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Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Insecticide Treated Net Utilization and its barriers among Under-Five Children in Adami Tulu District, Oromia Regional State, Ethiopia: A Qualitative study. *1-6*
2. Tuberculosis and HIV Co-infection among Patients on Tuberculosis Treatment at Fenote Selam District Hospital, Amhara Regional State, Northwest Ethiopia. *7-13*
3. Molecular Epidemiology of Bovine Tuberculosis in Cattle and its Public Health Implications in Gambella Town and its Surroundings, Gambella Regional State, Ethiopia. *15-27*

v. Fellows
vi. Auxiliary Memberships
vii. Process of Submission of Research Paper
viii. Preferred Author Guidelines
ix. Index

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Insecticide Treated Net Utilization and its barriers among Under-Five Children in Adami Tulu District, Oromia Regional State, Ethiopia: A Qualitative Study

By Bikila Lencha & Wakgari Deressa
Madawalabu University, Ethiopia

Abstract- *Background:* Despite the scaling-up of Insecticidal Treated Nets (ITNs) distribution in Ethiopia, its use among net owning households has not been satisfactory. Hence, in addition to scaling up of net distribution, periodic assessment of the use and barriers against the use of bed nets among high risk population is necessary.

Objective: The objective of this study was to assess the ITNs use and its barriers among under-five children in Adami Tullu District, Ethiopia.

Methodology: A small-scale qualitative approach was used. Purposive sampling was employed to get mothers with under-five children in all kebeles. Semi-structured discussion guide was used for focus group discussions which was conducted in Afaan Oromo (local language of the study area). Open code computer program was used for the analysis of the data. The data was coded; categorized and appropriate themes were developed. The data was summarized and presented along the main themes.

Keywords: *ITN use, under-five, barriers, children and malaria.*

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Insecticide Treated Net Utilization and its barriers among Under-Five Children in Adami Tulu District, Oromia Regional State, Ethiopia: A Qualitative Study

Bikila Lencha [α] & Wakgari Deressa [σ]

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Results: The mean age of the participants was 28.3(±4.4). Majority of the participants knew the cause of malaria. All of the participants agree with vulnerability of children and pregnant women. The participants said that ITN has no side effect as far as it is used appropriately. Rather, it protects against malaria and other insects like housefly and fleas. Lack of understanding, fear of side effect and using the net for other purposes like carrying harvest materials, using the net as a mattress and covering the toilet were among the barriers mostly discussed. Four main themes were identified from the focus group discussion i.e. perception about cause and prevention of malaria, perception about vulnerability of children, benefit and side effects of ITNS and barriers towards ITN utilization.

Conclusion and recommendation: Lack of understanding, fear of side effect and using the net for other purposes were identified as main barriers. IEC (Information, Education and communication) on the effective and continuous utilization of under-five children should focus on durability of LLINs and avoid misconceptions regarding longevity of the nets.

Keywords: ITN use, under-five, barriers, children and malaria.

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I. Introduction

Malaria remains a major public health and development challenge (1). It caused 216 million cases and 655,000 deaths worldwide in 2010, of which 81% of the cases and 91% of the deaths were from the Sub-Saharan Africa (1). Children under the age five years are most likely to suffer from the severe effects of malaria because they have not developed sufficient naturally acquired immunity to the parasite (2).

The use of Insecticide Treated Nets (ITNs) is one of the main malaria control strategies in Ethiopia to reach the national targets to achieve malaria elimination within specific geographical areas with historically low malaria transmission and achieve near zero malaria death in the remaining malarious areas of the country (3).

In Ethiopia, the Federal Ministry of Health (FMOH) conducted continuously mass distribution of LLINs between 2005 and 2007, targeting to distribute two LLINs per household in malaria endemic areas and further 15 million were distributed in 2010 and 2011 to replace LLINs distributed previously (4). Despite this rapid scale up of each kebele since 2005, it is unlikely that all LLINs are still in use after six years (5). Identification of awareness gaps, monitoring of behavioral changes on malaria disease recognition and use of preventive and control measures such as the use of ITNs are a priority area for the Government of Ethiopia with a special emphasis on identifying the barriers and increase the use of ITNs as per the national malaria guidelines (5).

Increase in ITN access (i.e. household ownership) does not necessarily translate to equal increase in utilization (6). Because, the success of ITN utilization depends on several factors: such as, willingness of people to use nets, inconvenience to hang the nets, educational background, place of residence, perception of the side effect associated with ITN use and colour of nets (5,6).

Hence, in addition to scaling up ITNs distribution, periodic assessment of the use and

barriers among high risk population is highly recommended (7). Thus, this study aimed at identifying utilization of ITNs use and its barriers among under-five children in a malaria-prone district of Eastern Shewa.

II. Methods

a) Study setting and participation

A qualitative study was conducted in Adami Tulu District of East Shewa zone in February 2014. Malaria transmission in the District is seasonal and epidemic type, peaking from September to December. Two species of *Plasmodium* are present in the area; *Plasmodium falciparum* (about 70%) and *P. vivax* (about 30%) (5). The source population was all under five-children and their mothers/ care takers.

b) Sample size and sampling methods

Out of the 47 rural villages, four of them were selected (Anano Shisho, Boccessa, Elka Chelemo and Gallo Heraphe) for malaria intervention trial and all of them were included in our study. Four groups of FGD participants were selected by the investigator purposively in consultation with the Kebele (small administrative units) leaders and health development army. All groups contain at least 6-8 women with at least one child less than five years old. This is helpful to handle the discussion easily and to avoid side conversation. Totally, 30 mothers with at least one under-five children participated. Purposive sampling was employed to get mothers with under-five children in all kebeles.

c) Data collection methods and instruments

Semi-structured discussion guide was developed after reviewing similar literatures and held with mothers of under-five children from each of the four kebeles to explore and understand perceptions and barriers towards use and non use of ITNs among under-five children. Based on the objectives of the study the main themes developed were perception about cause and prevention of malaria, perception about vulnerability of children, benefit and side effects of ITNS and barriers towards ITN utilization.

The discussion was facilitated by Principal investigator using a check-list and all the discussions were tape recorded. The note taker was assigned by the investigator. The recorded data was transcribed in Afaan Oromo and then translated into English word- for- word. Then it was supplemented with field note in any case there was a difficult of identifying the audiotape record to clarify the ambiguity. Translation into Afaan Oromo was verified by investigator.

d) Data quality control

FGDs were conducted in Afaan Oromo (local language of the study area). Correctness of transcription was checked for 5% of all the audio tapes. Minor corrections, such as incomplete responses to the questions, were checked in comparison with note and corrected on subsequent transcriptions. Principal investigator entered each transcript into Microsoft WORD as each interview ends. Participants gave their ideas freely throughout the discussion until the idea was saturated.

e) Data processing and analysis

Open code computer program was used for the analysis of the data by sorting information, looking for similarities, differences or contradictions. English transcripts were read and re-read to develop codes that identify important and common concepts related to the main themes of the study. The data was coded; categorized and appropriate themes were developed. Field notes and original transcripts were looked upon when more information/ clarity are needed during coding, analysis and write up. Finally, the data was summarized and presented along the main themes.

f) Ethical consideration

Ethical clearance was obtained from Ethical review committee of School of Public Health, Addis Ababa University. Formal letter have been written for Adami Tulu health office. The letter was written for each of respective kebeles from Woreda Health bureau and informed before going for data collection. Participants were informed that their participation is purely voluntary and assured of the confidentiality of all information.

III. Results

a) Sociodemographic characteristics of the participants

The mean age of the participants was 28.3 (±4.4) and ranges from 20-38 years. Half of the participants are literate (had formal education) and around three fourth of them were Muslims in religion. On average each mother has 1.5 children and 17(56.7%) of the participants are housewife in occupation (Table one).

Table 1 : Sociodemographic characteristics of the discussants

Variables	Frequency	Percent
Age		
20-29	15	50
30-38	15	50
Education level		
Literate	15	50
Illiterate (no formal education)	15	50
Religion		
Muslim	22	73.3
Orthodox	3	10
Protestant	5	16.7
Number of under-five		
One	17	56.7
Two	10	33.3
Three	3	10
Occupation		
Farmer	17	56.7
Housewife	12	40
Trade	1	3.3

The result of FGD is summarized in the following paragraph.

Majority of the participants knew the cause of malaria. All of the participants agree with vulnerability of children and pregnant women. Most of the participants agreed that cleaning environment and hanging the nets helps to prevent malaria. The participants said that ITN has no side effect as far as it is used appropriately. Rather, it protects against malaria and other insects like housefly and fleas. Lack of understanding, fear of side effect and using the net for other purposes like carrying harvest materials, covering the toilet were among the barriers mostly discussed. Majority of participants had misconception concerning ITN utilization throughout the year. Finally, education was explained as the best way to increase utilization among under-five children.

Four main themes were identified from the focus group discussion i.e. perception about cause and prevention of malaria, perception about vulnerability of children, benefit and side effects of ITNS and barriers towards ITN utilization

b) Perception about cause and prevention of malaria

Almost all of the FGD participants mentioned mosquito bite as a cause of malaria though they explained it in different ways. The stagnant water around the home, unclean environment and absence of toilet were raised as a reason for mosquito breed there and bite peoples. One of discussant said "*If there is stagnant water around the home; the mosquitoes will breed there and bites peoples at home. The same is true if there is no toilet around the home" (30 years, Muslim Mother of one child, Gallo Heraphe kebele)*

Majority of the FGD Participants discussed role of ITNs in prevention. In addition, they said that washing the clothes of children, keeping the household materials clean, burning the waste materials and digging toilet at distance from the home as a prevention ways. Draining the stagnant water around the home is considered as most important mechanism in preventing malaria.

"By draining water in front of the home, using net hanging correctly, Cleaning one own house, washing clothes of the children and cleaning eating materials." (38 years, Orthodox mother of one child in Boccessa kebele)

c) Benefits and side effects of ITNs

FGD participants discussed that ITNs could prevent malaria if used in appropriate manner and most of the side effect occur due to technical problems. In addition, it protects nuisance insects like fleas, housefly and others if hanged correctly and kept in a clean manner.

The discussants agreed that the side effects may occur depending on the way the net is being hanged. One of participants said "*specially, it needs great care for the children. If it burns you during the night time, you will feel it throughout the day. Specially, when the net is new, it should be hanged correctly since it has the chemical at the beginning. We can take care of U5 Children by correctly hanging the net and inserting under the bed or mat in all directions (35 years Muslim mother of 2 children in Gallo Heraphe kebele)*

The discussants further recommended inserting the net under the bed in all corners in order to avoid hotness. One of the participant said *"if treated with insecticide and hanged the net could prevent Malaria. The chemical may burn your body if hanged without making in the air; you feel discomfort and hotness during the hot season (Bonna). Therefore, you have to insert it*

under the bed in all corners." (30 years Muslim mother of 1 child in Elka Chellemo kebele)

Making the net in the air is suggested before use. Unless, it dangerous especially for children. One of the mother said *if children hold the net before making in the air, it may cause danger since the child eat by contaminated hand, unless the family wash the children immediately (25 years Muslim mother of 2 children in Elka Chellemo kebele)*

Majority of the mothers witnessed inappropriate use of the net in their community. One of the mothers said*, after taking the net some peoples uses it as mattress, bed cover and then it causes irritation which looks like scabies. But, the net is not given to wear during night rather to use it by hanging rectangular on the bed and inserting the four edges under the bed (28 years, Orthodox mother of 1 child in Boccessa kebele).*

d) Perception about vulnerability of children

All of the discussants agreed that malaria is more serious for under-five children and pregnant women. One of the mother said "*under-five year's old children and pregnant mothers are mostly affected because most of the time children's go to the stagnant water to play (20 Years, Protestant mother, 1 child Gallo Heraphe kebele)*

e) Barriers towards ITN utilization

The most common reasons for non use raised by FGD participants were dirty due to smoke, lack of understanding; perception of repeated wash decreases the chemical, living far away from the lake and fear of side effect especially for children.

