q214	Halkan halkan Hindheessa jala ni Raftuu?	A) Eyyeen	B) Iyyootti	
Q215	wayaa nafa guutuu haguugu halkan halkan ni uffattuu?	A) Eyyeen	B) Iyyootti	
Q216	Gosa biyyee naannoo jireenya keessanii maali? _____, _____			
Q217	Naannoo mana jireenya keessani godni babaqaaqee ni jiraa?	A) Eyyeen	B) Iyyootti	
Q218	Haala hanqina nyaataa:	Dheerina qaamaa _____m	Hanga qaamaa _____kg	MUAC _____ (daa immaniif)
Q219	Haala dhibee TB	A) Hin qabu	B) Ni qaba	C) Hin bekkaamu
Q220	Haala dhibee Busaa	A) Hin qabu	B) Ni qaba	C) Hin bekkaamu
Q221	Dhiiga namaa fudhattee ni beektuu?	A) Eyyeen	B) Iyyootti	C) Hin Yaadadhu

3	Amalootaan walqabatee			
Q301	Halkan halkan alatti ni waaritu?	A) Eyyeen	B) Iyyootti	
Q302	Halkan halkan alatti ni Raftuu?	A) Eyyeen	B) Iyyootti	
Q303	Imaltuu deemtani beektuu ji a darbe keessa	A) Eyyeen	B) Iyyootti	
Q304	Yoo Eyyeen Ta e gaafiin Q303 Essa Deemtan:	Aannaa _____	Ganda _____	Ollaa_____
Q305	Namni dhibee kanaan qabamee maatii keessa ni jiraa?	A Eyyeen	B) Iyyootti	
Q306	Saaphana siree ni qabdu	A) Eyyeen	B) Iyyootti	
Q307	Yeroo akkamii fayyadamttu:	A) Yeroo hunda	B) Darbe darbee	C) hin fayyadamu
Q308	Fageessituu ilbisaa ni fayyadamtuu	A) Eyyeen	B) Iyyootti	
Q309	Dhibee tiittee gurraattii ni bektuu?	A) Eyyeen	B) Iyyootti	
Q310	Yoo Eyyeen ta ee gaaffiin lakkofsa Q309, maaliin dhufuu danda a?	A) Tiittee gurraattii	B) Tiittee Busaa	
		C. waaqqa irraa	D hin beeku	
		E, Kan Biroo (Ibsi) _____		
Q311	Mallattoolee Dhibee tiittee gurraattii ni bektuu	A) Eyyeen	B) Iyyootti	
Q312	Yoo Eyyeen ta e gaaffiin lakkofsa Q311, maal faadha? _____, _____, _____			
Q312	Dhibee tiittee gurraattii Fayyuu ni danda'a jatee ni yaadduu?	A) Eyyeen	B) Iyyootti	
Q313	Dhibee tiittee gurraattii ittiifamuu ni danda'a jatee ni yaadduu?	A) Eyyeen	B) Iyyootti	
Q314	Yoo Eyyeen ta e gaaffiin lakkofsa Q313, maal faadha? _____			
Q315	Isin ykn maatiin kessan dhibee Tiittee guraatti ta'uu yoo shakkitan maal gootu?	A) Buufata Fayyaa geessu		
		B) Mana yaala Aadaa geessu		
		C) Bishaan amantaa/xabala bira geessu		
		D) Qoricha bituu		
		E,kan biroo (ibsi) _____		
Q316	Qoricha ammayyaa kan dhiibee Tiitteeitiif Oolu kana amantaa irraa ni qabdani?	A) Eyyeen	B) Iyyootti	
Q317	Yaada biroo dhibee tiittee gurraatti irratti yoo qabaatee? _____			

5	Bu'aa qorannoo laaboraatoorii		
Q501	Rk39 test Result	A. Positive	B. Negative
		C. Invalid	
Q502	Hemoglobin/ hematocrit	_____	
6	Clinical presentation (for case only)		
Q601.	Guyyaa dhibeen si jalqabe ____/____/____		
Q602	Mallattoolee dhibee tiittee gurraattii:		
	A)Ho'a qaamaa kan torbaan lammaa ol ta'ee C) Darra'u/humna dhabuu E) Hanga qaamaa hirdhisuu G) Qufaa I) Funuuna K) Dhukkubii garaa M) Ita'uu linfinoodii	B) laafachuu D) Fedhii nyaataa dhabuu F) Maashaa dadhabsiisuu H) Garaa kaasaa J) Cachexia, L) Pancytopenia N) Ita'uu rajjijjii	

Annex 4 RK39 Rapid Diagnostic Test Procedure

The utility of a rapid diagnostic test for visceral Leishmaniasis lies in its simplicity. Several brands of tests with rK39 antigen are available. Operators should always read the package inserted carefully and follow the manufacturer's instructions. This is especially important with regard to the type of specimen used. Some brands can be used only with serum, while others can be used with whole blood collected by finger prick.

Test procedure in general, the test procedure is as follows:

1. Remove the test strip from the pouch and place it on a flat surface.

2. Place a specified amount of patient specimen (serum or finger-prick blood) on the absorbent pad on the bottom of the strip.
3. Add the specified amount of buffer provided.
4. Read the result after 10–20 minutes according to the manufacturer's instructions.

Some brands require a slightly different procedure, for example:

1. Take a test tube or a U-bottom microtitre plate.
2. Add a specified amount of buffer to the tube or well.
3. Add a specified amount of specimen (blood or serum) to the tube or well and mix.
4. Immerse the test strip into the buffer-specimen mixture.
5. Read the result after 10–20 minutes according to the manufacturer's instructions.

Points to consider for optimizing use of rapid diagnostic tests:

Have a clear management plan to deal with positive and negative results.

Follow biosafety standards and precautions for handling blood and other body fluids.

Ensure proper storage conditions.

Do not use damaged or expired tests.

Adhere strictly to the manufacturer's instructions.

Use test kits within 1 hour of removal from the pouch.

Read the results within the time specified by the manufacturer.

Do not reuse a test

Interpretation of the test

Positive result: When both, control and test lines, appear, the sample tested has antibodies against recombinant K39 antigen of Leishmania. Even a faint line should be considered positive.

Negative result: When only the control line appears, there are no antibodies against recombinant K39 antigen of Leishmania present in the patient's sample.

Invalid result: When no control line appears, a fresh patient sample should be tested with a new strip.

Advantages and disadvantages of the rK39 test

Advantages

Simple to perform with minimal training.

Can be performed with finger-prick whole blood, serum or plasma.

Kits can be transported and stored at ambient temperature (up to 30 °C).

Results are available within 10–20 minutes

Disadvantages

Cannot distinguish between active cases and relapse in previously treated cases. Therefore, interpretation must always be accompanied by clinical case definition.

In patients with advanced HIV infection, a negative result does not rule out VL diagnosis.

10. Declaration

