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Prevalence of *Sarcocystis* infection in camels (*Camelus dromedarius*) slaughtered at Addis Ababa abattoir, Ethiopia

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Abstract

Camels are very important animals for the livelihood of pastoralists in arid and semi-arid environments, but are highly neglected and poorly studied. A cross-sectional study was conducted from October 2018 to May 2019 to estimate the prevalence, associated risk factors and tissue distributions of *Sarcocystis* in one-humped camels slaughtered at Addis Ababa abattoir enterprise (Akaki branch). Esophagus, diaphragm and heart tissue samples were collected from 166 slaughtered camels and examined histopathologically for presence of *Sarcocystis*. Of the total camels examined, *Sarcocysts* were detected in 51 (30.72%) camels. Relatively higher proportions of males (33.33%), young ones (33.33%), those originated from Minjar (35.71%) and camels with good body condition (43.39%) had *Sarcocystis* infection compared to other categories. However, except for body condition score ($p = 0.039$), other considered risk factors did not show significant difference with the prevalence of *Sarcocystis* infection. The infection rate of esophagus, diaphragm and heart were 19.87%, 14.46% and 13.25%, respectively. Moreover, multiple tissue infections by this parasite were detected in 26 (51%) camels of the 51 Sarcocystis positive camels. In general, the observed high prevalence of camel *Sarcocystis* infection in the present study, coupled with lack of information on the public health and economic significance, warrants further investigation and community awareness creation about the control measures.

Key words: Addis Ababa abattoir, *Camelus dromedarius*, Prevalence, Risk factors, *Sarcocystis*.

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INTRODUCTION

One-humped Camels (*Camelus dromedarius*) are contributing to the livelihoods of large human population in marginal and arid areas of Africa, particularly in the arid lowlands of Eastern Africa namely, Somalia, Sudan, Ethiopia, Kenya and Djibouti. The roles they play towards resilience to the present climate change make them the preferred domestic animal species and are towards an increase (Behnke, 2010).

Ethiopia has been considered to have a large camel population, which ranks third in Africa next to Somalia and Sudan (FAO-OIE-WHO, 1993). However, the exact data on camel population in the country appears to be debatable as reports show variable figures where estimates were as high as 4.8 million (Behnke, 2010) while official government livestock data showed 1.2 million (CSA, 2017). Camels are widely distributed in the Southern, Eastern and Northeast lowland areas of the country (Mirkena et al., 2018) and play an important role as a primary source of subsistence through milk and meat production, draught power, transportation service and source of cash (Kissi and Assen, 2017). Recently, camels have become one of the national export animals to the Middle East countries (Tefera and Abebe, 2012). Although camel production was challenged by several factors including diseases, infections with *Sarcocystis* species are widely known to cause considerable economic losses due to condemnation of edible organs and decreased meat production and quality (Romazanvoc, 2001).

Sarcocystis species are intracellular protozoan parasites of the phylum Apicomplexa which affect the skeletal and cardiac muscles of man and many species of animals worldwide (Fayer et al., 2015). The parasite requires two hosts to maintain its lifecycle, an intermediate or prey host (herbivores) and final or predator host (carnivores). One humped camels are the intermediate hosts for *Sarcocystis cameli* and become infected after ingestion of the sporulated oocysts passed in the feces of carnivores (mainly dogs) as the final hosts (Stojecki et al., 2012; Fayer et al., 2015). Although the economic and public health impacts of *Sarcocystis* infection in camel are not fully understood, some researchers

speculate that heavy infection with *Sarcocystis* spp can result in loss of weight, anemia, abortion and even death in cases of very heavy infection (Valinezhad et al., 2008; Hamidinejat et al., 2013).

Despite the huge potential of one humped camels and their role in the livelihoods of pastoral communities and national economy, still very little attention has been given to camels' production and health care (Megersa, 2010). Except one study conducted about a decade ago (Woldemeskel and Gumi, 2001), information on the current prevalence of *Sarcocystis* infection in camels is lacking at national level. Therefore, the present study was designed to estimate the prevalence, identify the associated risk factors and assess tissue distribution patterns of *Sarcocystis* infection in camels slaughtered at one branch of Addis Ababa abattoir enterprise.

MATERIALS AND METHODS

Study Area

The study was conducted from October 2018 to May 2019 at Akaki abattoir, which is owned by the Addis Ababa abattoir enterprise and located in Addis Ababa city, the capital of Ethiopia. Although the camel meat is not popular in Addis Ababa, camels are slaughtered for the Somali and other Muslim communities who live in the city. The camels slaughtered in the abattoir originated from Borana and Kereyu pastoral areas and Minjar-Shenkora district.

Borana pastoral area is located at approximately 600 km South of Addis Ababa at an altitude ranges from 970 m.a.s.l in the south bordering Kenya to 1693 m.a.s.l. in the Northeast. The area is characterized by an arid and semi-arid climate, with pockets of sub-humid zones. The rainfall in the area is bimodal where the average annual rainfall varies between 350 mm and 900 mm. The rainfall of the area is erratic by nature and there are four distinct seasons interspersed by long rainy season (expected between March and May) and the short rainy season (between October and November) (Galma, 2015).

Kereyu Pastoral area, circumscribed in Fentale and Boset districts, is located at about 250 km East of Addis Ababa at an altitude of 930 m above sea level. The Kereyu pastoralists occupy the arid lands around the Awash River down in the rift valley for pasture for their cattle, goats and camel (Tefera and Abebe, 2012). It has an average annual rainfall of 504 mm. The mean annual maximum and minimum temperature are 32.40 and 18.5°C, respectively. Pastoralism and agro-pastoralism are the main livelihood systems in the area.

Minjar-Shenkora is one of the districts in the Amahara Regional state of Ethiopia, located at the southern end of the North Shewa Zone at about 129 km East of Addis Ababa. The district is bordered on the east, south and west by the Oromia Regional state and on the northwest by Hagere Mariam. Its altitude ranges from 1040 to 2,380 meters above sea level. The average temperature ranges from 14 ºC to 27 ºC while the annual rainfall ranges between 780 and 900 mm. The district is known with its scattered bushes, shrubs and acacia trees (Setotaw et al., 2014).

Study Population

The study population included the total number of camels slaughtered at Akaki abattoir. Camels purchased from different markets were transported to the abattoir by trucks and kept at lairage for 3 to 4 days.

The camels in the pastoral area (their original sites) brows on bushes and shrubs, but may rarely consume grass when shrubs or trees are not available. The browse species includes the family *Chenopodiaceae*, *Acacia brevispica*, *Opuntia ficus indica*, *Dichrostachys ciniarea* and *Euphorbia tirucalli*. Rivers, ponds and wells are the main sources of water for camels. The watering sites are usually visited by large numbers of camels and other animals at a time from the surrounding as well as from distant areas. The pond and river water sources are also shared by wild animals (Mirkena et al., 2018).

Study Animals and Sample Size

The study animals were selected from the study population using convenient sampling method. Since the abattoir usually slaughters on average six to eight camels per day, sampling was made once per week for 6 months. Accordingly, a total of 166 apparently health camels were selected for this study irrespective of their origin, sex, body condition and age. Data about the age, sex, origin and body condition score of the selected animals were recorded before slaughtering. The age of the camels was estimated using rostral dentition (Bello et al., 2013) and then categorized as young (less than 5 years) and old (≥ 5 years of age) for ease of data analysis. The body condition score of the camels

were assessed according to Faye et al. (2001) and then grouped as poor (score 1), medium (score 2 and 3) and good (score 4).

Study Methodology

Post-mortem Examination.
Following slaughter and evisceration, the cardiac muscle, esophagus and diaphragm were examined for macroscopic sarcocysts using visualization, palpation and multiple incisions, when required. The pathological lesions were differentiated according to the guidelines on meat inspection for developing countries (Herenda et al., 1994).

Histopathological examinations
Representative tissue samples from oesophagus, diaphragm and heart of 166 slaughtered camels were collected and fixed with 10% neutral buffered formalin solution. The samples were labeled immediately and transported later to the parasitology and pathology laboratory of the Faculty of Veterinary Medicine, Hawassa University. In the lab, the specimens were trimmed, washed with water, dehydrated in ascending series of ethanol (70%, 80%, 85%, 90% and 99%), cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were prepared, stained with Hematoxylin-Eosin (HandE) stain and then examined under light microscope (Makhija, 2012).

Statistical Analysis
Data were entered into Microsoft Excel spreadsheet coded and then analyzed using STATA statistical software (STATA, 2013; window version 13.1). Association between various risk factors (sex, age, origin and body condition score) and the prevalence of *Sarcocystis* infection was estimated using chi-square independent test and one way ANOVA. In all the analysis, significance was set at $p< 0.05$.

RESULTS

The overall prevalence of *Sarcocystis* infection in camels in the current study was 30.72% (51 out of 166 camels). All of the cysts observed in the examined tissue were microscopic (Fig. 1 and 2) and had morphological difference on their wall, some were thick walled (Fig. 3a) and the majorities were thin walled (Fig. 3b).

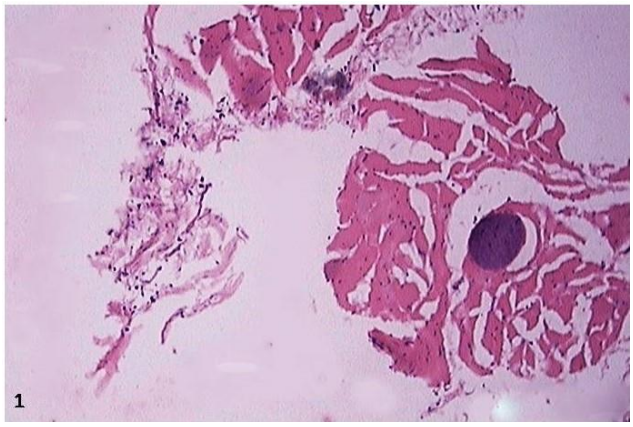

Figure 1. Cardiac muscle containing oblong shaped *Sarcocystis*, Haematoxylin and Eosin stain, 10X Objective magnification.

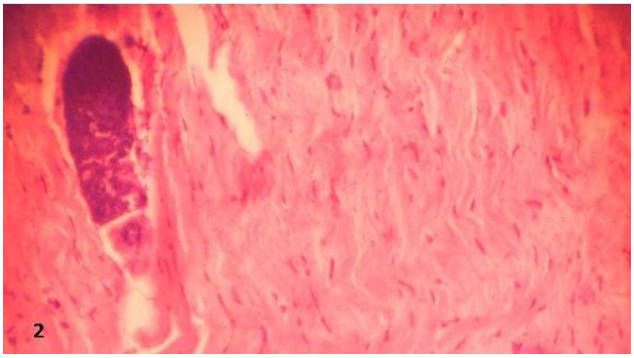

Figure 2. Muscle tissue from esophagus containing oblong shaped *Sarcocystis*, Haematoxylin and Eosin stain, 40X Objective magnification

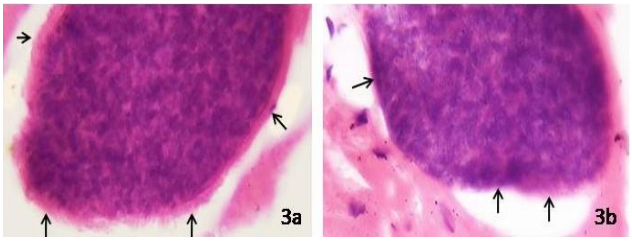

Figure 3. Higher magnification of *Sarcocystis* with thick wall (arrow) (a) and *Sarcocystis* with thin wall (arrow) (b). Haematoxylin and Eosin stain, 100X Objective magnification

Relatively higher prevalence of infection was observed in male camels (33.33%), young camels (33.33%), camels originated from Minjar (35.71%), and camels with good body condition (43.39%) than in other categories. However, with the exception of the body condition score ($p = 0.039$), the difference in the prevalence of *Sarcocystis* infection between or among the categories of the other considered risk factors (origin, age and sex) were not statistically significant ($p> 0.05$) (Table 1).