Concerning lack of understanding, "*There is a say in Afaan Oromo "Dooqni ofiis hin nyaatu, namas hin laatu"-peoples have nets in the house. But, they neither use for themselves nor give for others." (35years, Muslim mother of 2 children in Anano Shisho kebele.).* Another mother said the following about fear of side effect *"peoples fear that their children's might be affected due to hotness under the net (25 years, Muslim mother of 1 child in Anano Shisho kebele)*

Almost all of the FGD participants had wrong perception about the longevity of ITNs." *peoples are saying that the net is not protecting us from Insect bite after one or two wash because they think that chemical decreases during wash and become useless. Therefore, it is better if given with insecticide for the future (26years, Orthodox mother of 1 child in Boccessa kebele)*

FGD participants expressed different ideas concerning the relation between seasonality and ITN use based on their living village. Those mothers living near the lakes agreed that they use the net throughout the year. Those living away from the lake were not using the net during the dry season. *For example, mothers near Lake Dambal said "since we are living around the Lake, mosquito is always there even though it increases during rainy season. Thus, it is good to use throughout the year by washing." (32 years, Muslim mother of 1 child in Boccessa kebele)*

Another mother said*" Mostly we use when Malaria starts; during the rainy season when mosquito comes back. You will not find many mosquitoes during winter unless we hang the net to protect other insects (27 years, Protestant mother of 2 children in Gallo Heraphe kebele)*

Majority of the participants rose cooking in the house as the problem in using the net, since there is smoke in the house*, we wash it many times which makes the chemical non-fuctional.in addition, the rain comes down through the thatch and affect the net. The net had worn out with in short time. (25 years, Muslim mother of 1 child in Elka Chellemo kebele)*

All FGD participants raised lack of knowledge and misuse of the net as the main reason for not using among under five children. One of the participants said *""walaala"-due to lack of knowledge, even there are peoples who sell instead of protecting himself and his children from Malaria (30 years, Grade 5, Orthodox mother of 2 children in Boccessa kebele).*

Finally, the discussants agreed that health extension workers should educate the community about the procedures of hanging the net and appropriate utilization. One of the discussant said that *health extension workers have to educate the people on the hanging procedure and utilization and we have to use it properly in the future (25 years, Muslim mother of 1 child in Anano Shisho kebele)*

In addition, they agreed to teach each other ones they learned from health extension workers. The mother from Gallo Heraphe kebele said, *for example I had used the nets and seen its benefits for myself and my family. Therefore, I have to advice my neighbor on how to hang the nets and its benefits.*

At kebele level, the net should be distributed as it reaches all family members including U5 children's (25years, protestant mother of 1 child in Gallo Heraphe kebele).

IV. Discussion

This study, using a qualitative approach, has explored the knowledge of the cause and prevention of malaria, benefits and side effects of ITNs and barriers for ITNs use in Adami Tulu District of East Shewa Zone. In this study majority of the discussants said mosquito bite is a cause of malaria. Similarly, study among under-five Nigerian children in Nigeria found that the perception of the majority of caregivers about the cause of malaria is mosquito bite (8). This is very important because cross sectional study on Bioko Island, of Equatorial Guinea found that knowledge of prevention and transmission of malaria were associated to ITN utilization (9).

The knowledge towards malaria prevention using insecticide treated nets was reported to be low from couple of studies done in Ethiopia (10, 11). However, in our study, majority of the discussants agreed that insecticide treated nets can prevent malaria if hanged correctly. The possible reason for this difference could be increase in awareness creation by health extension workers.

In the present study, all of the discussants said that under-five children and pregnant women are at risk of malaria and should be given priority. Similarly, a study about ITNs usage against malaria in Tigray Region found that three fourth of the respondents claimed that children under-five years of age and pregnant women are at high risk of malaria and should be given priority to sleep under ITN in the household (12).

In our study majority of the discussant (those living away from the lake) agreed that they don't use the net throughout the year due to hotness, absence of mosquito during the dry season and other reasons. Similarly, a study carried out in 2011 in the highlands of western Kenya showed that, the vast majority of the population was not willing to use the net during dry season because of hotness, discomfort, low mosquito density and other reasons (13).

The perception that the ITN was no longer effective after two washes if not re-treated was the most important reason for not using ITN among households with under-five children (14). Similarly, in our study majority of the discussants had similar perceptions and recommended the net to be given with the chemical.

One of the mothers' said, *I have taken two nets two years ago, I removed and washed it since it gets dirty due to smoke .Then after, I didn't used it again since there is no chemical to retreat it* (30years Muslim mother of 1 child Elka Chellemo). But, the government is distributing long lasting insecticide treated nets which does not need re-treatment and can serve 3 to 4 years on average (4).

Majority of our discussants said that the nets get dirty due to smoke in the house and the peoples will wash it again and again. Then after, they will use it for other purpose other than the intended use. Similarly, the study in the southern nations, nationalities and peoples in Ethiopia identified possible reasons for not using ITNs among households like too old or torn, too dirty and unavailability of nets (15).

Lack of understanding was also found to be one of the main barriers against the net use. Most of the peoples use it for other purpose like carrying harvest materials like maize, covering the toilet and plant in the garden. Other similar studies also reported using nets for other purposes than sleeping under have been reported in a number of studies. These include drying of fish in Western Kenya, fish farming, curtains at home, ant traps in Uganda, and protecting dead bodies from house flies while making burial arrangements in Nigeria (16,18).

Limitations and strengths of the study

Due to logistical constraints the FGD was conducted among only 4 groups. Despite the limitations, this study gives an overview of the knowledge of discussants towards the cause and prevention, benefits of the nets and barriers towards the use of the nets. Fortunately, saturation was achieved with in these 4 groups. The investigators participated in the data collection, analysis and write up. The FGD was conducted among populations of different education level and from different geographical area.

V. Conclusions

Majority of the discussants identified mosquito bite as a cause of malaria. Under-five children and pregnant women were discussed as a vulnerable group in the community. The participants said that ITN has no side effect as far as it is used appropriately. Lack of understanding, fear of side effect and using the net for other purposes were identified as main barriers.

Health extension workers should have to teach the procedures of hanging and the benefits of the net ones distributed for the community. Continuous supervision of the health extension workers is important to avoid using the net for unintended purpose.

IEC (Information, Education and communication) on the effective and continuous utilization of under-five children should focus on durability of LLINs and avoid misconceptions regarding longevity of the nets.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

Both authors contributed in the design, analysis and interpretation of data. BL prepared the draft of the manuscript and WD reviewed the manuscript. Both authors read and approved the manuscript.

VI. Acknowledgements

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Tuberculosis and HIV Co-infection among Patients on Tuberculosis Treatment at Fenote Selam District Hospital, Amhara Regional State, Northwest Ethiopia

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Abstract- *Introduction:* Tuberculosis is the leading cause of death for HIV-infected patients, and HIV is the most important risk factor for developing active TB. The risk of death from TB is significantly higher in the HIV-infected population. The interaction between TB - HIV co-infected person is bidirectional and synergistic: HIV-1 infection predisposes to the development of active TB, and the course of HIV-related immunodeficiency is worsened by active TB infection. *Objective:* The aim of this study was to assess the level of TB - HIV co-infection at Fenote Selam district Hospital, Amhara, Northwest Ethiopia, 2014.

Methods and materials: Fenote Selam district hospital is 378 km from capital city of Ethiopia, Addis Ababa. The study was conducted in May 2014. To determine the level of TB-HIV co-infection, retrospective data review method was used. Medical records of all TB patients who were on anti tuberculosis treatment from March, 2009 to March, 2014 were included in the study. The patients' records in the five years were 651. Data were collect from patients' medical record. Ethical approval and clearance was obtained from Debre Markos University Medicine and Health Science College of Ethical Review Committee (ERC).

Keywords: *Tuberculosis, HIV, TB/HIV Co-infection, retrospective, TB treatment outcome.*

GJMR-F Classification : *NLMC Code: WF 200*

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Result: A total of 602 participants were included in the study. Of these patients, 133 (22.1%) were co-infected with HIV and 469 (77.9%) were only TB diagnosis patients. Age and treatment outcomes were significantly associated with TB-HIV co-infection.

Conclusion: Under one third of the patients were TB-HIV co-infected. This study showed that there was increasing rate of TB-HIV co-infection from 23.9% to 35% in the year 2009/10 to 2011/12 then decreasing from 35% to 12% in the year 2011/12 to 2013/14. The age groups 15-24 and 25-34 years old were 2.544 and 3.409 times likely to be risk of TB-HIV co-infection.

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I. Introduction

Globally, HIV kills more than 8000 people daily while more than 5000 people die of tuberculosis (TB) every day. It is estimated that one-third of the world's population are infected with TB, and 40 million people currently living with HIV/AIDS [1]. In addition, without proper treatment, 90% of HIV-infected individuals could die within months of contracting TB. Four million people infected with HIV have also TB disease worldwide making TB the major killer in HIV-infected patients[1].

Tuberculosis is the leading cause of death for HIV-infected patient and HIV is the most important risk factor for developing active TB [2; 3]. The double epidemic of TB and HIV is great concern, especially in sub-Saharan Africa where 80% of the burden of co-infection resides and health systems are already weak and overstretched [4]. The risk of death from TB is significantly higher in the HIV-infected population even if the organism is sensitive to and responsive to anti-TB medications [5]. The convergence of the tuberculosis (TB) and the HIV epidemics has posed new public health challenges [6].

The interaction of HIV and TB in co-infected persons is bidirectional and synergistic: HIV-1 infection predisposes to the development of active TB and the course of HIV-related immunodeficiency is worsened by active TB infection[7].

The HIV/AIDS pandemic is a major challenge to the control of TB in Ethiopia. The dual epidemic has a number of impacts on the health sector because it increases TB and HIV burden, demands for more care and worsens the situation of the already overstretched health care delivery system in the country. HIV increases susceptibility to infection with M. tuberculosis, the risk of progression to TB disease, and the incidence and prevalence of TB. The life time risk of HIV positive individuals to develop TB is 20-37 times greater than HIV negative individuals. It also increases the likelihood of re-infections and relapses of TB. In a population where TB/HIV is common, health services struggle to cope with the large and rising number of TB[8].

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Furthermore, 22 high burden countries (HBCs) with TB accounted for approximately 80% of the estimated number of new all form of TB cases which rising worldwide on the same year. Ethiopia was ranked seventh among the world 22 high burden countries (HBCs). In Africa, those countries with high rates of TB-HIV co-infection are the main focus of intensified efforts in directly observed treatment short course (DOTS) expansion[9].

Ethiopia adopted the DOTS strategy since 1997 after success of the pilot program with the development of the first combined Tuberculosis and Leprosy Prevention and Control Program manual. TB-HIV collaborative activities was piloted in 2004 and afterward scaled up nationally. The STOP TB strategy was launched by World Health Organization (WHO) in 2006 to achieve the millennium development goals (MDGs) for TB in 2015. Ethiopia also adopted this strategy to achieve the national TB-leprosy and TB/HIV targets [8].

The main reason of this study was to assess the level of TB-HIV co-infection and factors associated with TB-HIV co-infection at Fenote Selam district hospital, Amhara, Northwest Ethiopia, 2014.

II. Methods and Materials

Study settings: The study was conducted at Fenote Selam district hospital, which is about 378 km from capital city of Ethiopia, Addis Ababa, and data collection, was conducted in May 2014.

Study design: retrospective cohort study was employed to identify the level of TB-HIV co-infection and factors associated with TB-HIV co-infection.

Study population: the population of the study was medical records of all TB and TB-HIV co- infection patients who were on anti tuberculosis treatment at TB clinic from March, 2009 to march, 2014.

Sample size: Medical records of all TB patients who were on anti tuberculosis treatment from March, 2009 to March, 2014 were included in the study. There were a total of 651 patients in the TB clinic in a given five years. Of these, 602 of patients were included in the study. One hundred thirty three (133) of them were TB-HIV co-infected cases and the remaining 469 were diagnosis only TB cases.

Sampling technique and procedure: All TB diagnosed and TB-HIV co-infected patients' records from March 2009 to March 2014 were selected. All participants were included in the study up on fulfillment of the inclusion criteria. All socio-demographic characteristics and associated factors were recorded on a checklist.

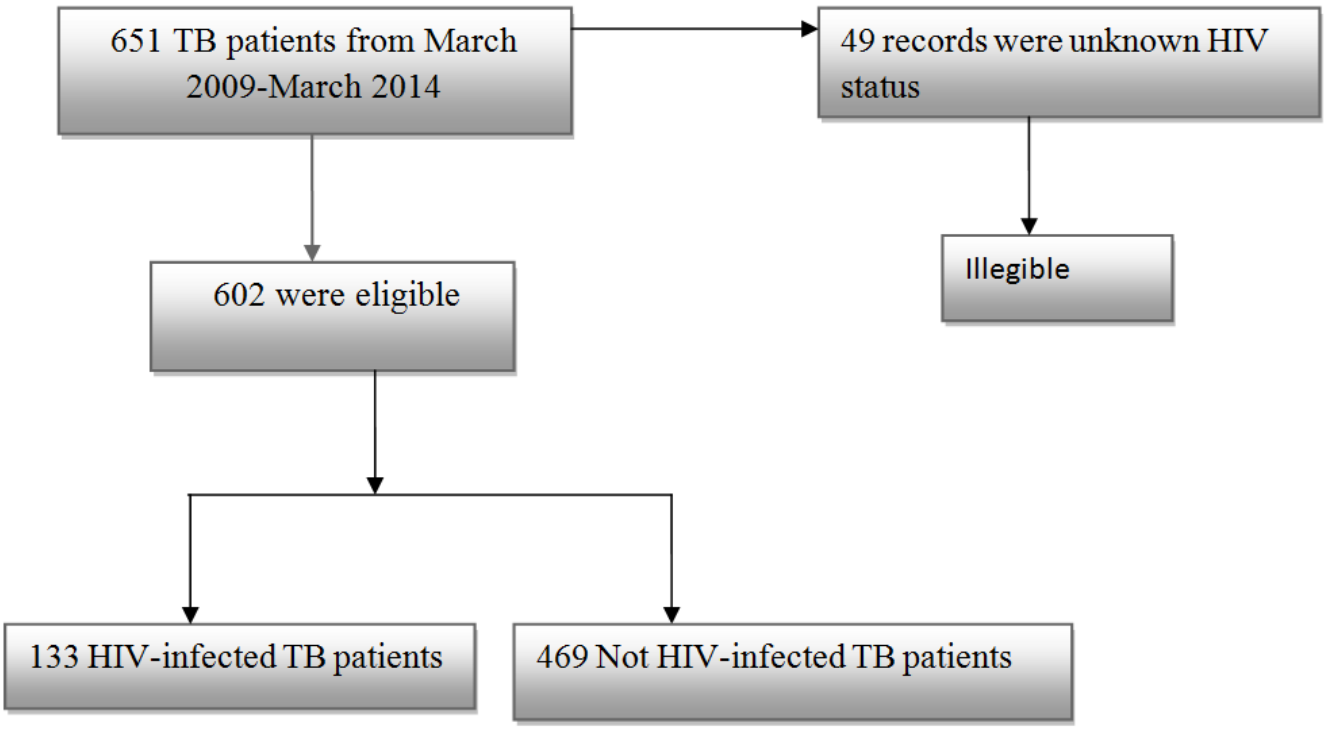

Figure 1 : Schematic presentation of sampling procedures

Data collection process: Checklists were adapted and modified by researcher then checked by linguistic experts and used to collect data on the socio-demographic variables and associated factors. Patients' identification and medical records were identified by researchers and code was given for each patient's identification and medical records. The log book of DOTS clinic includes the information like patients' age,

sex, body weight, address, TB type, TB category, treatment outcomes and HIV status. TB- HIV-infected patients and only TB infected patients were identified on the log book of DOTS clinic. The data were collected by two trained nurses. The supervisor was monitoring the data collection process. The data collections were taking three weeks.

Inclusion criteria: All TB patients' record in the TB clinic from March, 2009- March, 2014 were included in the study i.e. notified as tuberculosis cases from 2009 to 2014 in the study site.

Exclusion criteria: Unknown HIV status and incomplete records were excluded in the study.

Data quality control: Before study conducted, orientation was given for supervisor and data collector nurses concerning to standardized checklist, the purpose of the study, techniques and procedures of data collection methods. The researcher gave a guideline for data collectors and the supervisor for data collection process. The supervisor was monitoring the data collection process.

Data processing and Analysis: Before data entry, data were monitored and checked for its completeness by the supervisor and each completed checklist was coded on pre-arranged coding sheet by the researcher. Data were entered into Epi Info version 3.5.1, then exported and analyzed by using SPSS Windows software (version 16.0). Data were cleaned for its consistency by running simple frequencies and percentage. Then, printed frequencies were used to check for outlier and to clean data. Tables and graphs were used to present data frequencies and percentage. It also used to describe the study population in relation to relevant variables. Stepwise bivariate and multivariate logistic regressions techniques were used to analyze data and control the adverse effects of confounding variables. The crude and adjusted odds ratios together with their corresponding of 95% confidence intervals were computed. A P-value < 0.05 was considered to declare a result as statistically significant.

Ethical consideration: Ethical approval and clearance was obtained from Debre Markos University, medicine and health Science College ethical review board. A formal letter for permission and support were obtained to Fenote Selam district hospital administrator. Then, the hospital administrator wrote a letter to the TB clinic focal person.

III. Results

a) Socio-demographic characteristics of study participants

A total of 651 patients were registered in the TB clinic from 2009 to 2013/14. Of the total, 602 participants were included in this study. Of these patients, 133 (22.1%) were TB-HIV co-infected and 469 (77.9%) were only TB cases. The majority of participants were in the age groups of 15-24 and 25-34 years old which accounts 160(26.6%) and 163 (27.1%) respectively. Of the total participants 328(54.5%) were males. The urban participants were accounted about 367(61%) (Table1).

Table1: General characteristics of the study participants registered at Fenote Selam district Hospital TB clinic, Amhara, Northwest Ethiopia, 2014.

Characteristics of variables	Frequency, N (%) (n= 602)
Age	
0-14	51(8.5)
15-24	160(26.6)
25-34	163(27.1)
35-44	93(15.4)
45-54	80(13.3)
≥55	55(9.1)
Sex	
Male	328(54.5)
Female	274(45.5)
Address	
Rural	235(39)
Urban	367(61)
Type of TB	
PTB+	94(15.6)
PTB-	258(42.9)
EPTB	250(41.5)

TB treatment outcome	
Defaulted	39(5.6)
Failure	4(0.7)
Death	51(8.5)
Transfer out	32(5.3)
Cured	95(15.8)
Complete	381(63.3)
HIV status	
Sero- positive	133(22.1)
Sero-negative	469(77.9)
Total	602(100)

Trend of TB-HIV co-infection increased from 23.9% to 35% in the years 2009/10 - 2010/11 and decreased from 35% to 12% in the years 2011/12 -2013/14 (See figure 2 below).

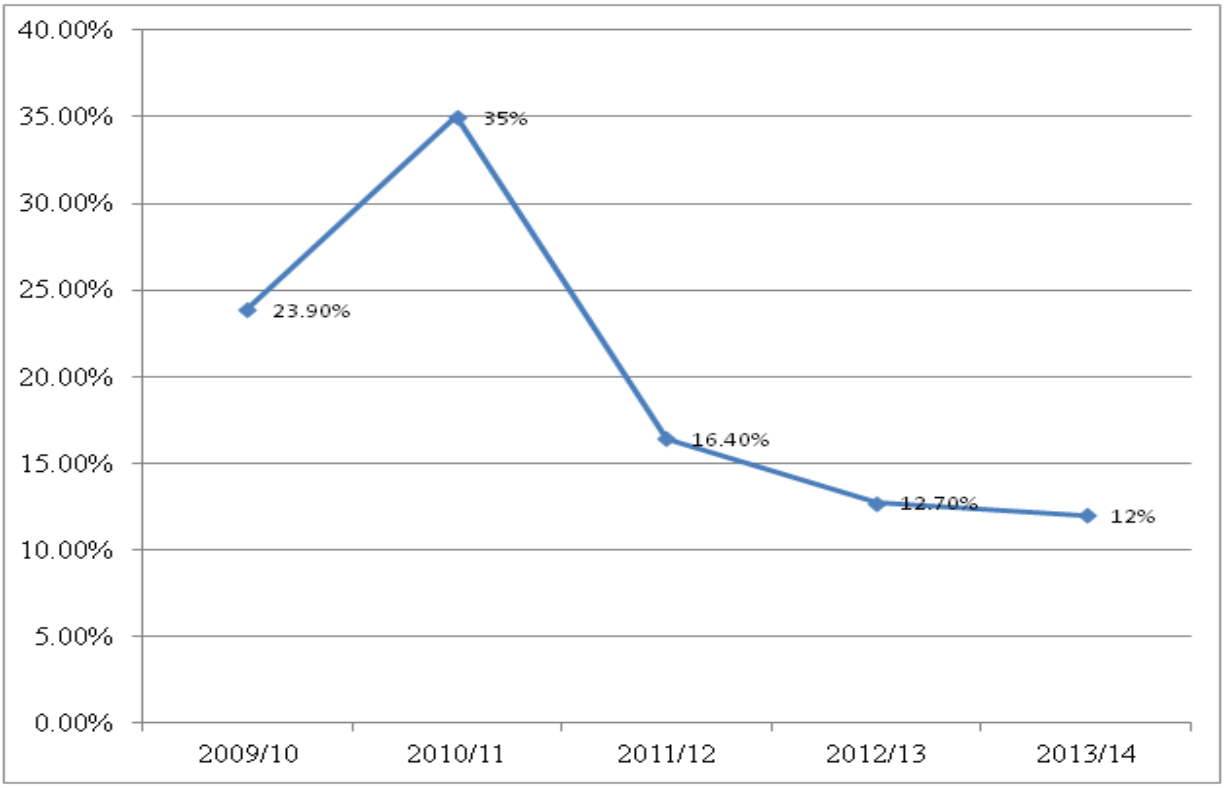

Figure 2 : Trend of TB-HIV co infection from 2009/10-2013/14 at Fenote Selam District Hospital, West Gojjam Zone, Amhara, Northwest Ethiopia, 2014

b) Associated factors

In bivarate analysis, age group from 15-24 and 25-34 years old were 2.544 and 3.409 times likely to be risk of TB-HIV co-infection ,respectively, as compare to age groups ≥55 years old. And also cured and died were 2.733 and 4.603 times risk for TB-HIV confection, respectively, as compare to completed treatment outcomes.