Table 1. Prevalence of *Sarcocystis* infection in camels by the putative risk factors

Variable		№ examined	№ (%)positive	**F**/χ2	p value
Origin	Borana	62	21 (33.87)	1.01	0.368
	Kereyu	62	15 (24.19)		
	Minjar	42	15 (35.71)		
BCS*	Poor	61	17 (27.87)	3.31	0.039
	Medium	52	11 (21.15)		
	Good	53	23 (43.39)		
Sex	Female	64	17 (26.56))	0.847	0.357
	Male	102	34 (33.33)		
Age group	Young	75	25 (33.33)	0.438	0.508
	Old	91	26 (28.57)		
Overall		166	51 (30.72)		

*BCS = body condition score

Out of the 166 tissues each of esophagus, diaphragm and heart tissue examined histopathologically, the cysts were observed in 33 (19.87%), 24 (14.46%) and 22 (13.25%) tissues, respectively. The cysts were observed concurrently in two or three tissues of 26 (51%) of the 51 *Sarcocystis* positive camels. There was no statistically significant difference (p = 0.0758) in detecting *Sarcocystis* between the tissues examined; the higher being in esophagus (Table 2, Fig. 2).

Table 2. Frequency and proportion of *Sarcocystis* in different tissues (n=498) of camels examined

Tissue /organ	No positive	Proportion	95% CI
Oesophagus	33	19.87	13.75–26.01
Diaphragm	24	14.46	9.05–19.86
Heart	22	13.25	8.04–18.46
Over all (Average)	79	15.86	12.76–19.37

DISCUSSION

The prevalence of *Sarcocystis* reported in this study (30.7%) is lower than the previous reports made in Ethiopia (45.5%; Woldemeskel and Gumi, 2001) and other countries like Saudi Arabia (88.35%; Fatani et al., 1996), Iran (83.6%; Valinezhad et al., 2008; 51.5%; Hamidinejat et al., 2013) and Iraq (91.6%; Latif et al., 1999). The difference in the prevalence of *Sarcocystis* in camels between the present study and other previous reports could be due to the variations in animal husbandry, the ecology and the sensitivity of the diagnostic methods used in each study. In Ethiopia, since there is no grazing grass land or pasture intentionally kept for camels and the camels are left to browse on the bush and shrubs, the chance of infection with sporulated oocyst/usually sporocysts/ is very minimal except at the watering points. However, the large number of sentinel dogs kept by the pastoralist and the wild carnivores such as hyenas, wolves, and jackals are known to contaminate the pastures and especially the drinking water with *Sarcocystis* sporocysts (Valinezhad et al., 2008).

The absence of macroscopic *Sarcocysts* in the present study could be partly explained by the inherent nature of the *Sarcocystis* species affecting camels. Moreover, since there is a very rare to no trend of keeping cats by most pastoralists in the country, there is no probability of detecting macroscopic sarcocysts as they are feline origin (Nourollahi-Fard et al., 2015). The microscopic cysts encountered during the current study were both thin and thick walled. In line with this, both thin-walled and thick-walled sarcocysts were reported by Fatani et al. (1996) and Dubey et al. (1989) from camels in Saudi-Arabia and Egypt, respectively. In contrary, Woldemeskel and Gumi (2001) identified only thin walled sarcocyst from camels originated from southern Ethiopia. Whether these morphologically distinct Sarcocysts represent two different species, as argued by Dubey et al. (1989) and Saeed et al. (2018), or they are strains of the same species named *Sarcocystis cameli* needs further investigation preferably on its ultrastructural and molecular features. Dubey et al. (2015) redescribed Sarcocystis and reported only two Sarcocystis species in camel, namely *Sarcocystis cameli* and *Sarcocystis ippeni*, which both appear thin-walled on light microscope but the former had thick wall and the later thin wall on transmission electron microscope (ultrastructural basis). On the other hand, reports about the

molecular criteria and immunodiagnoses of different species of *Sarcocystis* in camels are lacking (Valinezhad et al., 2008).

The prevalence was higher in males (33.33%) than in females (26.56%), young camels (33.33%) than old camels (28.57%) and in camels originated from Minjar (35.71%) than from Borena (33.87%) and Kereyu (24.19%) although these differences were not statistically significant. This finding is in line with several studies including the once conducted by Woldemeskel and Gumi (2001), Shekarforoush et al. (2006), Valinezhad et al. (2008) and Hamidinejat et al. (2013). These findings suggest the presence of other potential risk factors particularly associated with the husbandry system. In this regard, the prevalence of *Sarcocystis* infection in the current study among the three body condition scores was statistically significant ($p = 0.039$) and could be emanated from the difference in the husbandry and management systems. Camels with good body condition were more infected (43.39%) than camels with poor (27.87%) and medium (21.15%) body condition scores probably because they got additional feed and care by maintaining homesteads where dogs are freely roaming.

Of the three tissues/organs examined for the presence of sarcocyst, esophagus was the most frequently infected organ/tissue (19.87%), followed by diaphragm and heart. Similar report was also made by Shekarforoush et al. (2006), Woldemeskel and Gumi (2001) and Hamidinejat et al. (2013). In contrary, Fatani et al. (1996) reported that diaphragm is the most commonly affected tissue. The detection of the cyst concurrently in two or three tissues of the camels we examined (51%) probably confer more or less equal affinity *Sarcocystis* species for any organ with striated muscle. According to Valinezhad et al. (2008), these differences may be due to differences among *Sarcocystis* strains of various definitive host origin and the methods applied in the sarcocyst detection. In the diagnosis of *Sarcocystis* infection, the sensitivity is known to increase in the order of squash technique, histopathology and polymerase chain reaction. According to Calero-Bernal et al. (2015) the detection ratio of *Sarcocystis* infection can be improved two-fold by examining histological sections rather than squashed muscle.

CONCLUSIONS AND RECOMMENDATIONS

Results of this study showed a high prevalence of *Sarcocystis* infection in camel irrespective of their sex, origin and age group. Based on light microscopy, both thin and thick walled Sarcocysts were detected for the first time in Ethiopia. With the increasing curiosity of consumers for food safety and the increasing export competitors, the presence of this infectious agent could be a deterring factor for export of meat. Therefore, awareness need to be created among the pastoral community and actors involved in the value chain of camel production and marketing to minimize risks of economic loss associated with camels' infection/disease. Moreover, further molecular-based investigations and experimental trials with best animal models are needed to better clarify the encountered species/s and its/their zoonotic implication, if there is any. Further studies are also required to identify husbandry related risk factors by involving large number of camels slaughtered both in backyard and other abattoirs found in different parts of the country.

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Market Chain Analysis of Coffee in Sidama Zone, SNNPR, Ethiopia: The Case of Dale District

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Abstract

The study analyzed the market chain of coffee in Dale district, a major coffee producing district in southern Ethiopia. The main coffee marketing channels were investigated and the determinants of household coffee supply identified. Data were generated from 123 coffee producers and 36 coffee traders in the district using a formal survey. Nine coffee marketing channels were identified and channel I and channel II, representing 49.3% and 25.7% of the total produce, respectively, were the principal coffee marketing channels. The coffee marketing performance revealed that in channel I and channel II 36.9% and 34.13% of total gross marketing margin were added to coffee price, respectively. Out of the total gross marketing margin, 9.6% was captured by coffee assemblers, while 27.3% went to wholesalers in channel I, while out of the total gross marketing margin in channel II 34.13% goes to the wholesalers. The OLS model identified that sex of the household head, education level, coffee productivity, extension contact, price of coffee, and access to market information were the significant variables influencing coffee marketable supply positively. Whereas, the variables distance to the nearest market and non-farm income negatively affected the coffee marketable supply.

Key words: Marketable Supply, Marketing Channel, Marketing Margin, OLS model
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INTRODUCTION

Over one million farming households and about 25% of the total population of Ethiopia are dependent on the production and export of coffee. It also accounts for more than 25% of the GDP, about 40% of the total export earnings, absorbs around 25% of employment for both rural and urban dwellers and 10% of the total government revenue of the country (MoARD, 2008). Dale district is found in Sidama zone, Southern Nations, Nationalities and Peoples Regional State (SNNPR). In the district, most farmers (96%) grow coffee as the main source of income. In 2011 total of 59111.6 quintals of washed and 112499.56 quintals of unwashed coffee were produced and traded in Dale district (WoFED, 2013).

The Ethiopian current regulation requires all coffee to be inspected in central markets of Addis Ababa and Dire Dawa. Thus, coffee produced from different Zones of the country is required to be assembled and transported to the central markets in Addis Ababa and Dire Dawa. The Coffee Standard and Quality Inspection and Auction Centers (CSQIAC) of Ethiopia monitor the exportation of all coffee. In the supply chain, the National Coffee Board of Ethiopia (NCBE) is responsible for inspecting, organizing and coordinating the classification, grading and auction sale of the coffee supplied to central markets in Addis Ababa and Dire Dawa with its own operational rules, regulations, and modalities (ECX, 2009).

Coffee has been given greater attention by the country and it is the first cash crop with which the Ethiopia Commodity Exchange (ECX) started trading operations in April 2008. Agricultural markets in Ethiopia before 2008 had been characterized by high costs and high risks of transaction, with only one third of output reaching the market. Besides, small-scale farmers, who produce 95% of Ethiopia's output, came to market with little information and are at the mercy of merchants in the nearest market, and are unable to negotiate for better prices or reduce their market risks (ECX, 2009).

Ethiopian is the birth place of coffee with diversified landscape and suitable climate for growing large quantities of coffee. It is a country that produces high quality coffee with a distinct flavor including the highest valued coffee in the world called '*Mocha*' and the leading exporter of the famous *Arabica* coffee. However, the coffee sector is less developed and the export volume has not shown significant increase over the years. As a result, the country's export represents about three percent of the world exports and the coffee industry accounts for 2.5% of the country's gross domestic product (ECEA, 2012).

Transformation of the production system for domestic and export agricultural commodities requires the existence of efficient marketing system that can transfer the agricultural commodities from the point of production to the required market at the lowest possible cost.

The efforts to increases agricultural production and productivity should be accompanied by a well performing marketing system which satisfies consumer demands with the minimum margin between producers and consumer prices. Higher prices for producer encourage farmers to adopt new technologies and increase production (Amha, 1994).

Limitations to export coffee to distant but rewarding market emanate from low marketable output and high transaction costs. Dependable marketing system of coffee market is yet to develop in Ethiopia. Market infrastructures and marketing facilities are less developed. This in turn, reduces incentives to transactions (Hassano, 2012). Therefore, building the capacity of smallholders to actively engage in the market is one of the important tasks.

The coffee commodity chain faces its own complex set of problems, including various constraints on production, processing and marketing. For example, in specialty/gourmet segments of the international coffee market, Ethiopia occupies a unique place with an impressive selection of distinct coffee profiles. Many analysts have also proposed increasing the quantity of washed coffee as it sells at significant premiums over unwashed coffee (FDRE, 2003).

Market chain analysis is a modern approach to study problems of production and marketing. Analysis of the market of coffee based on market structure, conduct and performance considering the product and location specificity will, therefore, be useful to identify the bottlenecks and come up with possible solutions. Dale is one of the districts known for production of high quality coffee in Ethiopia; however, its coffee market is less studied. This study investigated the coffee marketing chains and factors affecting coffee supply in Dale district.

METHODOLOGY

Study Area

Dale district is one of the 19 districts in the Sidama Zone of SNNPR region and covers a total area of over 30,212 ha. The district capital, Yirgalem town is located at about 320 km south of Addis Ababa along the main highway to Moyale, located at $6^0$44" N and $38^0$28'' E longitude (WoFED, 2013).

The mean annual rainfall of the district ranges from 1041 mm to 1448 mm. Mean temperature ranges between 11^0c to 22^0c. The district is subdivided into 36 *Kebeles* (Smallest administrative unit)and all of them produce coffee (WoFED, 2013).

Average land holding of individual farm household is about 0.5 ha. Coffee, barley, wheat, *teff*, *enset* and vegetable crops are the common crops grown in the area. Coffee production is the main economic activity of the district with total area coverage of 15,367 hectare (WoFED, 2013).

Sampling and Data Collection

Dale district was purposely selected for its high production of coffee. Sample farm households were drawn from five purposively selected rural administrative *kebeles* of the district for their higher volume of coffee production (Table 1). The data were collected by using pre-tested semi-structured questionnaire. The survey questionnaires were administered through a simple random sampling based on proportional probability sampling technique. Yamane's sampling formula (1970) was used to determine the sample size.

$$n = \frac{N}{1 + N(e)^2}$$

Where: n= Sample size; N= Total number of coffee producer households; e = level of precision with 95% confidence interval.
Accordingly, 123 coffee producer households were selected.

Table 1. List of sampling Kebeles and sample size used for the study.

Name of Kebele	Coffee producer households	Sample households
Awada	3618	15
Gane	6633	27
Mesincho	6331	26
Moto	8441	34
Wenenata	5126	21
Total	30149	123

Yirgalem and Hawassa, areas with high volume flow of coffee produce, were the sites selected for the coffee trader's survey. There were a total of 36 coffee traders and all of them were covered in the traders' survey.