After controlling the confounder variables through multivariate logistic regression analysis, age groups 15-24 years (AOR: 2.586, 95% CI: 1.010, 6.617) and 25-34 years (AOR: 3.370, 95% CI: 1.332, 8.525) were independently associated with TB-HIV co-infection. Died (AOR: 4.326, 95% CI: 2.039, 9.176) and cured (AOR: 2.758, 95% CI: 1.456, 5.227) were independently associated with TB-HIV co-infection (Table 2).

Table 2 : Bivarate and multivariate logistic analysis of TB-HIV Co-Infection among Patients at Fenote Selam district Hospital TB clinic, Amhara, Northwest Ethiopia, 2014.

Characteristics	TB-HIV-infection N (%)		COR(95% CI)	P-value	AOR(95%CI)	P-value
	Yes	No				
Sex						
Male	71(24.3)	257(78.4)	1.00			
Female	62(25.4)	212(77.4)	1.059(0.719,1.558)	0.773		
Age					1.164(0.344, 3.937)	0.807
0-14	6(11.8)	45(88.2)	1.089(0.327, 3.622)	0.890	2.586(1.010, 6.617)**	0.048
15-24	38(23.8)	122(76.2)	2.544(1.011, 6.400)*	0.047	3.370(1.332, 8.525)**	0.010
25-34	48(29.4)	115(70.6)	3.409(1.369, 8.487)*	0.008	2.526(0.934, 6.828)	0.068
35-44	21(22.6)	72(77.4)	2.382(0.896, 6.329)	0. 082	1.719(0.604, 4.889)	0.310
45-54	14(17.5)	66(82.5)	1.732(0.621, 4.830)	0.294	1	
≥55	6(10.9))	49(89.1)	1.00	----		
Address						
Rural	54(23)	181(77)	1.088(0.734, 1.611)	0.675	1.00	
Urban	79(21.5)	288(78.5)	1.00		1.434(0.929, 2.214)	0.104
Treatment Outcome				0.423	1.527(0.682, 3.419)	0.303
Default	9(23.3)	30(76.9)	1.381(0.627, 3.042)	0.713	1.694(0.169, 16.952)	0.654
Failure	1(25)	3(75)	1.534(0.157, 14.975)	0.002	2.758(1.456, 5.227)**	0.002
Cured	16(50)	16(50)	2.733(1.462, 5.107)*	0.000	4.326(2.039, 9.176)**	0.000
Death	20(21.1)	75(78.9)	4.603(2.194, 9.656)*	0.472	1.210(0.688, 2.129)	0.509
Transfer out	19(37.3)	32(62.7)	1.227(0.702, 2.146)		1	
Complete	68(17.8)	313(82.2)	1			
Type						
PTB+	34(39.1)	60(63.8)	2.324(1.377,3.925)*	0.002	1.490(0.713,3.114)	0.289
PTB-	50(22.3)	208(80.6)	0.986(0.636,1.530)	0.950	0.974(0.611,3.1.553)	0.912
EPTB	49(21.8)	201(80.4)	1		1	

IV. Discussion

This study showed that the prevalence of HIV co infection among TB patients was 22.1%. This is nearly similar to a study done by Daniel G D et.al. [10]. However, this finding is higher than a report by WHO in 2012(13%), report of WHO in 2012 for Ethiopia (8%) [11] , in northwest Ethiopia (Dabat) 11.4 %[12]. On the other hand, this is lower than the studies conducted in sub-Saharan African countries which were 70%[13], a study conducted in Nigeria (44.2 %)[14] and study conducted in Debre Markos, Ethiopia 44.8% [15]. This discrepancy might be due to a preference of patients towards quality of care at referral hospital, especially, when the disease becomes serious and complicated, as compare to care in district hospital. The other possible reason might be due to fear of stigma and discrimination that they may seek care away from their village in the bigger town.

In this study, that treatment success was 71.9%. In line with this, a report showed in India, treatment success of TB-HIV co-infection was 74.5%[16]. This may be due to similar in socio-economical status of the study participants and universal implementation of DOTS program throughout the world.

Gender had no significant difference on TB-HIV co-infection. Similarly, the study conducted in Addis Ababa, Ethiopia, showed that no significant difference between male and female[17]. This could be due to similarity in socio-cultural perspective of study participants.

In contrast, the study conducted in Dabat, Ethiopia[12], Northwest Ethiopia[18] and São Paulo state [19] indicated that TB–HIV co- infection case was higher in men than women. Besides, Tabarsi et al. found that the TB-HIV co-infected intravenous drug user were males[20]. This attributed that males are economically active and influential in the community and they may smoke, drink and have multi-sexual partners.

This study showed that 8.5 % of patients were died. This is lower than study conducted in Malaysia 23.3% [21], Thailand 29 % [22], and in Vietnam 26% [23]. This may be due difference sample size and may be due to socio-cultural and economical difference.

This study showed that there is no significant difference in the rate of HIV infection among type of TB. Similarly, study conducted in Ethiopia showed that there was no significant difference among type of TB [10; 24]. However, other studies indicated that rates of HIV infection among smear-negative and EPTB cases were higher than smear-positive cases[25; 26]. This could be due to the relatively low prevalence of HIV infection in the catchment area of the study population. Another possible explanation could be under diagnosis of TB or

referral of some smear-negative and EPTB suspected cases due to limited diagnostic facilities.

This study revealed that study participants in age groups of 15-24 and 25-34 years old were independently associated with outcome variables. This is similar to previous study conducted at Debre Markos referral hospital [15]. The fact is that these age groups are more active for sexual activity and they are vulnerable to HIV infection than any other part of the population.

According to the study conducted at Debre Markos referral hospital showed that TB-HIV co-infection was 49.2% in 2008/9, 42.7% in 2009/10, 39.3% in 2010/11, 32.9% in 2011/12, 44.6% in 2012/13, and 44.8% in 2013. This result showed that there was a decreasing trend from 2008 to 2011 and some increasing trend from 2012 to 2013 of TB-HIV co-infection [15]. This study revealed that the trend of TB-HIV co- infection was increased from 23.9% to 35% from 2009/10 to 2011/12, respectively; then decreased from 35% to 12% from 2011/12 to 2013/14. This is due to intensive provision of INH prophylaxis to prevent opportunistic infection of TB as soon as they detect HIV positive. The other possible explanation may be due to the current strategic plan of the ministry of health in Ethiopia, which expands health facilities across the country and increased awareness of the community through health education and mass media on prevention and control of HIV.

V. Conclusion

The overall result of TB-HIV co-infection was 22.1%. The age groups of15 -24 and 25-34 years old which accounts 27.2% each, TB-HIV co-infection was strongly associated with death, defaulted and TB categories. The age groups 15-24 and 25-34 years old were 2.544 and 3.409 times likely to be risk of TB-HIV co-infection as compared to the age groups ≥55 years old. Died and cured were independently associated with TB-HIV co-infection as compared to completed treatment outcome.

This study showed that there was increasing rate of TB-HIV co-infection from 23.9% to 35% in the year 2009/10 to 2011/12 then decreasing from 35% to 12% in the year 2011/12 to 2013/14. The main reason is that provision of INH prophylaxis to prevent opportunistic infection of TB as soon as they detect HIV positive. The other reason may be due to expanded health facilities across the country, the increased awareness of the community through health education according to current strategic plan of the federal ministry of health of Ethiopia. Generally this study shows relatively lower TB-HIV co-infection than a number of other studies held in different countries in sub-Saharan African.

VI. Recommendations

The author of this study suggested that health education should be given for the community on mode of transmission of TB, prevention of HIV infection, impact of TB-HIV co-infection on TB treatment outcomes and productivity of population and national country. Besides this, regional health bureau, zonal health department, higher officials should create networking the health facilities each other to identify treatment outcome of the referred patients. In addition, the data registration system of patients should be improved to include patients' personal practice like smoking habits, alcoholism and chronic illness. This should also be collected by health professionals as they interview patients.

VII. Acknowledgement

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Molecular Epidemiology of Bovine Tuberculosis in Cattle and its Public Health Implications in Gambella Town and its Surroundings, Gambella Regional State, Ethiopia

By Jemberu Alemu, Gezahegne Mamo, Gobena Ameni & Mahendra Pal
Gambella University, Ethiopia

Abstract- A cross sectional study was conducted from December 2014 to May 2015 in Gambella town municipal abattoir and health centers to investigate the prevalence of BTB, isolation and molecular characterization of its causative agents and to assess its public health implications in Gambella, Ethiopia. Postmortem examination, bacteriological culturing, RD deletion typing and spoligotyping were used for investigation. The overall prevalence of BTB in cattle was 13.2% (66/500) on the basis of detailed postmortem examination. Statistical significant difference was observed in the prevalence of BTB among different body conditioned animals ($\chi 2$ = 39.105, P=0.000and breeds ($\chi 2$ = 24.996, P=0.000). Molecular characterization of 11 mycobacterial isolates from human patients using RD9 deletion typing showed that all were M. tuberculosis and further spoligotyping of the isolated revealed that SIT289, SIT134, SIT1634, SIT142 and one new strain not found in the spoligotype databases.

Keywords: bovine tuberculosis, molecular epidemiology, RD typing, spoligotyping, public health.

GJMR-F Classification : NLMC Code: WF 200

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Jemberu Alemu [α], Gezahegne Mamo [σ], Gobena Ameni [ρ] & Mahendra Pal [ω]

Abstract- A cross sectional study was conducted from December 2014 to May 2015 in Gambella town municipal abattoir and health centers to investigate the prevalence of BTB, isolation and molecular characterization of its causative agents and to assess its public health implications in Gambella, Ethiopia. Postmortem examination, bacteriological culturing, RD deletion typing and spoligotyping were used for investigation. The overall prevalence of BTB in cattle was 13.2% (66/500) on the basis of detailed postmortem examination. Statistical significant difference was observed in the prevalence of BTB among different body conditioned animals ($\chi 2$ = 39.105, P=0.000and breeds ($\chi 2$ = 24.996, P=0.000). Molecular characterization of 11 mycobacterial isolates from human patients using RD9 deletion typing showed that all were M. tuberculosis and further spoligotyping of the isolated revealed that SIT289, SIT134, SIT1634, SIT142 and one new strain not found in the spoligotype databases. Of these M. tuberculosis strains identified SIT 289 and SIT134 were found in cluster with 45.5% (5/11) cluster rate. Lineage of the human isolates indicated that 27.3% (3/11) Euro-American, 9.1% (1/11) Indo-oceanic family in TB-insight database. Interestingly, one isolate from animal taken from cranial mediastinal lymph node was confirmed to be M. tuberculosis using RD4 deletion typing and spoligotyping, in which the isolate was identified as SIT523 with indo-oceanic lineage family. Awareness of cattle owners for BTB was found to be insufficient (22%) and the result also revealed the presence of potential risk factor for zoonotic transmission. In conclusion, isolation of M. tuberculosis in cattle and occurrence of various strains of M. tuberculosis in the communities warrants further investigation on the transmission of the disease in Gambella Region.

Keywords: bovine tuberculosis, molecular epidemiology, RD typing, spoligotyping, public health.

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I. Introduction

Tuberculosis is communicable Mycobacterial disease of human and animals, caused by members of *Mycobacterium tuberculosis* complex (MTBC) (Smith *et al.,* 2006; Pal, 2007; Malama*et al.,* 2013). Although, recent studies indicated that M. *tuberculosis* has been isolated from cattle (Ameni *et al.,* 2011) and *M. bovis* from humans infected with bovine tuberculosis (Zeweld, 2014), *M. tuberculosis* is specifically adapted to humans while *M. bovis* is most frequently isolated from domesticated cattle (Smith *et al.,* 2006), In spite of variation in host specificity, the members of MTBC are characterized by 99.9% or greater similarity at nucleotide level and are virtually identical at 16s rRNA sequence (Brosch *et al.,* 2002).

Bovine tuberculosis is a contagious disease, which can affect most warm blooded animals, including human being (Radostits *et al.,* 2007). Organisms are excreted in the exhaled air, in sputum, feaces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes of infected animals (Radostits *et al.,* 2007). In cattle, exposure to this organism can result in a chronic disease that jeopardizes animal welfare and productivity and in some countries leads to significant economic losses by causing ill health and mortality (Ewnetu *et al.,* 2012). Moreover, human TB of animal origin caused by *M. bovis* is becoming increasingly evident in developing countries (Russel, 2003; Mamo *et al*., 2013).

Bovine tuberculosis diseased animal loses 10 to 25% of their productive efficiency; direct losses due to the infection become evident by decrease in 10 to 18% milk and 15% reduction in meat production (Radostits *et al.,* 1994). Apart from effects on animal production, it has also a significant public health importance (Müller *et al.,* 2013). Currently, the disease in human is becoming increasingly important in developing countries, as humans and animals are sharing the same micro environment and dwelling premises, especially in rural areas, and susceptibility of AIDS patients to tuberculosis (Shitaye *et al.,* 2007). It is estimated that *M. bovis*

causes 10 to 15% human cases of tuberculosis in countries where pasteurization of milk is rare and bovine tuberculosis is common (Ashford *et al.,* 2001; Berg *et al.,* 2015).

In developing countries like Ethiopia, the socio economic situation and low standard living area for both animals and humans are more contributing in TB transmission between human to human and human to cattle or vice versa (Ameni *et al.,* 2010a; Ejeh *et al*., 2013). Human infection due to *M. bovis* is thought to be mainly through drinking of contaminated or unpasteurized raw milk and under cooked meat. The high prevalence of TB in cattle, close contact of cattle and humans, the habit of raw milk and meat consumption, and the increasing prevalence of HIV may all increase the potential for transmission of *M. bovis* and other *Mycobacteria* between cattle and humans (Shitaye *et al*. 2007).

Bovine tuberculosis is an endemic disease of cattle in Ethiopia, with a reported prevalence of 3.5–5.2 % in abattoir (mostly zebu) and 3.5–50% in crossbreed farms (Shitaye *et al*., 2007; Demelash *et al*. 2009; Regassa *et al.,* 2010; Berg *et al*., 2011). Nevertheless, the available information is limited due to inadequate disease surveillance and lack of better diagnostic facilities (Cosivi*et al.,* 1998; Asseged *et al*., 2000). In particular, information on genotypic characteristics of *M. bovis*, a strain affecting the cattle population in Ethiopia, is limited (Biffa *et al*., 2010a). Such information is critical to monitor transmission and spread of the disease among cattle (Berg *et al.,* 2011).

The World Health Organization 2009 report indicated that the status of TB in Gambella Region was the highest from all the Ethiopian Regions, with the notification rate (new and relapse) 261- 421/100, 000 (WHO, 2009). This was one of the bases of the present study.

Gambella regional state has large livestock populations. Despite, the large number of livestock population in the region, there is no information on BTB. Despite the fact that bovine tuberculosis is a public health threat and also leads to economic losses, in Ethiopia research on and control of animal tuberculosis has not received much attention like human tuberculosis (Chukwu*et al.,* 2013).Thus the present study was designed to determine the prevalence of bovine tuberculosis in Gambella town municipal abattoir and identifying risk factors associated with bovine tuberculosis, to isolate and molecular characterization of *Mycobacterial* isolates from slaughtered cattle and from human pulmonary TB patients and to investigate the potential risk factors for zoonotic transmission of mycobacterial infections.

II. Materials and Methods

a) Study Area

The study was conducted in Gambella town municipal abattoir and Gambella hospital of Gambella regional state, southwest Ethiopia from December 2014 to May 2015. The Gambella People's Regional State is located south west Ethiopia between the geographical coordinates of 6^0 28'38" to 8^0 34' North Latitude and 33^0 to 35^0 11'11" East Longitude, 766 km far from Addis Ababa which covers an area of about 34,063 km^2. The Region is bounded to the North, North East and East by Oromya National Regional State, to the South and Southeast by the Southern Nations and Nationalities People's Regional State and to the Southwest, West and Northwest by the Republic of south Sudan (Behailu *et al.,* 2011). The mean annual temperature of the Region varies from 17.3^0C to 28.3^0C and absolute maximum temperature occurs in mid-March and is about 45^0C and the absolute minimum temperature occurs in December and is 10.3^0C. The annual rainfall of the Region in the lower altitudes varies from 900-1,500mm; at higher altitudes it ranges from 1,900-2,100mm. The annual evapotranspiration in the Gambella reaches about 1,612mm and the maximum value occurs in March and is about 212 mm (Tilahune, 2012). Based on the 2013/ 2014 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the Gambela Region has total population estimation of 406,000 (CSA, 2013/2014) and livestock population of Gambella 253,389 cattle, 39,564 sheep and 83,897 goat (CSA, 2010/2011).

The Gambella town municipal abattoir: - The abattoir which is administered under Gambella town municipality is the only source of inspected beef for all inhabitants of the town. The average number of animals slaughtered per day during the study period was about 25 with all 100 % of the slaughtered animals being cattle. The overall abattoir sanitary environment is below the requirements of good hygiene practices (GHP) in slaughterhouses. The internal and external facilities and sanitary conditions of the slaughter house were very poor. Neither place for disposal of condemned carcasses nor facilities for wastewater treatment exist and it is not friendly with the environment. The abattoir workers had no clothing, boot, apron and other accessories. Three assistant meat inspectors were delivered services only during ante mortem and no one was carried out post mortem examination during the study period in such a ways the population is endanger of meat born zoonosis and sanitation problems.

b) Study Population and Study Design

According to the available logistics and time a total of 500 apparently healthy animals slaughtered in the abattoir of Local Nuer, Horro and Felata breed cattle were included as study population for the stated objectives and the major sources of cattle for this

abattoir were Gambella town and its surroundings, Mettu, Gore, Bure, Sibo and Gumero. In addition, 50 Acid fast bacilli (AFB) positive sputum samples from human TB patients attending the health facilities in Gambella town were included.

A cross sectional study with systematic random sampling was carried out in abattoir to examine the carcass and sample suspected TB lesions from slaughtered cattle at Gambella town municipal abattoir. Similar cross sectional study and purposive sampling was carried out to collect samples from all AFB positive TB patients attending Gambella Hospital. Both sputum and extra pulmonary TB samples mainly fine needle aspirate from suspected human case was taken in the course of the study period for isolation and molecular characterization of the causative agents.

c) Sampling, Sample Size Determination and Study Methodologies

All animals coming to the slaughter house from different areas during the study period were considered for sampling. The sample size calculation was based on 50% prevalence assumption (since there was no study on bovine tuberculosis in the area), 95% CI and d=0.05 (Thrusfeild, 2005).

$$n = \frac{Z^2 \times p_{expe} (1-p_{expe})}{d^2}$$

Where n= required sample size

$P_{exp.}$ =expected prevalence
d=Desired absolute precision (5 %)
Z= Normal distribution constant

Therefore, the sample size calculated was 384, but to increase the precision using thumb rule by 20% and the total animals supervised were 500.

The sample size for the questionnaire survey used was 100 for livestock owners, and abattoir workers. For human case, a total of 50 acid fast positive patients were interviewed about their association with cattle, habit of consumption of meat and milk and other relevant information related to tuberculosis.

d) Ante and postmortem examination

Physical examination of the animals were carried out before they were slaughtered. Body temperature, pulse rate, respiratory rate, condition of superficial lymph nodes and visible mucus membranes were examined and recorded for individual animals to be slaughtered. Breed, source or origin and sex were also recorded. Age was estimated as described by Amstutz (1998) and Body Condition Scoring (BCS) chart was made based on the description by Nicholson and Butterworth (1986). Detailed postmortem examination (inspection, palpation and incision) of the carcass, lungs, liver and kidneys together with mesenteric, hepatic lymph nodes and lymph nodes of the head was undertaken in accordance with the method developed by Ethiopian meat inspection and quarantine division of the Ministry of Agriculture (Hailemariam,1975; Ameni*et al.,* 2007). Lymph nodes were incised into a size of 2 mm to facilitate the detection of tuberculous lesions from each animal. These include Mandibular, Retropharyngeal, Bronchial, Mediastinal, and Mesenteric lymph nodes. The animal was classified as lesioned (infected) when tuberculous lesion was found, and if not as non lesioned (not infected). The severity of gross lesions in individual lymph nodes and other organs were scored as follows; 0= no gross lesion, 1= small lesion at one focus, 2= small lesions at more than one focus and 3= extensive necrosis as developed by Ameni *et al. (*2006). The cut surfaces were examined under bright light for the presence of abscess, cheesy mass, and tubercles (Corner *et al*., 1990). In the presence of suspected tuberculous lesion, tissue samples were collected in sterile universal bottles containing 0.85% normal saline for culture kept at -20^{0}crefrigerator. The samples were transported under cold chain by ice box with packed ice to the Akililu Lema Institute of Pathobiology for culture and further processing in three week basis.

e) Isolation of mycobacteria

Tissues with suspected lesions were collected and subjected to bacteriological culture examination. The tissue specimen or sputum collected from AFB and gene Xpert positive patients for culture were collected individually in to sterile universal bottles in normal saline and then labeled and kept frozen (–20 °C) at Gambella regional hospital before being transported to Aklilu Lema institute of Pathobiology laboratory Addis Ababa.