Data Analysis

Descriptive statistics

Descriptive statistics including means, percentages, ranges, ratios, standard deviations and variances were used to examine the socioeconomic and institutional characteristics of coffee producers and traders in the marketing channels, and the structure, conduct and performance of coffee markets in the study area.

Market concentration

The concentration ratio is expressed in terms of percentage of the market controlled by the biggest four firms. Four firms (CR4) concentration ratio is the most typical concentration ratio for judging the market structure (Kohls and Uhl, 1985). A CR4 over 50% is considered as a tight oligopoly, between 25% and 50% as a lose oligopoly and less than 25% no oligopoly. We used this method for coffee market concentration analysis. The problem associated with this index is the arbitrary selection of "r" (the number of firms that are taken to compare the ratios).

$$MS_i = \frac{V_i}{\sum V_i}$$

Where, MS_i = market share of buyers i.

V_i = amount of product handled by buyer i.

$\sum V_i$ = Total amount of product

$$C = \sum_{i=1}^{r} S_i$$

Where, C = concentration ratio handle

S_i = percentage share of i^{th} firm

r = number of largest firm for which the ratio is going to be calculated

The degree of coffee market concentration analysis was carried out for Yirgalem and Hawassa towns taking the annual volume of coffee purchased in 2013/14.

Marketing margin

According to Mendoza (1995) computing the total gross marketing margin (TGMM) is always related to the final price paid by the end buyer and is expressed as a percentage. In addition, the producers gross marketing margin (GMM) and net marketing margin (NMM) were calculated.

$$\text{TGMM} = \frac{\text{Consumer price} - \text{producer price}}{\text{Consumer price}} \times 100$$

$$\text{GMM}_p = \frac{\text{Price paid by consumer} - \text{Gross marketing maregin}}{\text{Price paid by consumers}} \times 100$$

$$\text{NMM} = \frac{\text{Gross marketing margin} - \text{Marketinn cost}}{\text{Price paid by consumer}} \times 100$$

Another parameter related to marketing margin is the producer's share. It is calculated as:

$$PS = \frac{\text{Producer price}}{\text{Consumer price}} \text{ or } 1 - \frac{\text{Marketing margin}}{\text{Consumer price}}$$

Econometric model

Since all the sampled coffee farmers of the study area supply coffee to the market, OLS model was fitted to the survey data to identify the determinants of coffee supply to the market. Following Green (2003) formula, the OLS model is specified as:

$$Y_i = \beta X_i + U_i$$

Where,

Y_i=a vector coffee supplied to the market by the sample farmers

β = a vector of estimated coefficient of the explanatory variables

X_i= a vector of explanatory variables (Access to credit, Access to market information, Sex of household head, Age of household head, Area allocated for coffee, Coffee productivity, Extension contact, Household education level, Household family size, Size land holding, Coffee price in 2013, Nearest market distance, Non–farm income).

Ui = disturbance term

RESULTS AND DISCUSSION

Sample Characteristics

Average age of sampled household was 39.5 ± 12.7 years and average family size was 5.5 ± 2.7 persons per household (Table 2). With respect to education level, average number of years of schooling was 8.3 ± 2.2 years. In the study area, demand for credit is influenced by availability of cash on hand. The sampled household accessed credit both from formal and informal sources. The average amount of credit taken by the farmers was 6 335.6 birr.

The government deployed at least two development agents (DA's) in each *kebeles* and built Farmers'

Training Centers (FTC) in attempt to fill the required knowledge gap and achieve food self-sufficiency in the country. In the study area the average number of extension contact by the respondent was 18.65 times per year.

Sampled households also reported that they have to walk on average for an hour to reach to the nearest market center (Table 2). In all the selected *kebeles* market is available for six days per week, except on Sundays. Farmers obtained information on price before they sold their coffee was obtained from various sources and 20.3% of coffee producing sampled households reported that they get the information from the nearby market, 14.6% from the central market and 17% from both.

Table 2. Distribution of sampled households.

Variables	Mean	Standard deviation	Minimum	Maximum
Distance from nearby market (walking hour)	1	0.48	0.5	2
Years of farm experience	26.7	11.32	7	35
Age	39.54	12.66	20	65
Family size	5.54	2.73	5	15
Education (schooling years)	8.29	2.20	0	12
Number of extension contact per year	18.65	18.5	52	2
Amount of credit taken (birr)	6,335.6	2,234.62	10,000	1,500

Coffee Marketing Channels

Coffee marketing channels is the sequence of intermediaries through which coffee passes from farmers to ultimate consumers (Mendoza, 1995). Generally, in the study area nine channels were identified.

I. Producers → Assemblers → Wholesalers → ECX → Exporters → Export
II. Producers → Wholesalers → ECX → Exporters → Export
II. Producers → Primary cooperatives → Union → ECX → Exporters → Export
IV. Producers → Assemblers → Retailers → Domestic consumers
V. Producers → Domestic consumers
VI. Producers → Wholesalers → ECX →Retailers (Rejected coffee) → Domestic consumer
VII. Producers → Assemblers → Wholesalers → ECX → Retailers (Rejected coffee) → Domestic consumer
VII. Producers → Primary cooperatives → Union → ECX → Retailers (Rejected coffee) → Domestic consumer
IX. Producers → Informal traders → Domestic consumers

Among these, channel I and channel II, which represented 49.3% and 25.7% of the total produce, respectively, were the principal coffee marketing channels (Fig. 1).

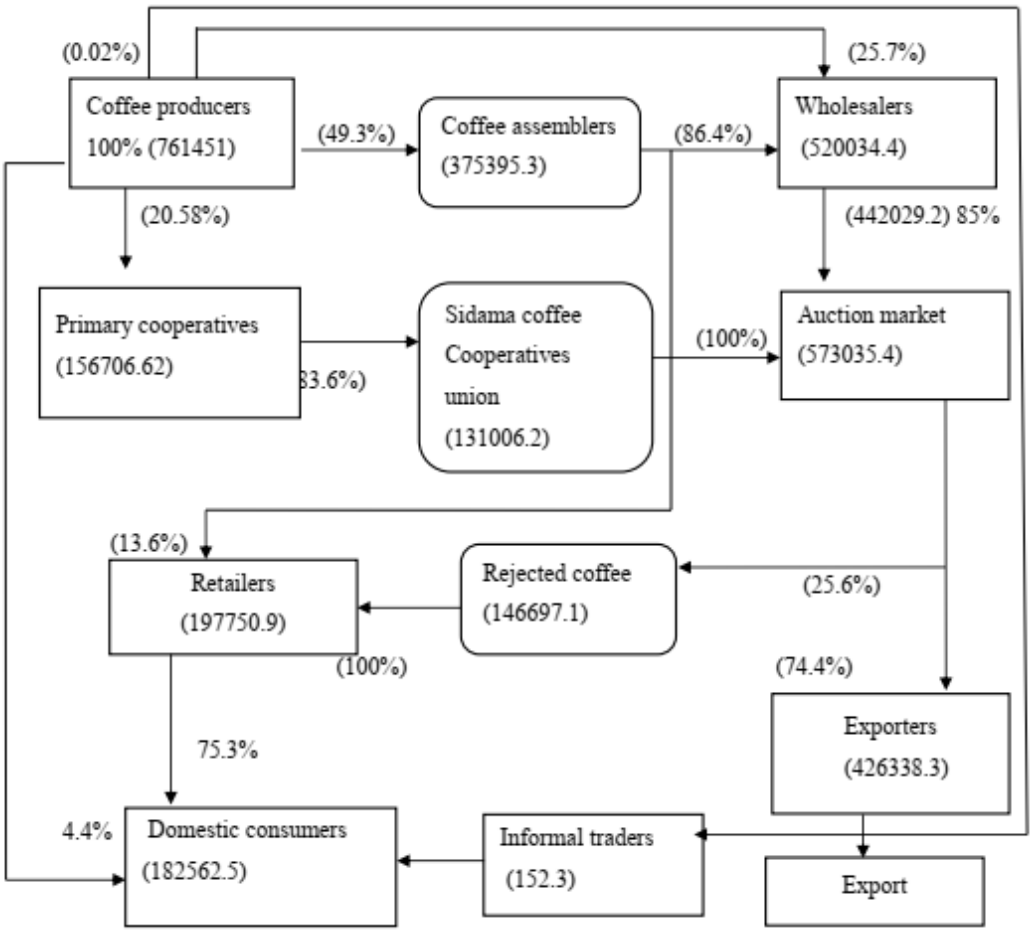

Figure 1.Coffee marketing channels of the Dale district, southern Ethiopia.
(Values in the parenthesis represent the volume of coffee product in kg.).

Degree of Market Concentration

Coffee markets at Yirgalem and Hawassa were strongly oligopolistic in the hands of few coffee traders (Table 3). CR4 measures concentration ratio showed that the top four or 19.05% of the traders controlled 65.3% of the coffee market in Yirgalem and 26.67% of the coffee traders controlled 69% of the coffee market in Hawassa.

The strongly oligopolistic market in both towns indicated that there is market imperfection because few traders seem to have monopolized the coffee market.

Table 3.Traders concentration.

Market centers	Concentration ratiofor the largest four firms (CR4) (%)	Traders (%)	Market structure
Hawassa	65.3	19.05	Tight oligopoly
Yirgalem	69.0	26.67	Tight oligopoly

Labor cost which includes (weeding, pruning, harvesting, cost of food item during group work, loading and unloading, etc.) was the principal cost of coffee growers and consisted about 57.5% of the total cost (Table 4). Cost of transport (farm to home, home to market or sometimes market to home when the price is very low) was 15.4% and the second major cost of producers followed by cost of land, materials and tax which amounted to 12.4%, 12.2% and 2.5%, respectively.

Table 4. Cost of actors in Channel I and Channel II (birr / feresulla*).

Cost items	**Producers**	**%**	**Assemblers**	**%**	**Wholesalers**	**%**	**Producers**	**Wholesalers**	**%**
Labor	58.4	57.5	6.46	23.4	5.26	11	58.4	5.26	9.7
Transportation	15.6	15.4	6.20	22.5	20.00	41.6	15.6	20.00	37.1
Land rent	12.6	12.4					12.6		
Packaging materials	12.4	12.2	6.20	22.5	3.72	7.8	12.4	3.72	6.9
Tax	2.5	2.5	2.5	9.1	2.41	5	2.5	2.41	4.5
Commission	-	-	-	-	1.43	3	-	1.43	2.6
Wage	-	-	-	-	2.64	5.5	-	2.64	4.9
Pulping and hulling charge	-	-	-	-	4.16	8.7	-	4.16	7.7
Depreciation	-	-	-	-	2.09	4.4	-	6.50	12
Other miscellaneous expenses	-	-	6.2	22.5	6.25	13	-	7.86	14.6
Coffee purchasing price	-		202.7		233.4		-	211.57	
Cost	101.5		27.56		47.96		101.5	53.98	
Selling price	202.7		233.4		321.2		211.57	321.2	
Profit	101.2		30.7		87.8		110.07	109.63	

*One *feresula* approximately equals to17 kg

There was a difference in gross marketing margin between coffee assemblers ((C – P)*17) (birr 521.9)) and coffee wholesalers ((W – C)*17) (birr 1492.6) in channel I (Table 5)). However, the gross margin of wholesalers was higher ((W – P)*17) (birr 1863.71)) in channel II due to the direct transaction with farmers. The Woreda Agriculture Office reported that a law was recently developed to encourage the direct transaction between coffee growers and wholesalers in order to improve quality of coffee and farmers benefit.

The total wholesalers gross margin in channel I (birr 1492.6 was lower than in channel II (birr 1863.71). The producers share from the auction market was 63.1% in channel I and 65.87% in channel II (Table 5). This result supports the theory that the share of producers decreases as the number of market agents increases.

Table 5: Summary of market share for channel I and channel II.

Marketing agent	Selling price (birr/feresulla)		Gross share from wholesale price (%)	
	Channel I	Channel II	Channel I	Channel II
Producers (P)	202.7	211.57	63.1	65.87
Assemblers (C)	233.4	-	9.6	-
Wholesalers (W)	321.2	321.2	27.3	34.13

The gross marketing margins of 36.9% and 34.13% were added to coffee price in channel I and channel II, respectively (Table 6). Out of these, 9.6% was gross margin of coffee assemblers, while 27.3% was that of wholesalers in channel I and in channel II. The gross margin of wholesalers was about 34.13%.

Table 6: Distribution of marketing margin.