The specimens were labeled and pooled together, kept in universal bottle containers, and then transported in ice pack box to Aklilu Lemma Institute of Pathobiology, Addis Ababa Ethiopia, within three week basis by airplane. There the samples were processed for isolation of *M. tuberculosis* complex according to the standard methods (Ameni *et al.,* 2007).

f) Identification and characterization of mycobacteria

Initial identification of mycobacterial species from animal tissue was based on the rate of growth, pigment production, and colony morphology as described in OIE (2009). When visible colonies were observed, Ziehl Neelsen staining was performed to confirm the presence of acid-fast bacilli. AFB positive isolates were prepared by mixing two loops full of colonies in 200 mL distilled water, heat-killed at 80^{0}C for 1 hour using water bath, and stored at -20^{0}C until molecular characterization was perform and were subjected to PCR based on amplification of a multi copy DNA target sequence for identification of *M. bovis* and *M. tuberculosis* (Debebe *et al.,* 2013).

g) RD deletion typing

For RD9 deletion typing of culture positives of sputum; RD9 intR: CTG GAC CTC GAT GAC CAC TC, RD9 flankF: GTG TAG GTC AGC CCC ATCC and RD9 flankR: GCC CAA CAG CTC GAC ATC primers to check for the presence of RD9 locus was used; The HotStarTaq Master Mix system from Qiagen was used for PCR, with primers described previously (Ameni *et al.,* 2013).

The primers used were RD4Flank int: 5´-ACAC GCTGGCGAAGTATAGC-3´; RD4flankR: 5´-AAGGCGA ACAGATTCAGAT-3´ and RD4falnkF: 5´-CTCGTCGAAG GCCACTA AG-3´. The mixture was heated in a Thermal Cycler (Applied Bio-systems; Gene AMP 9700) for 15 minutes at 95°C and then subjected to 35 cycles of one minute duration at 95°C, one minute at 55°C, one minute at 72°C and 10 minutes at 72°C. The presence of RD4 (RD4 is intact in *M. tuberculosis*, *M. africanum*) gives a product size of 335 bp (RD4 intF + RD4flankR), and its absence (*M. bovis*) gives a product size of 446 bp (RD4flankF + RD4flankR).

h) Spoligotyping

Spoligotyping was carried out using the commercially available kit according to the manufacturer's instructions and as previously described by Kamerbeek *et al. (*1997).

i) Questionnaire survey

The roles of various risk factors in the occurrence and spread of bovine TB among cattle, and between cattle and people, were assessed by a questionnaire. Structured questionnaire was distributed to TB patients, cattle owners, and abattoir workers to assess the perception of stakeholders on the occurrence of bovine tuberculosis, livestock constraints, socioeconomic status, herd composition, awareness on the potential risk of zoonotic transmission of bovine tuberculosis.

j) Data Management and Analysis

Prevalence was calculated as the proportion of suspected lesion positive animals from the total number of animals visited (Thrusfield, 2005). Data related with age, sex, breed, origin and body condition of each animal was recorded on a data sheet during antemortem examination. Presence or absence of TB like lesions and affected tissues were recorded during postmortem examination. The recorded data was entered and stored in Microsoft Excel computer program and analyzed by STATA version 11 (STATA Corp. College station, TX). The variations between different factors were also analyzed using multi variable logistic regression and chi-square ($\chi 2$) was used for association of different risk factors. A p-value <0.05 was considered statistically significant, 95% confidence interval was considered and Odds ratio analysis was used.

In molecular epidemiology study of isolates from human pulmonary tuberculosis patients and animals tissue, the spoligotype patterns were converted in to binary and octal formats and entered to the online spoligotypedatabase, http://www.pasteur guadeloupe.fr:8081/SITVIT Demo/index.jsp to determine the shared international spoligotype (SIT) number and the results were compared with already existing designations in the international spoligotyping database (SpolDB4.0 database). Those isolates with no designated SIT number were considered as new to the database. Two or more isolates with identical spoligotype pattern were considered as clustered while those with single SIT were considered as non-clustered isolates. TB-lineage and family were determined using SPOTCLUST database, http://tbinsight.cs.rpi.edu/about_spotclust.html

k) Ethical Considerations

Ethical clearance was obtained from the Ethical Committee of Gambella regional health office (Ref. number of 16/3776/7) and working permission was gotten from the hospital higher managers and the municipality.

III. Results

a) Prevalence of Bovine Tuberculosis

The overall prevalence of bovine tuberculosis in slaughtered cattle of Gambella municipal abattoir was 13.2% (66/500: 95% CI, 10.22-16.18) based on the occurrence of gross tuberculous lesions.

Table 1: Univariate and Multivariable logistic regression analysis of tuberculous lesion with various host related risk factors in Gambella municipal abattoir.

Risk factor	Number examined	Number positive	Crude odds ratio (95% CI)	Adjusted odds ratio (95%CI)
Age (year)				
<5	24	2	1	1
5-8	188	24	1.61(0.36-7.28)	1.26(0.25-6.43)
>8	288	40	1.77(0.40-7.84)	1.08(0.22-5.37)
Sex				
Male	346	36	1	1
Female	154	30	2.08(1.23-3.53)	1.05(0.52-2.15)
BCS				

Good	89	29	1	1
Medium	173	22	2.17(1.089-4.31)	3.54(1.45-8.64)*
Poor	238	15	7.19(3.62-14.26)	12.16(4.58-32.24)*
Breed				
Nuer	301	39	1	1
Horo	185	19	0.77(0.43-1.38)	0.67(0.09-4.97)
Felata	14	8	8.96(2.95-27.198)	6.43(1.96-21.04)*
Origin				
Gambella town & surroundings	328	47	1	1
Sibo	45	3	0.598(0.18-2.04)	3.0(0.29-30.67)
Gore	29	5	0.85(0.19-3.88)	5.88(0.49-71.13)
Mettu	49	6	0.83(0.34-2.08)	4.9(0.57-42.38)
Gumero	33	3	1.25(0.45-3.43)	6.94(0.82-59.00)
Bure	16	2	0.43(0.13-1.434)	2.01(0.22-18.47)

**Statistically significant*

b) Gross Pathology Results

Gross lesions were observed in the lymph nodes and lung of the slaughtered cattle and the majority of the lesions were considered typical of tuberculous lesions characterized by central round, oval, or irregular, often coalescing areas of caseous necrosis and mineralization (calcification) (Figure 1). Whenever gross lesions suggestive of TB were detected in any of the tissue, the tissue was classified as having lesions.

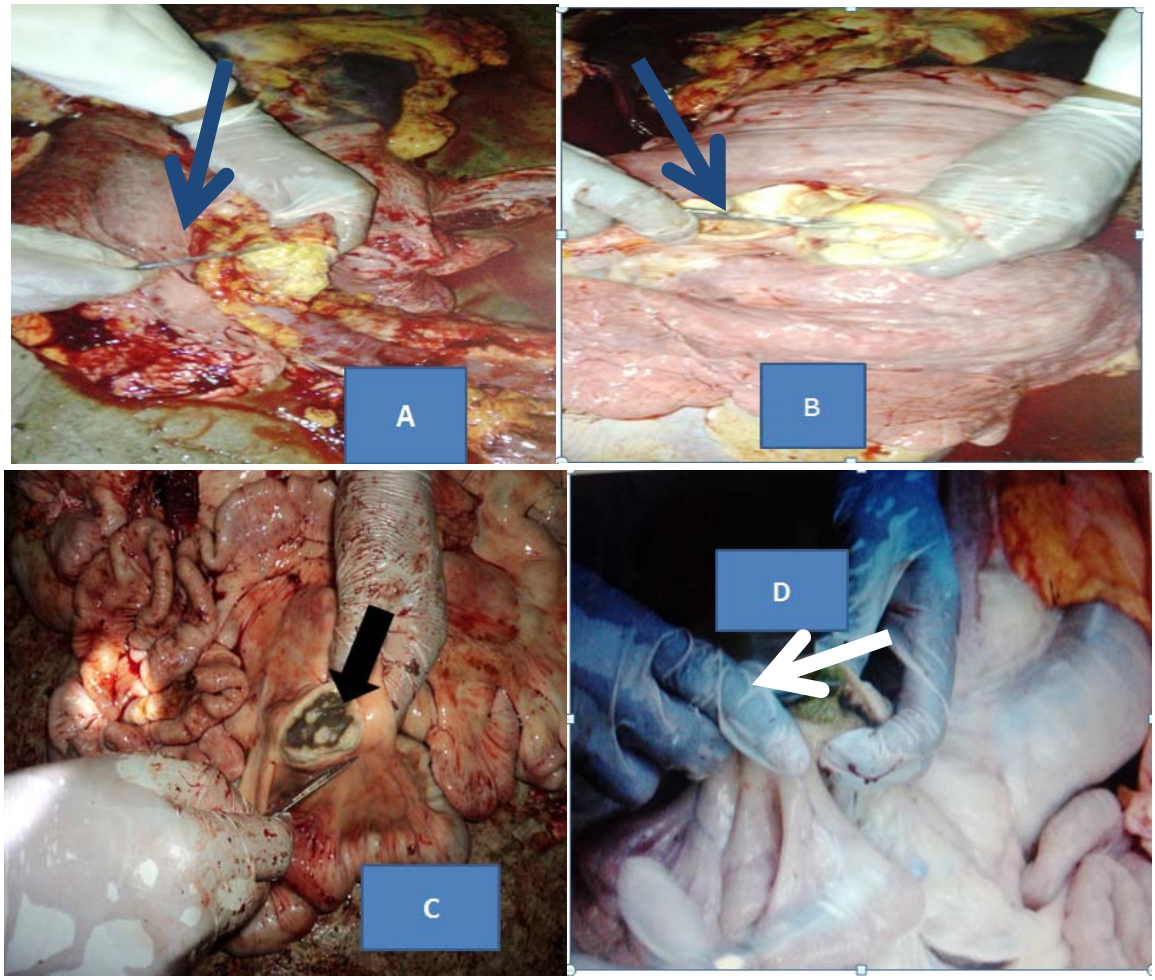

Figure 1: Typical TB lesions of cattle slaughtered in Gambella town municipal abattoir, A= granulomatous lesion from mediastinum B=Caseous and granulomatous necrosis in lung C&D= calcified and granulomatous lesion in mesenteric lymph nodes.

The frequency and distribution of lesions according to organ level and anatomical site is indicated in (Table 2).

Table 2: Distribution of lesions in different anatomical sites with their respective frequency of occurrence.

Anatomical site	Organ affected	Frequency (%)
Head	Mandibular lymph node	5(6.1%)
	Retropharyngeal lymph node	15(18.5%)
Thoracic	Bronchial lymph node	8(9.8%)
	Mediastinal lymph node	19(23.2%)
	Lung	14(17.1%)
Abdominal	Mesenteric lymph nodes	21(25.6%)
Total		82(100%)

Table 3 : Mean pathology scoring of lesion from lung and lymph node of bovine tuberculosis in cattle slaughtered at Gambella municipal abattoir.