Channel I	Value (%)
TGMM (Complete distribution channel)	36.9
GMM (Collector)	9.6
GMM (Wholesaler)	27.3
GMM_p (Producers share)	63.1
Channel II	
TGMM (Complete distribution channel)	34.13
GMM (Wholesaler)	34.13
GMM_p (Producers share)	65.87

Determinants of Household Coffee Market Supply

Among the thirteen hypothesized variables only eight variables namely sex of the household head, education level of household head, quantity of coffee produced, access to extension service, price of coffee in 2013, distance to the nearest market, non-farm income and access to market information were found to be the significant (Table 7).

Sex: Both men and women took part in the production and marketing of coffee. Sex of the household head had significantly positive influence on market supply of coffee ($P \leq 0.05$) (Table 7). The positive sign implies that if the household is male headed it leads to increase in coffee supply to the market by 0.049 kilogram. Tshiunza et al. (2000) who studied about determinants of cooking banana in Nigeria found that male headed household tends to produce more cooking banana for market than female headed and explained that males have relatively better labor advantage to produce and supply more volume.

Education level: This variable is positive in the model and statistically significant at ($P \leq 0.05$) (Table 7). One additional year of formal education level leads to an increase in marketable supply of coffee by 0.013 kilogram. The positive and significant relationship may indicate that formal education determines the readiness to accept new ideas and innovations, and easy to get supply, demand and price information which enhances farmers' willingness to produce more and increase volume of sales. Zekarias et al. (2012) studied market chain analysis of forest coffee in south western Ethiopia and found that education level has significant positive effect.

Coffee productivity: Households with high level of productivity supplied more to the market. Coffee productivity affects the volume of coffee supplied to the market positively and highly significantly ($p \leq 0.01$) (Table 7). The model shows that a one kilogram increase in coffee productivity per hectare resulted in 0.334 kilogram increase in the volume of market supply of coffee. A study by Zekarias et al. (2012) on market chain analysis of forest coffee in south western Ethiopia found that quantity of production has significant effect on volume of market supply.

Extension contact: The extension service positively and significantly ($p \leq 0.01$) affected the volume of coffee product supplied to the market (Table 7). Increase in frequency of extension agent contact by one increased the amount of coffee supplied to the market by 0.005 kilogram. This suggests that extension service avails information regarding technologies which improves production of coffee that positively affects the volume coffee supplied by the household to the market. Gecho (2005) and Musema (2006) found that access to extension service on improved maize, red pepper and improved haricot bean seed positively and significantly affected marketed supply of each of the commodities.

Price of coffee in 2013: The model revealed that the price of coffee had positive and significant ($P \leq 0.05$) effect on the volume of coffee supplied to the market (Table 7). The positive and significant relationship indicates that the rise by one birr in the preceding year price of coffee supplied to the market increases the supply by 0.169 kilogram in the following year.

Distance to the nearest market: It was argued that distant markets increase producers marketing cost which in turn reduces the volume of supply to the market. The model output from the current study indicates that the variable affected supply volume of marketed coffee negatively (Table 7). An increase in one hour walking time to the nearest coffee market led to a decrease in the quantity coffee supplied by 0.123 kilogram. The variable was also statistically significant ($P \leq 0.05$). Earlier study by Hassano (2012) also revealed that market distance affect marketed supply of coffee negatively in Nensebo district of Oromia, Ethiopia.

Table 7. OLS results of determinants of coffee market supply.

Variables	Coefficient	Standard error	t-ratio	p-value
Constant	2.767***	0.246	11.24	0.000
Sex	0.049**	0.024	2.07	0.041
Age	0.001	0.002	0.59	0.554
Education	0.013**	0.006	2.01	0.047
Coffee productivity	0.334***	0.036	9.13	0.000
Extension	0.005***	0.002	3.32	0.001
Lagged price	0.169***	0.035	4.78	0.000
Credit	0.018	0.034	0.51	0.609
Market distance	-0.123**	0.058	-2.29	0.024
Family size	-0.007	0.005	-1.40	0.164
Farm size	0.012	0.044	0.30	0.768
Coffee land	0.056	0.057	0.97	0.333
Non-farm income	-0.001***	0.000	-2.63	0.010
Market information	0.084**	0.033	2.52	0.013

Dependent variable=quantity supplied, N=123, R^2=0.9733, Adjusted R^2= 0.9701***, ** and * shows the values statistically significant at 1%, 5% and 10% respectively.

Non–farm income: Increase in non-farm income as compared with farm income sources will tend to minimize agricultural activities and shift the focuses on those non-farm activities which benefit the farmers more. This leads to decrease in their volume of farm output or coffee produced and supplied to the market. The model indicated that this variable affected supply of coffee negatively and significantly ($p \leq 0.01$) (Table 7). One birr increase in non-farm income resulted in decrease of coffee product volume supplied to the market by 0.001 kilogram.

Market information: The variable's coefficient is positive and statistically significant ($p \leq 0.05$) (Table 7). The coefficient also indicated that access to market information increased the marketable supply of coffee by 0.084 kilogram. Earlier study by Hassano (2012) also indicated that access to market information affected market supply of coffee positively in Nensebo district of Oromia, Ethiopia.

CONCLUSION AND IMPLICATIONS

The study identified nine major marketing channels. The marketing costs and margin analyses showed that coffee producers incurred the highest cost followed by wholesalers. The coffee assemblers bear the lowest cost (birr 468.52). About 36.9 % of the total gross marketing margin was added to coffee price in channel I. Out of the total gross marketing margin about 9.6% was accounted for gross margin of coffee assemblers and 27.3% for the wholesalers. Hence, the study pointed out that all marketing participants of the commodity operated at a margin and all the marketing agents profited through the channel.

The coffee wholesalers obtained significant annual total net benefit than producers and coffee assemblers. The estimated annual net benefits of a typical coffee producer, coffee assemblers and coffee wholesalers in Dale district were birr 4887.96, birr 2016.54 and birr 255354.21, respectively. This implies that coffee marketing is relatively highly determined by wholesalers.

Eight variables namely sex of the household head, education level of household head, quantity of coffee production, price of coffee in previous year, distance to the nearest market, non-farm income, access to extension service, and access to market information significantly affected the market supply of coffee at household level.

Since the coffee market in the study area is oligopolized, government should attract other traders to enter into coffee trade by improving the existing credit system and giving different incentives in order to make the market more competitive.

Existence of informal traders in the coffee market discourages the legal traders to expand their business or enter into the market (for new traders). Hence the government should take action to protect the legal traders from unfair competition with informal traders by putting mechanisms which prevent informal traders not to participate in the market and eventually convince them to join the formal and legal market. Besides, due attention should be given to improve communication networks in different coffee production sites and marketing centers of the study area.

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Morphological Characterization and Genetic Variability among Bambara Groundnut (*Vigna subterranean* L.Verdc.) Accessions of Ghana

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Abstract

Bambara groundnut (*Vigna subterranean* L. Verdc.) is an indigenous African crop cultivated for the subterranean pod that is rich in protein. Yield of Bambara groundnut in Ghana is variable and unpredictable. The current study was undertaken to morphologically characterize and estimate the extent of genetic variability, heritability and genetic advance among 25 Bambara groundnut accessions collected from major growing areas in Ghana. The experiment was laid in a randomized complete block design with three replications. The accessions differed significantly ($p<0.01$) in number of days to emergence, 100 seeds weight, biological yield, harvest index, number of pods with two seeds per plant, number of pods per plant, economic yield and yield per plot. The genotypic coefficient of variation (GCV) values were near to phenotypic coefficient of variation (PCV) values for economic yield, harvest index, shelling percentage, number of pods with two seeds per plant, number of pods per plant, economic yield, percentage germination, number of days to emerge, and yield per plot, that indicates high contribution of genotypic effect for phenotypic expression of these characters. High heritability coupled with high genetic advance as percentage of the mean was obtained for economic yield, harvest index, number of pods with two seeds per plant, number of pods per plant, percentage germination, number of days to emergence, and yield per plot; reflecting the presence of additive gene action for the expression of these traits. The presence of genetic variability among accessions, heritability of the traits and the additive gene action for their expression shows the possibility to improve Bambara groundnut through selection.

Key words: Genetic Advance, Heritability, Selection, Variability

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INTRODUCTION

Bambara groundnut (*Vigna subterranean* L. Verdc.) is an indigenous African crop, cultivated primarily for the subterranean pods, rich in protein which helps to alleviate nutritional disorder in human and livestock (Massawe et al., 2002). Bambara groundnut has a number of production advantages in that it can yield on poor soils with little rainfall as well as produce substantial yields under better agronomic conditions (Anchirnah et al., 2001). It contributes soil nitrogen for other crops by fixing atmospheric nitrogen through symbiosis with *Rhizobium* bacteria and therefore it is beneficial in crop rotations and intercropping (Karikari et al., 1999).

In Ghana, the crop is mostly grown in the coastal savannah, transition and guinea savannah agro – ecologies, where rainfall is low as compared to the high rainfall areas of the country (Berchie et al., 2010). West Africa as a whole produces 45% – 50% of the annual world production of Bambara groundnut estimated at 330,000 tons although most countries including Ghana do not collect accurate statistics on internal production, and marketing of the crop (Obeng-Aseidu et al., 2000). Bambara groundnut has been characterized by variable and unpredictable yields for reasons that have not been identified due to limited research carried out on the crop (Massawe et al., 2002). Although there has been an increase in the number of scientific reports on Bambara groundnut in Ghana (Adu-Dapaah and Sangwan, 2004; Berchie et al., 2010; and Obeng-Aseidu et al., 2000), few studies have been made on the genetic diversity of the accessions in Ghana.

Knowledge of the genetic variability available among accessions of Bambara groundnut is needed as a guide for breeders and other scientists working on the improvement of this underexploited legume crop. Therefore, the present study was conducted to morphologically characterize and assess genetic variability, heritability, and genetic advance of Bambara groundnut accessions collected from major growing regions of Ghana.

METHODOLOGY

Description of the Study Area and Accessions

The study was conducted on the multipurpose nursery of the College of Agriculture, University of Education (Ashanti Mampong campus) in Ghana. Ashanti Mampong is located at latitude 07° and 04° north and longitude of 01° and 2°41' west at an elevation of 1457.7 m above sea level. Twenty-five Bambara groundnut accessions (Table 1) were collected from the major growing areas in Ghana, that is the Northern, Upper East, Upper West, Ashanti and Brong Ahafo regions. The accessions were collected from farmers and traders at the major markets in the regions. The accessions were named using the first letter of the town, where they were collected and a number to differentiate them if there were more than one accession collected from that town.

Table 1: Collection area and description of 25 Bambara groundnut accessions, collected from the major growing areas in Ghana

S/N	Name	Source	Region	Description
1	B1	Bawku	Upper East	Dark brown with red mottling and white eyes
2	B2	Bawku	Upper East	Plain red with white eyes
3	B3	Bawku	Upper East	Cream with dark black stripes and white eyes surrounded with black colour
4	B4	Bawku	Upper East	Cream with white eyes surrounded by red colour
5	B5	Bawku	Upper East	Cream with white eyes surrounded by pale blue colour.
6	B6	Bawku	Upper East	Plain brown with white eyes
7	N1	Navorongo	Upper East	Cream with white eyes surrounded by pale blue colour.
8	E1	Ejura	Ashanti	Brown with white eyes surrounded by black eyes
9	E2	Ejura	Ashanti	Brown with black mottling and white eyes
10	E3	Ejura	Ashanti	Cream with black mottling and white eyes with thin black colour surrounding it
11	E4	Ejura	Ashanti	Coffee with white eyes
12	A1	Attebubu	BrongAhafo	Cream with red mottling and white eyes surrounded by thin red colour
13	T1	Tatale	Northern	Cream with red mottling and white eyes with thin red colour.
14	A3	Attebubu	BrongAhafo	Cream with white eyes surrounded by black colour.
15	A2	Attebubu	BrongAhafo	Cream with red mottling and white eyes surrounded by pale blue colour.
16	N2	Navorongo	Upper East	Brown with red mottling and white eyes surrounded by dark red colour.
17	N3	Navorongo	Upper East	Plain coffee with white eyes
18	W1	Wa	Upper West	Dark red with white eyes
19	N5	Navorongo	Upper East	Cream with black eyes
20	N4	Navorongo	Upper East	Light coffee with white eyes surrounded by black colour
21	T2	Tatale	Northern	Cream with white eyes surrounded by black colour
22	T3	Tatale	Northern	Plain brown with white eyes
23	T4	Tatale	Northern	Light coffee with white eyes surrounded by black colour
24	T5	Tatale	Northern	Completely cream with white eyes
25	W2	Wa	Upper West	Completely black with white eyes

Experimental Design and Field Management

The research was conducted from September to December 2014 using randomized complete block design with three replications. A planting distance of 50 cm between rows and 25 cm within rows, with one seed per hill and 30 plants per plot was used. The total plot size was $3.75m^2$ (2.5m x1.5m), with five rows in a plot and six plants per row. The planting area was ploughed and harrowed. Hand weeding was carried on as and when the weeds appear. All the plots were watered with equal amount of water, whenever there was no rain for four consecutive days starting from the first day of emergence. The crops were harvested, when the leaves begun to turn yellow which is a sign of pod maturity.