Tissue	Number examined	Number positive (%)	Mean ±SE)
Lung	500	14(2.8)	1.86±0.231
Mandibular	500	5(1)	2.6±0.245
Bronchial	500	8(1.6)	2.25±0.366
Mediastinal	500	19(3.8)	1.5±0.159
Retropharyngeal	500	14(2.8)	1.93±0.245
Mesenteric	500	22(4.4)	2.14±0.168

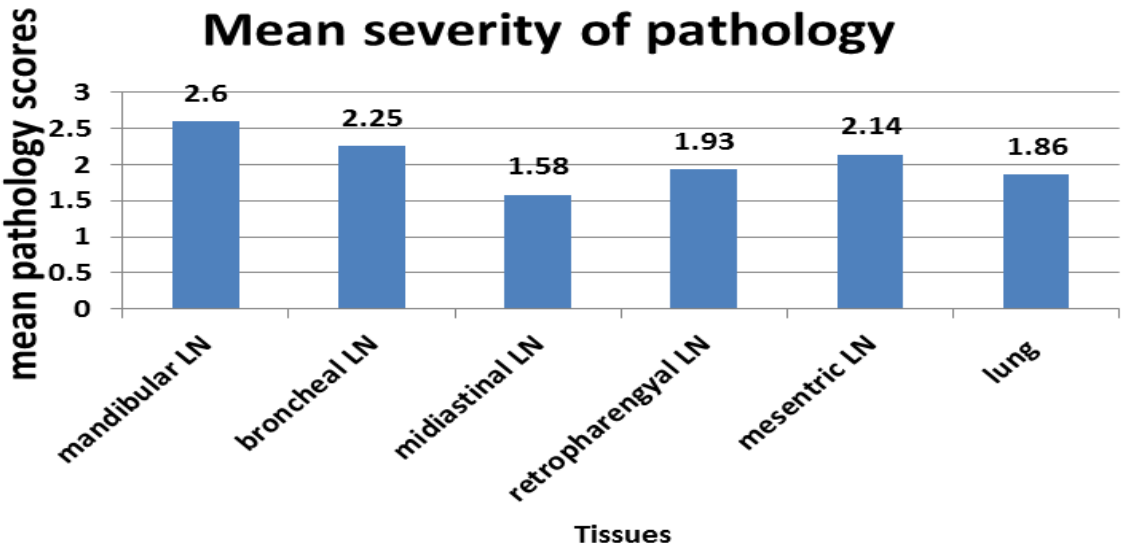

Figure 2 : Mean severity of lesion in lung and different lymph nodes.

c) *Mycobacteriological Culture Result*

Out of 82 tissue samples 14(17.07%) showed a growth on LJ medium and out of 50 sputum samples and one FNA sample, 17(34%) of sputum samples had showed growth on LJ media while the FNA sample did not grow (Table 4).

Table 4: Bacteriological results on LJ media.

Type of Specimen	Number of sample	Growth on LJ-pyruvate	Growth on LJ-glycerol	Growth on both	Total growth (%)
Sputum	50	8	9	5	17(34)
FNA	1	-	-		0
Animal tissue	82	5	10	1	14(17.07)
Total	133	13	19	6	31(23.3%)

d) Molecular Characterization of Mycobacterial Isolates

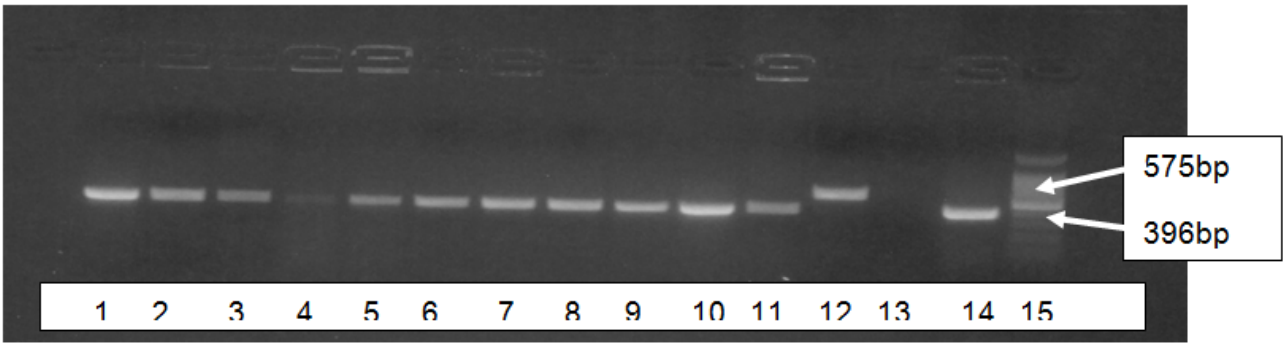

Figure 3 : Electrophoretic separation of PCR products by RD9 deletion typing of 11 mycobacteria isolates from human TB patients. 15-ladder (100bp), 14-*Mycobacterium tuberculosis* control, 13-distilled water negative control, 12-*Mycobacterium bovis* control and 1 to 11 are samples from TB patients.

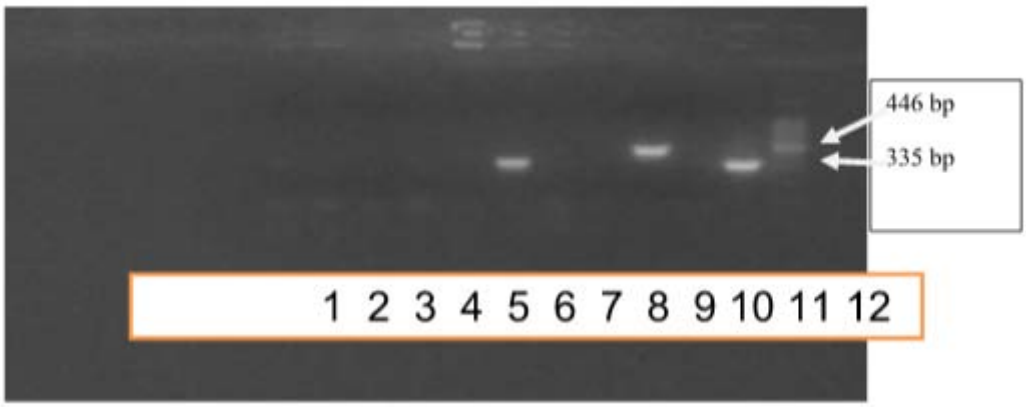

Figure 4 : Electrophoretic separation of PCR products by RD4 deletion typing of 8 mycobacteria isolates from tissue sampled culture. 12- Ladder (100bp), 11- *Mycobacterium tuberculosis* control, 10-Distilled water negative control, 9-*Mycobacterium bovis* control and 1 to 8 are samples from tissue culture positives.

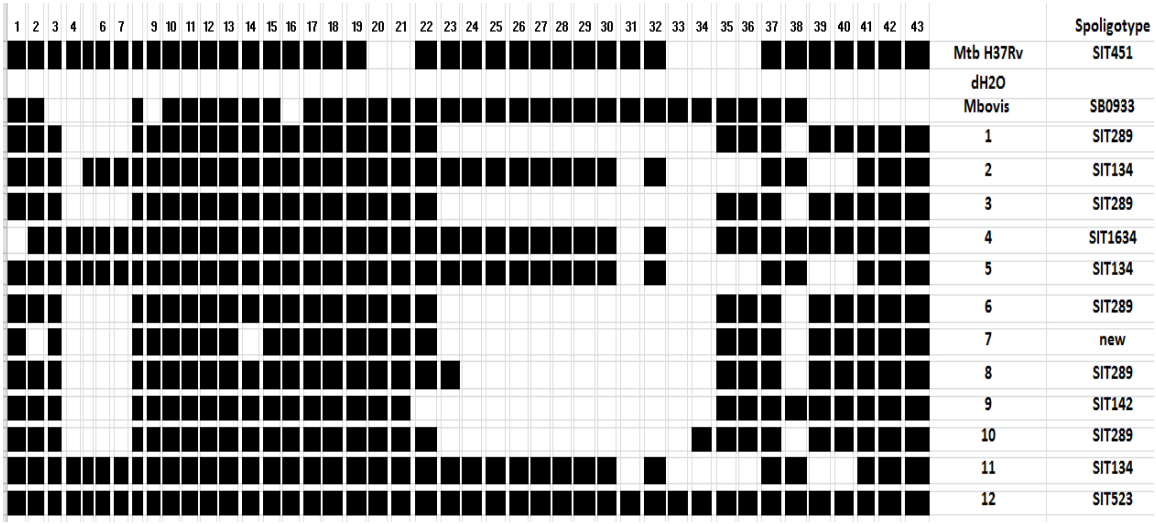

Figure 5 : Spoligotype patterns of mycobacterial isolates recovered from sputum of human patient and tuberculosis lesions in cattle (1 to 12). The filled boxes (blacks) represent the presence of spacers, and the empty boxes represent the absence of spacers.

Table 5 : Lineages of the isolates.

	Specimen	Spoligotype	Octal number	Lineage
1	Sputum	11100001111111111111111000000000000111011111	703777740003571	Unknown
2	Sputum	11111111111111111111111111111110100001100111	777777777720631	Euro-American
3	Sputum	11110000111111111111111000000000000111011111	703777740003571	Unknown
4	Sputum	11111111111111111111111111111110100111111111	777777777723771	Indo-Oceanic
5	Sputum	11111111111111111111111111111110100001100111	777777777720631	Euro-American
6	Sputum	11100001111111111111110000000000000111011111	703777740003571	Unknown
7	Sputum	10100001111110111111110000000000000111011111	503757740003571	Unknown
8	Sputum	11100001111111111111110000000000000111011111	703777740003571	Unknown
9	Sputum	11100001111111111111100000000000000111111111	703777700003771	Unknown
10	Sputum	11100001111111111111110000000000000111011111	703777740003571	Unknown
11	Sputum	11111111111111111111111111111110100001100111	777777777720631	Euro-American
12	Animal Tissue	11111111111111111111111111111111111111111111	777777777777771	Indo-Oceanic

e) *BTB Awareness and risk factor Assessment*

Table 6 : Client's (farmers, abattoir workers TB patients) awareness of bovine tuberculosis and its mode of transmission

knowledge examined in questionnaire	Responders out of 100 (%)
Had noticed respiratory problems in their cattle	30(30%)
Aware of bovine tuberculosis (TB)	22 (22%)
Know that cattle transmit bovine TB to humans	15 (15%)
Know that humans transmit TB to cattle or vice versa	0 (0%)
Know that milk is a source of infection	23(23%)
Know that meat is a source of infection	17(17%)
Drink raw milk	37(37%)
Eat raw meat	45(45%)
Use the same watering point with animals	48(48%)
Share the same house with animals	30(30%)

IV. Discussion

Tuberculosis remains a major global health problem causing high morbidity and mortality among millions of people each year (WHO, 2014). Tuberculosis caused by *M. bovis* is clinically indistinguishable from tuberculosis caused by *M. tuberculosis* and globally the proportion of human tuberculosis caused by *M. bovis* is estimated to 3.1% of all forms of which 2.1% of pulmonary and 9.4% of extra pulmonary forms (Cosivi *et al., 1998*).