Data Collection and Analysis

Data on traits, viz. percentage germination, number of days to emergence, days to 50% flowering, number of stems per plant, hundred seeds weight (g), biological yield (g), harvest index, shelling percentage, number of pods with two seeds per plant, number of pods per plant, economic yield and yield/ plot (kg) were recorded based on the International Plant Genetic Resources Institute Descriptors for Bambara groundnut (IPGRI 2000).

The values were determined as follows: Days to Emergence as the number of days from planting to the day when 50% of the seedlings emerged on a plot; Percentage Emergence as the number of seeds that germinated on a plot from the time of sowing to two weeks after sowing were counted and expressed as a percentage of the total number of seeds that were sown; Days To 50% Flowering as the number of days from emergence to the day when 50% of the plants flowered on a plot; number of stems per plant as the average number of stems per plant from three randomly picked plants at the time of harvesting; number of pods with two seeds per plant as the number of pods with two seeds from each of the three selected plants and an average number estimated, 100 seed weight (g) as the weight of 100 seeds selected at random from each plot and weighed with an electronic scale; shelling percentage calculated as; $100 \times \frac{\text{weight of seeds (g)}}{\text{weight of pods (g)}}$ for the pods of three selected plants for each plot; yield per plot (g) as the weight of all shelled seeds from a plot by using an electronic scale; Economic yield (g) after harvest, shelled seeds from three selected plants from each plot were oven dried at 80^0C to constant mass and weighed with an electronic scale; biological yield (g) after harvest, three selected fresh plants from each plot including the pods were oven dried at 80^0C to constant mass and weighed with an electronic scale; harvest index (%) was calculated as the ratio of the economic yield to the biological yield expressed as a percentage.

The data collected for each of these characters were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) software version 9.2 (SAS, 2008). Means were separated with Duncan multiple range testing (DMRT) at 5% probability levels.

Estimation of Variance Components

The variability present between accessions was estimated from grand mean to each character, phenotypic and genotypic variance and coefficient of variation. The genotypic and phenotypic variances were determined from mean square values of the ANOVA for each trait according to Prasad et al. (1981), as follows:

$\sigma^2e = MSe$,

$\sigma^2g = \frac{MSg - MSe}{r}$

$\sigma^2p = \sigma^2g + MSe/r$

Where σ^2e is environmental variance, σ^2g is genotypic variance, σ^2p is phenotypic variance, MSe is mean square of error, MSg is mean square of genotype, and r is number of replications. Genotypic Coefficient of Variability, Phenotypic Coefficient of Variability, Broad sense heritability and genetic advance, and genetic advance as the percent of the mean were computed using the variance components (Burton 1952; Johnson et al. 1955; and Kumar et al. 1985). The formulae are as follows:

$PCV = \frac{\sqrt{\sigma 2p}}{\bar{x}} * 100$

$GCV = \frac{\sqrt{\sigma 2g}}{\bar{x}} * 100$

$H = \sigma^2g/\sigma^2p * 100$

$GA = KH * \sigma p$

$GAM\ (\%) = GA/\bar{x} * 100$

Where PCV is phenotypic coefficient of variation, σ^2p is phenotypic variance, GCV is genotypic coefficient of variation, σ^2g is genotypic variance, H is Heritability in the broad sense, and $\bar{x}$ is grand mean of the character under study, GA is genetic advance, K is the selection differential (K = 2.06 at 5% selection intensity), σP is the phenotypic standard deviation of the character, and GAM (%) is the genetic advance as percentage of the mean.

RESULTS AND DISCUSSION

Analysis of Variance

The genotype mean squares for traits viz. germination percentage, number of days to emerge, hundred seeds weight, biological yield, harvest index, number of pods with two seeds per plant, number of pods per plant, economic yield, and yield per plot were highly significant (Table 2).

Table 2. Analysis of variance for different quantitative characters in Bambara groundnut

Traits	Genotype mean square (df 24)
Germination percentage	901.1 **
Number of days to emerge	0.72 **
Number of days to 50 % flowering	14.24 ns
Number of stems per plant	2.69 ns
Hundred seeds weight	190.96 **
Biological yield	205.06 **
Harvest index	188.04 **
Shelling percentage	6.71 ns
Number of pods with two seeds per plant	7.59 **
Number of pods per plant	83.51 **
Economic yield per plot	29.42 **
Yield per plot	1353.7 **

** Significant at p<0.01.

The observed high significant difference in the traits between the genotypes reflected the existence of large variability among tested genotypes and this variability can be further utilized in the Bambara groundnut improvement program. An efficient genetic improvement of a crop cultivar depends on the availability and knowledge of genetic variability in the agro-morphological characteristics. Where there is no more usable variability, in most crop plants as the result of continuous selection, use of induced variation has become an option (Vuledzani *et al*., 2015). However, in crop plants like Bambara groundnut, where no much selection has been made and variation has not been depleted, use of available variation in the accessions could be good resource for plant breeders to improve the crop.

Range and Mean Values of Agro-morphological Characters

Wide range of variability was observed between genotypes for the traits studied (Table 3). Accessions N4 and B6 had the highest germination (99%), followed by accession B3 (97%), while accession T5 had the lowest germination (20%). Accessions with low germination percentage had delayed seedling emergence. Days to emergence ranged from 5.3 to 12.3 among the tested accessions. Similar results with significant differences in germination and emergence were reported by Berchie *et al*. (2010), in which case they demonstrated the importance of priming Bambara groundnut seeds to break the seed coat to achieve uniform emergence. This shows that the variation in germination and subsequent emergence in Bambara groundnut is related to the inherent variation in seed coat hardness between accessions. Where the difference in seedling emergence between accessions is significant, either use of seed priming or using accessions known to have less hardness in seed coat might result in early emergence to attain uniform emergence and subsequent field establishment.

Genotype B3 attained maximum number of pods with two seeds (5.0), and yield per plot (104 g). Genotype B1 had maximum value in number of pods per plant (25.6), harvest index (47.5%) and economic yield (15 g). The highest values in 100 seed weight (63.12 g) were observed in genotype T5. The significant variations among the accessions for the majority of the morphological traits of economic importance is a sign of the presence of high degree of usable genetic variation giving room for selection of superior ones.

Table 3. Estimates of range and mean for Agro-morphological traits for accessions of Bambara groundnut in Ghana

Traits	**Unit**	**Mean**	**Range**
Germination	percent	80.99	20.00 -99.00
Days to emerge Days	days	8.10	5.33-12.33
Days to 50 % flowering	days	34.20	30.00-37.33
Stems per plant	number	8.50	7.00-10.30
Hundred seeds weight	gram	47.08	36.10-68.9
Biological yield	gram	8.58	4.33-15.33
Harvest index	percent	23.52	15.09-47.39
Shelling percentage	percent	68.08	50.80-84.3
Pods with 2 seeds per plant	number	2.17	00.00-5.00
Number of pods per plant	number	16.70	7.00-27.00
Economic yield per plot	gram	8.58	4.33-15.33
Yield per plot	gram	55.99	16.76-104.00

Estimation of Variance Components

Variables recorded viz. genotypic variance (σ^2g) ranged between 0.06 and 721.0; phenotypic variance (σ^2ph) 0.28-73.22; environmental variance (σ^2e) 0.89-451.2; phenotypic coefficients of variation (PCV) 11.16-78.62; and genotypic coefficients of variation (GCV) 0.89-66.18 (Table 4). The PCV values were higher than the GCV values for all the parameters indicating environmental influence on the expression of the traits (Jonah *et al*., 2013). However, GCV values were near to PCV values for the characters like economic yield, harvest index, shelling percentage, number of pods with two seeds per plant, number of pods per plant, percentage germination, and number of days to emerge, indicating high contribution of genotypic effect for phenotypic expression of these characters.

According to Rosmaina *et al*. (2016), PCV and GCV values greater than 20% are regarded as high and values between 10% and 20% to be medium, whereas values less than 10% are considered to be low. Accordingly, high PCV and GCV were recorded for economic yield, harvest index, shelling percentage, number of pods with two seeds per plant, number of pods per plant, percentage germination, number of days to emerge and yield per plot, while traits with moderate PCV and GCV were hundred seeds weight, and biological yield. High values of PCV and GCV indicated the existence of substantial variability for such characters and selection may be effective based on these characters.

Table 4. Estimation of component of variances: genotypic (σ^2g), environmental (σ^2e), and phenotypic (σ^2p); phenotypic (PCV) and genotypic (GCV) coefficient of variation, broad sense heritability (H) and genetic advance as percentage of mean (GAM%) in the agro-morphologic traits of 25 accessions of Bambara groundnut in Ghana

Traits	σ^2g	σ^2e	σ^2p	GCV (%)	PCV (%)	H (%)	GAM (%)
Hundred seed weight (g)	46.73	63.72	16.93	14.52	16.92	73.43	25.60
Economic yield (g)	162.20	235.43	73.22	48.34	78.62	68.94	95.65
Biological yield (g)	48.75	68.44	19.66	18.69	22.23	71.30	32.46
Harvest index	42.23	62.77	20.53	27.65	33.72	67.34	46.62
Shelling percentage	721.0	2237	22.36	34.37	60.45	0.032	0.41
Number of pods with two seeds/ plant	2.05	2.56	0.48	66.18	73.34	81.20	90.5
Number of pods / plant	19.2	27.8	8.7	26.33	31.65	68.94	44.98
Percentage germination	265.7	300.4	34.76	20.12	21.39	88.46	38.93
Number of days to emergence	2.93	3.24	0.28	21.32	22.31	91.30	41.96
Number of days to 50 percent flowering	0.18	4.92	4.75	6.47	22.23	3.58	0.478
Number of stems /plant	0.06	0.89	0.89	0.89	11.16	0.63	0.15
Yield per plot	329.9	451.2	121.3	33.25	38.64	73.22	58.25

Heritability and genetic advance

Heritability values ranged between 0.03% for shelling percentage and 91.30% for days to emergence. The genetic advance as a percentage of the mean also ranged from 0.15 for number of stems per plant to 95.7% for economic yield (Table 4). The response of a character to selection is more reliable, when heritability estimates and genetic advance are combined (Ibrahim and Hussein, 2006). Traits such as economic yield, harvest index, pods with two seeds, number of pods per plant, days to emergence and yield per plot recorded higher heritability values of 68.9%, 67.3%, 81.2%, 68.9%, 91.3%, and 73.2%, respectively, which is accompanied by high genetic advance as percentage of the mean of 95.65, 46.62, 90.5, 44.98, 41.96, and 58.25, respectively. This can be considered as a favourable attribute and an indication of additive gene effect (Percy and Turcotte, 1991). Where additive genes are less affected by environment, phenotypic selection for these traits will be effective (Rao and Patil, 1996).

High heritability values accompanied by low genetic advance were observed for hundred seed weight

(73.43% and 25.60%), biological yield (71.30% and 32.46%), and percentage germination (88.46% and 38.93%). In contrast, low heritability values accompanied by low genetic advance were observed for shelling percentage (0.032% and 0.41%), days to 50% flowering (3.58% and 0.478%) and number of stem /plant (6.63% and 0.15%). This indicates that in the latter case the traits are being exhibited due to favourable environmental influence rather than genotypic. Selection for such traits may not be rewarding due to the presence of high levels of dominance gene effect hence limited scope of selection (Rao and Patil, 1996).

CONCLUSION

From the results of this study it can be concluded that Bambara groundnut accessions significantly differed in important agro-morphological traits viz. percent germination, number of days to emergence, 100 seeds weight, number of pods with two seeds per plant, number of pods per plant, harvest index and economic yield. Most of these traits viz. days to emergence, number of pods per plant, number of pods with two seeds per plant, harvest index, economic yield, and yield per plot exhibited high heritability and high genetic gain as percentage of the mean reflecting the presence of additive gene action for the expression of these traits, hence improving these characters could be achieved through selection.

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An Abattoir Based Study of Bovine Tuberculosis in Adama and Bishoftu Abattoirs, Central Ethiopia

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Abstract

A cross-sectional study was conducted from November 2014 to August 2016 at Adama Municipal Abattoir and Bishoftu ELFORA Export Abattoir, central Ethiopia. The study aimed at estimating the prevalence and distribution of lesions of bovine tuberculosis (BTB) in organs on the basis of TB like gross lesion. Postmortem examinations were conducted on1896 cattle slaughtered at Adama (n=1266) and Bishoftu (n=630) abattoirs. The body condition scores, origins and ages of the animals were recorded during ante mortem examinations. The prevalence of BTB lesions was 4.2% (80/1896). Out of the 80 TB like lesions cultured, 26.3% of them were found to be culture positive *Mycobacterium* species. Higher proportion (52.5%) of TB like lesion was recorded in the respiratory pathway followed by lymph nodes of the head region (26.25%), mesenteric (7.5%), prescapular (7.5%) and hepatic lymph nodes (6.25%). Prevalence was higher in animals slaughtered at Bishoftu ELFORA export abattoir compared to Adama municipal abattoir. Animals coming to both abattoirs were from different origins and varied with their body condition score. ELFORA export abattoir slaughtered large proportion of lean animals not for export purposes but for local supermarkets while Adama municipal abattoir slaughtered fattened animals, which might account for difference in BTB prevalence. In conclusion, the lesion prevalence was low in cattle slaughtered at both abattoirs. Given the zoonotic importance BTB, this finding suggests the need for monitoring the prevalence and launching a feasible and practical control strategy of BTB.

Key words: Abattoir; Bovine Tuberculosis; Ethiopia, Prevalence; TB lesions.

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INTRODUCTION

Bovine Tuberculosis (BTB) is a chronic bacterial disease characterized by progressive development of tubercles in any tissue/organ of the body (Hlokwe et al., 2013; Pal et al., 2014). It has been reported from 176 countries as one of the important bovine diseases causing great economic loss (Awah-Ndukum et al., 2013). TB remains a major global health problem and causes ill health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide after the human immunodeficiency virus (HIV). TB can be difficult to diagnose based only on the clinical signs. Regular surveillance by skin test, bacteriology and molecular methods is not feasible due to lack of resources. Thus, conventional abattoir inspections continue to play a key role for national surveillance of BTB.

Ethiopia's increasing human population, coupled with expanding urbanization and higher average income is putting an increasing pressure on the meat supply. To meet this demand, millions of food animals slaughtered every year throughout the country. In 2007, for example, a total of 18.8 million cattle, sheep, goats and camels slaughtered at municipal abattoirs, primarily for domestic consumption (FAO, 2009). For this reason, close monitoring of meat hygiene, including proper implementation of meat inspection procedures during slaughter, should be a vital part of the national public health protection program.

BTB is characterized by the formation of nodules called tubercles whose location depends largely on the route of infection. In calves, BTB usually transmitted by ingestion and lesions involve the mesenteric lymph nodes with possible spread to other organs. In older cattle, infection usually transmitted by the respiratory tract with lesions in the lung and dependent lymph nodes (Carter and Wise, 2004). Recently, there has been increasing reports of human cases due to *M. bovis* especially in patients with HIV (Russell, 2003). Thus, a greater degree of transmission of infection with bacteria between

human and domestic animals could occur (Taracha et al., 2003).

In industrialized countries, animal TB is controlled and eliminated with milk test and slaughter, which has in turn drastically reduced the incidence of the disease caused by *M. bovis* in both cattle and human. In developing countries however, animals' TB is widely distributed, as control measures are not applied or applied sub optimally. In Ethiopia, where extensive production system predominates animals are kept in the same house with their owners; cow dung is used for painting of the wall and floor of houses as well as sources of energy for cooking. All these practices do exacerbate the chance of spread of TB to human (Asseged, 1999). The nationwide distribution of the disease and associated economic loss has not been fully determined due to lack of good diagnostic facilities (Asseged et al., 2004).

The primary reason for post mortem examination of carcasses in slaughterhouses is for the protection of public health and containment of disease spread among livestock. The knowledge of TB in cattle slaughtered provides useful information and is a proxy indicator for the epidemiology of the disease in the cattle population from which the slaughtered cattle are originated. Furthermore, it could serve as a good indicator of risk to humans through consumption of infected meat. Apart from providing data for regulatory programs, carcass examination also provides clues as to whether the infection is in its early stage or has reached the transmissible stage. This provides better programmatic awareness with subsequent development of targeted guidance on how to reduce the risk of TB spread within the specific geographic area, as well as opportunities to trace back the source of infection to the herds. Hence, having the knowledge of distribution, prevalence and risk factors of the disease are fundamental to look for effective control strategy. Therefore, the objectives of this study were to estimate the prevalence of bovine tuberculosis at Adama municipal abattoir and Bishoftu ELFORA export abattoir, central Ethiopia and to assess the distribution of tuberculous lesions in organs of slaughtered animals.

MATERIALS AND METHODS

Study Area

A cross sectional study was conducted from November 2014 up to August 2016 in Adama and Bishoftu towns of East Shewa Zone of Oromia Regional State, Central Ethiopia (Fig. 1). East Shewa, human population is 1,919,994 and cattle 1,031,652 (CSA, 2013). Adama and Bishoftu towns are the major towns in East Shewa zone. Adama town is located at 8.54^0N 39.27^0E, 99 km southeast of Addis Ababa along the road that connects Addis Ababa to Dire Dawa - Djibouti. Adama is among the largest metropolitan city in Ethiopia with estimated 450,000 human population and many resorts that make it suitable for conference and tourism. Adama abattoir slaughtering has the capacity of about 150 cattle and 500 sheep and goats per day. However, to fulfill the standard abattoir level it lacks many facilities including drainage system, sterilizers of the equipment, adequate light, workers' clothes. The inspection system is not detailed and only performs routine inspections.

Bishoftu is one of the major towns in east Shewa zone Oromia regional state with human population of 104 215 (World City Population, 2020). This town is located about 45 km southeast of Addis Ababa. Bishoftu is an important town where most governmental institutions including national air force, national and international research centers and others Universities and colleges are located. It has three export abattoirs and one municipal abattoir. In addition, it has many private intensive and extensive dairy, poultry and swine farms. This highly populated town is main supplier of animals, poultry and swine meat and its products such as milk, egg and others to Addis Ababa supermarkets. Bishoftu export abattoir has slaughtering capacity of 200 cattle 1500 sheep and goats. Cattle slaughters in Bishoftu abattoir were mostly emaciated cattle (mainly for local consumption) compared to Adama municipal abattoir that slaughter fattens animals. It is export abattoir and entails all conditions that ideal export abattoir requires. Bishoftu has a relative humidity varying between 70% and 80% during the rainy season and 40% to 50% during the dry season.

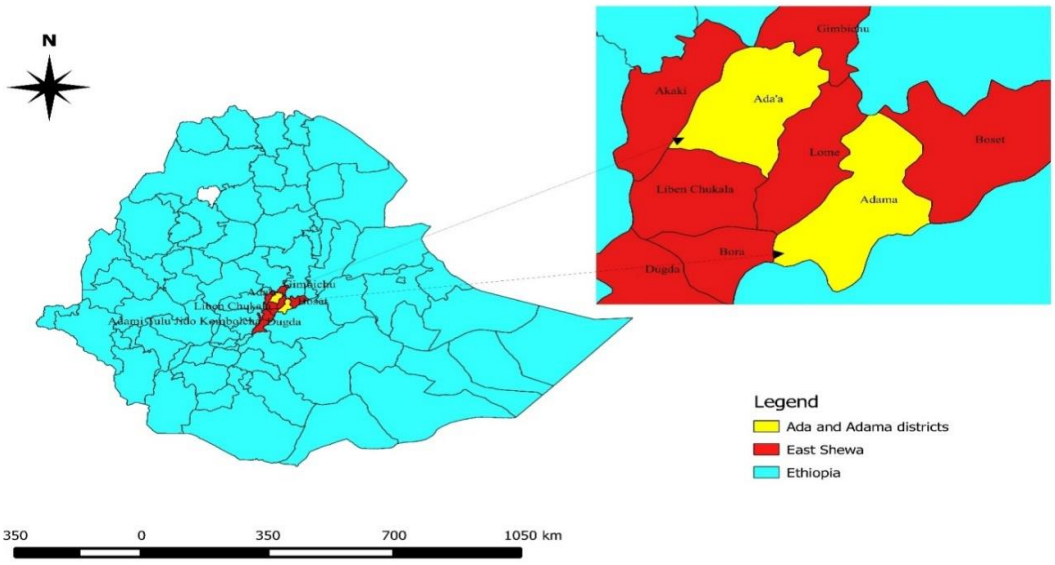

Figure 1: Map of Ethiopia showing Location of study area

Study Subjects

The study subjects comprised of abattoir slaughtered cattle at Adama and Bishoftu abattoirs East Shewa Zone, Central Ethiopia. Animals presented to these Abattoirs were from the surrounding Adama and other different sites. These animals were transported to abattoir by vehicles.

Sampling and sample size determination method

The sample size was calculated according to Thrusfield (2005) by taking 6.79% of expected prevalence reported by Terefe (2014), and 5% accepted error at 95% confidence interval. The general formula is:

$$\text{Required sample Size} = \frac{Z_{1-\propto/2}{}^2 \text{p}(1-\text{p})}{\text{d}^2}$$

Where

$Z_{1-\alpha/2}$ is standard normal variant (1.96 for 5% accepted error).
P = expected prevalence
d = desired absolute precision

Accordingly, the estimated sample size was 80 cattle for each month which is 960 cattle each year for two years. A total of 1,896 cattle were sampled during the study period (from November 2014 up to August 2016).

Post-mortem examination

Cattle and cattle related variables such as age, breed, sex and body condition were recorded during ante-mortem inspection. Postmortem examination was carried out as described previously by Vordermeier et al. (2002). Each of the seven lobes of the lungs were thoroughly inspected and palpated for suspicious gross TB-like lesions. Similarly, mandible, retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, and mesenteric lymph nodes were sliced into 2mm size sections and then be inspected for the presence of visible lesions according to the protocol described earlier by Vordermeier et al. (2002).

Body condition scoring (BCS) and age determination

The body condition scores, origins and ages of the animals were recorded before slaughtering during ante mortem examinations. Body condition scoring was made using a method developed for Zebu cattle (Nicholson and Butterworth, 1986), accordingly, based on observation of anatomical parts such as vertebral column, ribs, and spines. The study animals were classified as lean (1), medium (2 and 3) or good (4 and greater). Animals coming to the abattoir were apparently healthy and leanness considered as thinness. Age of the study animals was determined by using the dental eruption and wear as described by De-Lahunta and Habel (1986) and for the present

study, animals were categorized as young age ≤2 and adult age > 2.

Collection of suspected tuberculous lesions in slaughtered cattle

The tissues showing macroscopic lesions suggestive of BTB were collected from slaughtered cattle carcasses during the postmortem inspection at Adama Municipal and Bishoftu ELFORA Export abattoirs. From 1896 inspected carcasses, from which BTB suspected lesions were collected in sterile plastic bags and transported in cold chain to Akililu Lemma Institute of Pathobiology (ALIPB) for culturing. At the TB lab of the Institute, the samples were kept at –20°C until processed for culturing.

Mycobacterium culture of suspected tuberculous lesions

The suspected TB lesions were incubated using pyruvate and glycerol enriched LJ slants following standard operation procedures (RNTCP, 2009). All the bovine specimens were processed in biosafety level 2 cabinet. Ziehl-Neelsen staining and microscopic demonstrations of acid-fast bacilli were used to confirm successful inoculation and growth (WHO, 1998).