Ethiopia is one of the countries with highest number of livestock resource in Africa and animal tuberculosis is known to be endemic and wide spread in the country. However, in spite of high prevalence both human and animal tuberculosis in the country, the emphasis given on bovine tuberculosis to the Gambella region is very little and so far no research were carried out on BTB in Gambella Region. Infection of cattle with *M. bovis* constitutes a human health hazard as well as an animal welfare problem. Furthermore, the economic implications in terms of trade restrictions and

productivity losses have direct and indirect implications for human health and the food supply (Zeweld, 2014).

In the present study an attempt was made to determine the prevalence of bovine tuberculosis in Gambella town municipal abattoir and identifying risk factors associated with bovine tuberculosis, to isolate and molecular characterization of *Mycobacterial* isolates from slaughtered cattle and from human TB patients and to investigate the potential risk factors for zoonotic transmission of mycobacterial infections from animal to human and vice versa.

Based on detailed post mortem inspection the prevalence of BTB in slaughtered cattle was found to be 13.2%, which is moderately high and this result was comparable with other pervious research reports carried out on cattle originated from extensive and pastoral production system of Ethiopia; 11.50% by Abdurohaman (2009) in Butajira, and 11% by Mamo *et al. (*2013) in Afar, but less than a result from 19.8% record from cattle slaughter in rural Tanzania (Cleaveland *et al.,* 2007). The result of the present prevalence study was higher than findings by various other authors Biffa *et al.,* 2010a who reported 4.2% prevalence in cattle slaughtered at in Yabello municipal abattoir and 4.5% at Hosaana abattoir by Teklu *et al.,* (2004). In addition, the result were also higher than previously reported by other researcher in Northern and cental parts of the country (Nemomsa *et al. (*2014) (9%); Zeru *et al. (*2013) (6.4%); Romha *et al. (*2013) (5.8%). This difference in prevalence of tuberculous lesions could be due to the difference in origin or types of production system and breed of animals that are slaughtered in the abattoirs.

In respective of small sample size due to wondering of the Felata breed from place to place, association of breed with prevalence of BTB showed a statistically high significant difference among different local breed of cattle, (P = 0.000) animals which might be related to the genetic difference of the breeds, Other previous studies also showed different breeds could result in difference in susceptibility to BTB infections (Ameni *et al.,* 2007).

There is a statistically significant difference in the prevalence of the disease (P = 0.000) between BCS, the prevalence being the highest in poor body condition (32.6 %) as compared to medium (12.7%) fatty (good) animals (6.3 %) respectively which in agreement with study resulted by Nemomsa (2014). This could due to related to the weak protective immune response in poor body conditioned animals as compared to good one that may result extensive lesions and wasting of the body condition as well as its chronicity nature of the disease. The present result is consistent with previous reports which indicated that animals with good BCS have relatively good immunological response to the infectious agent than animals with medium BCS (Radostits *et al.,* 1994; Radostatit *et al* 2007).

In this study, gross tuberculosis lesions were found most frequently in the lymph nodes of thoracic cavity (50%), mesenteric lymph node (25.6%), followed by lymph nodes of the head (24.4%).This finding is significantly different from previous studies done in Ethiopia (Tamiru *et al.,* 2013) where 70 and 70.7% TB lesions were reported in lungs and associated lymph nodes, respectively. However, the distribution of TB lesion in the current study significantly similar with reports from Mexico (Ndukum *et al.,* 2010) where 49.2% of lesions involved the thoracic lymph node. The result, therefore, indicate that the primary route of infection was through the respiratory route which can also spread to other parts of the body as described previously (Radostits *et al.,* 2007).

In this study, culture positivity in primary culture media was found low and confirmed in 23.49% (31/133) despite slightly lower than that reported by Ameni *et al.* (2007), 56% culture positivity. This low isolation rate of mycobacteria may have resulted from reduced sensitivity of culture arising from prolonged storage at field sites and the freeze-thaw cycles that occurred during transportation and contamination of tissue samples (OIE, 2009). Furthermore, the presence of caseous and/or calcified lesions and even lesions resembling tuberculous lesions may not always found to be of mycobacterial origin; they can be caused by any other intracellular organisms or parasites, or viable mycobacteria may not be present in calcified lesions (Corner, 1994).

In the present study, interestingly, *M. tuberculosis* strain SIT523 was isolated and characterized with spoligotyping from cattle cranial Mediastinal lymph node tissue and the result implies the occurrence of reverse zoonosis in the study area where human strains could be transmitted to cattle. The transmission to cattle could be through different routes including ingestion of feed contaminated with infected sputum and/or urine from *M. tuberculosis* infected farmers. Humans suffering from active TB are the most probable source of *M. tuberculosis* in animals, with infection spread via sputum, and rarely urine or faeces (Thoen and Steele, 1995) or respiratory route as in rural area of Ethiopia, grazing cattle are commonly brought into the farmers' households at night where they may become infected via aerosol transmission from humans (Ameni *et al.,* 2013). Previous studies in Ethiopia had confirmed transmission of *M. tuberculosis* from farmers to their cattle, goat and camel (Berg *et al.,* 2009; Ameni *et al.,* 2011; Gumi *et al.,* 2012; Mamo *et al.,* 2012) supporting the result of the present study. Moreover, in a study conducted in India from extra pulmonary tissue of tuberculous cattle, 15–28% of the animals were discovered to be infected with *M. tuberculosis* (Aranaz *et al.,* 1996). Another study from Nigeria also identified one tissue isolate as *M. tuberculosis* by spoligotyping and VNTR (Prasad *et al.,* 2005). Even though human to cattle

transmission of *M. tuberculosis* has been reported, it is generally held that disease in cattle due to *M. tuberculosis* is less severe than that caused by *M. bovis* and the identification of *M. tuberculosis* in cattle by itself is intriguing (Tsegaye *et al.,* 2010). On to this, the identification of *M. tuberculosis* from cattle tissues requires further investigation.

In molecular characterization of isolates from human tuberculosis patients, *M. tuberculosis* was the predominant species causing TB in human and the genetic diversity of the isolate on the spoligopattern was 45.45%, which was higher than previous reports in other part of Ethiopia where 39% of spoligotype based genetic diversity where reported in Afar PTB patients (Mamo *et al.,* 2013). The difference might be related to difference on geographic and sociocultural difference among the studied population which might affect the transmission pattern of the organism. The most common spoligotype identified from TB patient was the SIT 289, in agreement with previous study (Ameni *et al*., 2011*)* which also reported the same SIT289 strains in pulmonary TB patients of central Ethiopia. In the present study, the predominant lineage was unknown according to TB-insight database analysis. Similar, unknown lineage had been previously reported form patients from Northwestern Ethiopia (Belay *et al.,* 2014*)* and this indicates the need for further investigation.

In the present study, the questionnaire survey of the respondents showed that 22 % of them were aware of BTB with no knowledge about zoonosis of the disease. This disagrees with report from Tamiru *et al.,* (2013) 80.7% of them were aware of BTB with low level knowledge about zoonoses of the disease. This result was comparable with the study on assessment of the knowledge of cattle owners about BTB in WuchaleJida district, Ethiopia showed that 38.3% (36 of 94) of the respondents knew that cattle can have tuberculosis, and 30.8% (29 of 94) recognized that BTB is zoonotic (Ameni *et al.,* 2003). Ameni *et al.,* (2007) have indicated that lack of understanding regarding the zoonotic of BTB, food consumption behavior and poor sanitary measures is the potential risk of BTB to public health. The proportion of BTB contributes to total tuberculosis cases in humans depends on the prevalence of the disease in cattle, consumer habits, socio-economic conditions, level of food hygiene (Ashford *et al.,* 2001) and medical prophylaxis measures in practice (Tigre *et al.,* 2011). According to the result of the present study, 45% consume unpasteurized or raw milk. Similarly, studies conducted in different parts of Ethiopia indicated the habits of raw milk consumption. The current result on habit of milk consumption was lower than 85.7% report from Jimma town, Ethiopia (Tigre *et al.,* 2011). Study conducted in WuchaleJida district indicated 52.1% (49 of 94) households' has habit of consuming raw milk (Ameni *et al.,* 2003), which is significant when compared with the current result. No one of the respondents in our study were found to be aware about the transmission of the disease from cattle to human and vice versa.

In our study, keeping cattle in close proximity to their house and calves in their house was a common practice of households. This indeed can facilitate transmission of the causative agent from animal to human or vice versa. According to Bogale (1999), conditions such as customs of consuming raw milk, keeping cattle in close proximity to the owner house and using cow dung for plastering wall or floor and as source of energy for cooking do exacerbate the chance of spread of tuberculosis as zoonosis in Ethiopia.

V. Conclusions and Recommendations

The result of the present study has shown that bovine tuberculosis was prevalent in cattle slaughtered at Gambella municipal Abattoir with moderately high prevalence (13.2%). This study also revealed that a high proportion of tuberculous lesion in the thoracic cavity lymph nodes and which implies that respiratory route was the major means of transmission. Isolation and molecular characterization of one *M. tuberculosis* isolate (SIT523 strain) from animal tissue sample suggested the occurrence of transmission of the agent between the communities and animals that implies reverse zoonosis. The high genetic diversity (45.5%) of the human *M. tuberculosis* isolates (SIT289, SIT134, SIT1634, SIT142, and the new one) and presence of clustering of the isolates might indicate the recent transmission pattern and circulation of the agents in the study communities. Lack of awareness regarding BTB and its routes of transmission in the study population was high and existence of habits of consumption of raw animal product and sharing of the same microenvironment with their livestock could be potential risk factors for zoonotic transmission of the disease. On the basis of findings of the present study, the following recommendations are forwarded: Further study should be conducted with larger sample size and geographic coverage to elucidate the role of *M. tuberculosis* complex in human and animal, With the finding of promising result on molecular characterization using few samples; a broader study to investigate the molecular epidemiology in human and animal tuberculosis is essential, Public health awareness campaigns should be launched and needed to raise community awareness about the risk of BTB transmission through consumption of raw/under cooked meat; and the zoonotic implication of BTB/, route of reverse zoonosis are of extreme importance for effective implementation of TB control measures, Establishment of collaboration between physician and veterinarians to trace back positive patient to get profile of their cattle in the slaughterhouse across

the region so as to estimate the regional prevalence of BTB as well as identification and characterization of the *M. tuberculosis* complex, and evaluation of their pathogenicity in bovine is essential.

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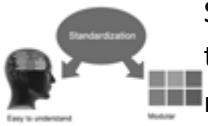

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The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

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Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

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References

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27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

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Key points to remember:

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- Please note the criterion for grading the final paper by peer-reviewers.

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Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
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Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
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Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- In spite of position, each table must be titled, numbered one after the other and complete with heading
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The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
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	A-B	**C-D**	**E-F**
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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

C

Chelemo · 2

E

Epidemiology · 14, 17, 33, 36, 37

F

Falciparum · 2

G

Gambella · 17, 19, 21, 22, 24, 26, 29, 30, 32, 33

M

Molecular · 17, 27, 33, 37

P

Plasmodium · 2

R

Radostits · 18, 30, 31, 36

T

Tabarsi · 12, 14
Tuberculosis · 7, 8, 9, 14, 17, 22, 28, 33, 35, 36, 37

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