Briefly, suspected tuberculous cattle specimens were cut into tiny pieces and then homogenized separately in 0.85% saline in sterile blenders to obtain fine pieces. Frozen samples were allowed to thaw to room temperature before processing. The cattle tissue homogenates were decontaminated with equal volumes of sterile 4% NaOH; mixed well by shaking for a few seconds and allowed to stand for 10 minutes at room temperature before neutralization with 1 mol/L HCl using phenol red as the indicator. Neutralization was achieved when the suspension changed to a yellowish colour; which was centrifuged at 3,000 rpm for 15 min. The supernatant was discarded leaving about 2ml and spread generously (~ 0.3 ml) on the LJ slants as follows: 2 LJ medium enriched with glycerol and 2 LJ medium enriched with Pyruvate. Incubation at 37°C for up to 12 weeks with weekly observation for growth of colonies was done. On observation of visible growth, a few colonies were gently mixed into one drop of sterile saline and smeared on a clean, grease-free microscopic slide, heat-fixed using the Bunsen burner flame without burning and stained by the ZN method to confirm the presence of acid-fast bacilli. The smeared slide was flooded with ZN carbon fusion, gently steamed without boiling with the Bunsen burner flame from the underside for 5 min. It was then rinsed gently until all free stain was washed away. The slide was flooded with 3% acid-alcohol decolorizing solution for 2 – 3 minutes until the red color disappeared, then rinsed again with water and the excess water drained. The slide was then flooded with Methylene blue counter stained for 1 minute, rinsed thoroughly with water, and excess water drained from the slide and the smear allowed to air dry without blotting. The smear was examined under a microscope (100 x oil immersion objective) for the presence of acid-fast bacilli. The presence of bacilli in 100 immersion fields was recorded as positive. Smears in which no acid-fast bacilli were seen in 100 fields were considered negative.

Growth of colonies on LJ media

Cultures with growth/colonies on LJ medium were checked for being AFB using ZN staining. Isolates which are AFB positive were harvested and kept into two separate nunc tubes (one contained 1 ml freezing media and the other 0.3 ml dH_2O). Isolates in freezing media were kept at -20^oC. Isolates in dH_2O were heated in a water bath at 80^oC for 50 minutes to release the mycobacterium genomic DNA material. Released mycobacterium genomic DNA material were kept at -20^oC until used for molecular characterization (Lowenstein, E. 1933).

RESULTS

The prevalence of BTB was 4.22% (80/1896) on the basis of gross TB lesions. There was statistically significant difference ($p<0.001$) between origin of animals (Table 1), as well as between abattoirs and body condition score ($p<0.001$) of the animals. Bishoftu abattoir slaughtered significantly more number of cattle with TB lesions because the animals that they slaughter were emaciated due to TB & they slaughter these animals for supermarkets or retailers and Adama municipal abattoir has lower TB cases because the animals they slaughter were fatten animals for butchers' house (Table 2).

Table 1. Association of BTB lesion prevalence with origins of animals in abattoir slaughtered cattle carcasses

Origin of animal	No of cattle inspected	No of carcasses positive (%)	χ^2	P-value
Southeast	1018	16(1.6)	45.9	0.000
Northeast	878	64(5.6)		
Total	1896	80(4.2)		

Table 2. Association of BTB lesion prevalence with BCS of animals andthe two abattoir

Variable	No of cattle inspected	No of lesion positive carcasses (%)	χ^2	P-value
Abattoirs				
Adama municipal	1266	32(2.5)	26.9	0.000
Bishoftu export	630	48(7.6)		
BCS				
Lean	460	33(7.2)	19.4	0.000
Medium	440	23(5.2)		
Good	996	24(2.4)		
Total	1896	80 (4.2)		

Distribution of tuberculosis lesions

The distribution of TB lesions in different tissues of cattle was presented in Tables 3. Identifying each study animal organs is marked with identifying number, so during collection of the organs there was no problem to identify which organ from which animal. About 78.75% of the lesions were observed in the lung and associated lymph nodes. The lung region contributes a higher percentage of tubercle lesions than the head and the gastrointestinal area.

Table 3. Percent of distribution of tuberculosis lesion in organs and lymph nodes

	Postmortem	
Organs	**Number**	**(%)**
Lung tissue	14	17.5
Bronchial LN	17	21.25
Mediastinal LN	11	13.75
Retropharyngeal LN	17	21.25
Mandibular LN	4	5.0
Mesenteric LN	6	7.5
Prescapular LN	6	7.5
Liver tissue	5	6.25
Overall	80	100

Tuberculosis lesions were present in different tissues of cattle (Table 3). About 52.5% of the lesions were observed in the lung and associated lymph nodes. The lung region contributes a higher percentage of tubercle lesions than the head and the gastrointestinal area (Table 4). Figure 2 and 3 also show BTB lesions in thoracic cavity and the lung tissue.

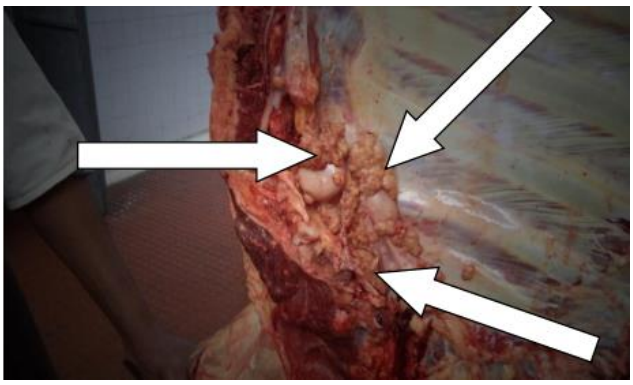

Figure 2. Tuberculosis lesions in the thoracic cavity of adult cattle at ELFORA abattoir, in Central Ethiopia.

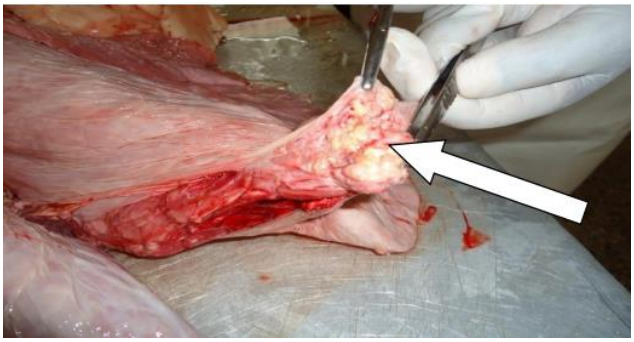

Figure 3. Tuberculosis lesions in lung tissues of adult cattle at ELFORA abattoir, in Central Ethiopia.

Table 4. Pooled TB lesions distributions among lymph nodes by regions.

Anatomic sites	**Lesions**	**Relative proportion***
Lymph nodes around head	21	26.25
Lung and associated lymph nodes	42	52.5
Mesenteric lymph nodes	6	7.5
Prescapular LN	6	7.5
Liver and hepatic lymph nodes	5	6.25
Total	80	100

Isolation of mycobacterium from tissue

The suspected TB lesions were collected from lung tissues and lymph nodes of different regions and a total of 80 samples were cultured using pyruvate and glycerol enriched LJ slants following standard procedures under biosafety level 2 cabinet. Then Ziehl-Neelsen staining and microscopic demonstrations of acid-fast bacilli were used to confirm successful inoculation and growth. The presence of at least three acid-fast bacilli in 100 immersion fields was recorded as positive but fields without bacilli were considered negative.

Accordingly, the growth of MTC species were observed in 26.3% (21/80) of tissue samples of BTB-like lesions on primary culture.

DISCUSSION

The abattoir survey revealed that 4.22% of inspected cattle were harboring TB suspected lesion with 26.25% (21/80) AFB positive culture yield. Abattoir prevalence in present study is comparable to a previous report of 4.2% in Yabello municipal abattoir (Demelash et al., 2009), 4.5% in Hosanna in cattle (Teklu et al., 2004). Culture positivity in present study is comparable with the results reported (Biffa et al. 2010) with 25.9% culture positivity from Addis Ababa, Adama, Hawassa. Statistically a significant difference was observed in the prevalence of the lesion ($p<0.05$) in terms of body condition scores (BCS), where the prevalence was found to be higher in animals with lean or poor BCS (7.2%) compared to medium (5.2%) and good (2.4%) body conditioned animals. The present result is consistent with the previous reports, which indicated that animals with good BCS have relatively strong immunological response to the infectious agent than animals with poor or medium BCS. It also indicates the wasting nature of the disease and as a result affected animals are chronically in poor body condition (Radostits et al., 2007).

The abattoir survey revealed that 4.22% of inspected cattle were harboring TB suspected lesion. Abattoir prevalence in present study is comparable to a previous report of 4.2% in Yabello municipal abattoir (Demelash et al., 2009), 4.5% in Hosanna in cattle 4.22% TB suspected lesions 26.25% (21/80) yielded AFB positive cultures. Culture positivity in present study is comparable with the results reported Biffa et al. (2010) with 25.9% culture positivity from Addis Ababa, Adama, Hawassa, Yabello, Melge-Wondo. In the present study, larger proportion (52.5%) of TB lesion was recorded in the respiratory pathway of the lung and associated lymph nodes followed by lymph nodes around head (26.25%), mesenteric lymph nodes (7.5%), prescapular lymph nodes (7.5%) and liver and hepatic lymph nodes (6.25%). This finding is lower than 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 was comparable with the results of (Alemu et al., 2016) who observed 50% of lesions in the lymph nodes of thoracic cavity. The observation of the largest proportion of TB lesions in the respiratory pathway was consistent with the reports of previous findings (Asseged et al., 2004; Mihreteab and Indris, 2011). This finding indicated that inhalation might be the principal route of TB infection in cattle. Therefore, during post-mortem examination more emphasis should be given to inspection of lungs, and lymph nodes around the lung and head region.

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CONCLUSIONS

In conclusion, BTB the abattoir survey result of the present study has shown that bovine tuberculosis was prevalent in cattle slaughtered at Adama municipal abattoir and Bishoftu ELFORA export abattoirs with moderately low prevalence (4.2%). However, higher proportion of BTB lesions recorded in the lung and associated lymph nodes implies that the respiratory route might be the principal route of TB infection and attention should be given on detail inspection of the lungs and associated lymph nodes during post-mortem examination.

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Prevalence of Tuberculosis and Human Immunodeficiency Virus Co-infection and Associated Factors among People Living With Human Immunodeficiency Virus at Burayu and Holeta Districts, Oromia Region, Ethiopia

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Abstract

Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) are the double burden diseases of the world. The African continent takes a great share of TB-HIV cases worldwide. This retrospective study was conducted to assess the prevalence of TB-HIV co-infection and associated factors in Burayu and Holeta health centers. A ten years retrospective study was conducted by reviewing files of HIV/AIDS patients attending HIV clinics in the two health centers (2008-2017). Data were coded, cleaned and analyzed using SPSS version 20 statistical software. A P-value <0.05 was considered statistically significant. Among 2937 people living with HIV/AIDS, 13.3% (95% CI: 12.07-14.53) were TB-HIV co-infected, the majority were males and in the age group of 15-45years. $CD4^+$ cell count <200cells/mm^3 (χ^2 = 58.22 P<0.001), WHO clinical stage III and IV (χ^2 = 119.3; P<0.001), antiretroviral drug adherence (χ^2 = 92.31; P< 0.001) nutritional status (χ^2 = 89.4; P < 0.001) were significantly associated with HIV-TB co-infection. The prevalence of TB among HIV patients at two health centers was moderately high. Therefore, TB screening among HIV-positive patients is mandatory. In addition, community mobilization on early case detection and health education on TB-HIV co-infection should be encouraged.

Keywords: Tuberculosis, HIV/AIDS, People living with HIV/AIDS, TB-HIV Co-infection

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INTRODUCTION

Tuberculosis (TB) is one of the 10 top global health problems which is a cause for the illness of 10 million and the death of 1.6 million people (WHO, 2018). The largest number of new TB cases was from Southeast Asia and Western Pacific regions (62%), followed by Africa (25%) (WHO, 2018). Globally, 36.9 million people were living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), out of which 25.7 million were in Africa (UNAIDS, 2018).

Ethiopia is among the 30 countries highly affected by tuberculosis, ranking 10th globally, and 4th in Africa (WHO, 2016). Ethiopia is also heavily affected by TB-HIV co-infection, being the 7th globally and 2nd in Africa (WHO, 2014). Understanding the prevalence and predictors of TB-HIV co-infections in the local context is critical to determine the burden and associated factors of the co-infection and design intervention strategies accordingly. Associated factors that contribute to TB-HIV co-infection are numerous (Mohammed et al., 2011). Therefore, in countries like Ethiopia where TB and HIV are the major public health problems, understanding the associated factors in local settings is vital to assess the status of TB-HIV co-infection and improve the management accordingly. Therefore, this study was designed to assess the prevalence of TB-HIV co-infection and associated factors among people living with HIV/AIDS (PLWHIV) that were attending the HIV clinics in Holeta and Burayu Health Centers.

MATERIALS AND METHODS

Study area

The study was conducted in health centers found in Burayu and Holeta towns located 31 and 15 Km West of Addis Ababa, respectively. According to the 2010 report of the Central Statistical Agency (CSA), the population of Holeta and Burayu towns were 57,621 and 93,437, respectively. There were two public health centers and 11 private clinics in Burayu and two public health centers and 10 private clinics in Holeta (Fig. 1).

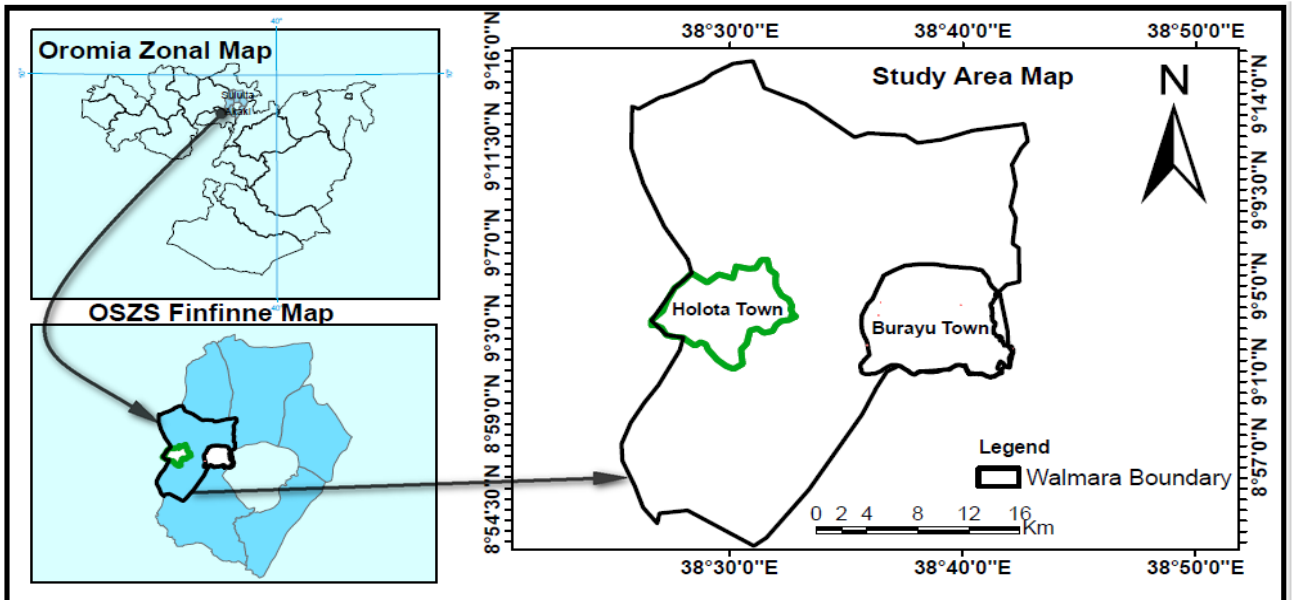

Figure1. Map of the study area.

Study design

A health facility-based retrospective study was conducted by reviewing 10 years (2008-2017) records of PLWHIVA at the directly observed treatment short-course (DOTS) clinic of two health centers. The selection of the two Health Centers was based on their high patient inflow with full information, which let as to get sufficient data within the study period. Records of cases with complete demographic and clinical data were included in the study, while those whose documents were incomplete, inconsistent, lost, or transferred were excluded from the study. The reviewed documents contained basic information such as patient's age, sex, weight address, TB type, and HIV status. About 2941 records from 2008-2017 on logbooks at the DOTS clinic of the two health centers were reviewed and 2937 were included in the study, while four records with incomplete data on logbooks were excluded.

Data collection and analysis

Socio-demographic characteristics (age, sex, marital status, residence, education and occupation) and TB-HIV infection associated factors (nutritional status, WHO clinical stage, CD4 count, adherence status) data of the cases were retrieved from the TB log and recorded in a pre-prepared data collection checklist adopted from an other study (Yeitayih et al., 2012). Data quality was assured by using a pre-tested data collection checklist and trained data collectors. Completeness and consistency of data were checked by data clerks and investigators before and after data entry. Data were entered, cleaned and analyzed using SPSS version 20 statistical software. Data were summarized using frequencies and proportions to describe the study population relevant variables. Chi-square test was used to determine the association between TB-HIV co-infections and relevant variables and P values of less than 0.05 were considered as statistically significant.

Ethics approval

Ethical clearance was obtained from the Institutional Review Board of Hawassa University. A formal letter of permission was obtained from the Heads of Holeta and Burayu District Health Offices. The consent for extracting data from records was obtained from Burayu and Holeta District Health Offices, Health Center heads and the DOTS clinic coordinators. The patients' clinical records were reviewed anonymously, and all information obtained from clinical records was kept confidential.

RESULTS

Totally 2937 records of PLWHIVA were reviewed and the majority (1789, 61%) were females, 2226 (75.8%) urban dwellers and 703 (39.6%) in the age group of 15-30 years. The majority (67.7%) of the cases was unemployed or daily laborers and 37.9% were single. Most (60.1%) of the participants had normal nutritional status and good adherence to antiretroviral therapy (ART) (62.9%). Most (66.7%) of the PLWHIVA were at WHO clinical stages I and II and (63.7%) of the cases had a CD4 count greater than 200 cells/mm^3 (Table1).

Table1. Socio demographic and clinical characteristics of PLWHIVA, Burayu and Holeta Health Centers (2008-2017).

Characteristics		Frequency	Percentage
Sex	Male	1148	39.1
	Female	1789	60.9
Age(years)	≤ 15	310	10.5
	16 – 30	1162	39.6
	31 – 45	1111	37.8
	>45	354	12.0
Residence	Urban	2226	75.8
	Rural	703	23.9
Nutritional status	Normal	1766	60.1
	Moderate malnourished	978	33.3
	Severe malnourished	143	5.0
ART adherence	Good	1848	62.9
	Fair	965	32.9
	Poor	103	3.5
WHO clinical stages	stage1	911	31.0
	stage 2	1050	35.7
	stage 3	929	31.6
	stage 4	47	1.6
CD4 cell count	< 200 cell/mm^3	1067	36.3
	200 – 500 cell/mm^3	1108	37.7
	Above 500 cell/mm^3	762	25.9
Educational status	No formal education	689	23.5
	Primary education completed	1143	38.9
	Secondary education completed	873	29.7
	Tertiary education completed	232	7.9
Marital Status	Single	1112	37.9
	Married	1043	35.5
	Separated/Divorced	325	11.1
	Widowed/widower	457	15.6
Occupation	Government Employee	209	7.1
	Industry workers	957	32.6
	Students	213	7.2
	Unemployed	1031	35.1
	Others	229	7.8

Trends of HIV/TB co-infection

The trend of TB-HIV co-infection showed variations during the study period. From 2008-2010 TB-HIV co-infection was gradually decreased from 12.9 - 9.9% and starting 2011 the co-infection progressively increased and reached 17.1% in 2013. Then, constantly decreased down to 7.7% in 2017 (Fig 2).

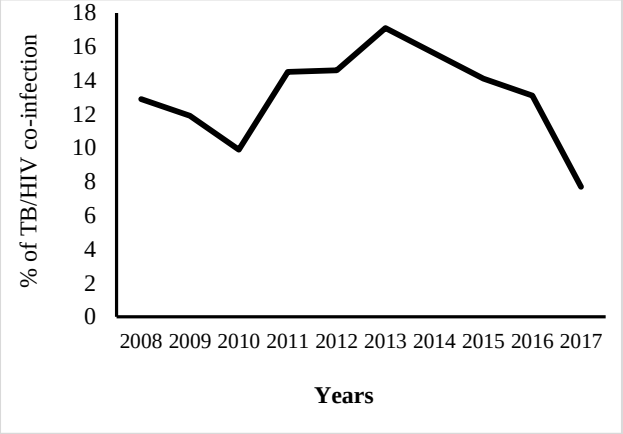

Figure 2. Trend of TB-HIV co-infection among PLWHIVA at Holeta and Burayu Health Center (2008-2017)

Among 2937 PLWHIVA, 391 [13.3%; (95% CI: 12.07-14.53)] were TB-HIV co-infected and the majority were in the age range of 15-45 years. The prevalence of TB-HIV co-infection among males was 210 (18.3%) (Table 2). TB-HIV co-infection was significantly associated with nutritional status, WHO clinical stage, ART drug adherence, CD4 cell count, educational status, and occupation ($P< 0.05$). Individuals in WHO stage III and IV were significantly exposed to the co-infection than stage I and II ($\chi^2 = 119.3$; $P < 0.001$). TB-HIV co-infection was significantly higher in case of $CD4^+$ cell count <200 cells/mm^3 as compared to CD4 cell count above 500 cells/mm^3 ($\chi^2 = 58.22$ $P < 0.001$). More TB-HIV co-infection was recorded in the case of severely and moderately mal-nourished people and the association was statistically significant ($\chi^2 = 89.4$; $P < 0.001$). The majority of co-infection was observed in cases with poor adherence and the association was statistically significant ($\chi^2 = 92.31$; $P< 0.001$).

DISCUSSION

Tuberculosis is a well-recognized opportunistic infection in patients with HIV/AIDS and is the leading cause of morbidity and mortality among people living with HIV/AIDS. Therefore retrospective or prospective assessment of the status of TB among PLWHIVA is very essential for public policy, planning, and development of collaborative activities accordingly. Hence, in this study, the overall prevalence of TB-HIV co-infection was 13.3%, which was in coherence with studies conducted in Southern, Central, North West Ethiopia, and Nigeria 13.9%, 12%, 11.4%, 14.1%, respectively (Asnake et al., 2017, Solomon et al., 2018, Sebsibe and Takele, 2013, Tony et al., 2015). The finding was less than the studies done in Southern, Central, North East, and North West Ethiopia 18.2 %, 36.9%, 20.3%, 24.4% and 44.8%, respectively (Sintayhu et al., 2015; Abel et al., 2018; Seada and Tewelde 2015; Daniel et al., 2015; Ahemed et al., 2013) and in other countries such as Cameroon and India 51.6% and 17% (Pefura et al., 2012; Purushottam et al., 2013), respectively. But, it was higher than investigations conducted in South West, North West Ethiopia, and Tanzania 8.1%, 7.5%, 8.5%, respectively (Kebede and Wabe, 2012; Yeitayih et al., 2012; Ngowi et al., 2008). These variations in the magnitude of TB/HIV co-infection among people living with HIV/AIDs may be associated with differences in coverage level of highly active antiretroviral treatment (HAART), diagnostic procedures used, the difference in TB diagnosis, under-reporting, epidemiology of TB in different countries and study methodology applied.

Residence of the study participants had no association with TB-HIV co-infection. However, reports from North West Ethiopia (Sebsibe and Takele, 2013) Cameroon (Pefura et al., 2012) indicated that TB-HIV co-infection occurs more among urban dwellers. This might be associated with overcrowded settlement and low-quality life status of most of the people in the urban areas of developing countries (Amare et al., 2009). In contrast, in South and North West Ethiopia (Mohammedaman et al., 2018; Sebsibe and Takele, 2013) most of the cases were among rural residents. Males were more exposed to co-infection; this could be usually due to the migration of the adult male population to the study area for a job and enrolled as laborers in private institutions and factories. Moreover, it may be because males are more involved in the consumption of alcohol and smoking and have risky sexual behavior (Aweke et al., 2016). Similar findings were documented in North West and South Ethiopia (Yeitayih et al., 2012; Mohammedaman et al., 2018), Nigeria, India, Brazil and Europe (Babatunde et al., 2016; Magna and Sitikantha, 2016; Bráulio et al., 2008; Pimpin et al., 2011). In contrast, in North West, North East, South Ethiopia (Sebsibe et al., 2013; Daniel et al., 2015; Mohammedaman et al., 2018) and Sub-Sahara Africa countries females are more exposed (Sia et al., 2014). Participants in the age group 15-45 years were more affected with TB-HIV co-infection, similar findings were obtained elsewhere in Ethiopia, Malawi and Cameroon (Yeitayih et al., 2012; Tweya et al., 2013; Sume et al., 2008). This might be because this age group was sexually active and involved in various superfluous

daily activities, which increase the frequency of the exposure.

Table 2.Chi-square analysis of factors associated with TB-HIV co-infection among PLWHIVA at Holeta and Burayu Health Centers (2008-2017)

Characteristics		Total	HIV-TB co-infection		χ^2	P-value
			Yes	No		
Sex	Male	1148	210	938	40.49	<0.001
	Female	1789	181	1608		
Age(years)	≤ 15	310	35	275	2.47	0.47
	16 – 30	1162	166	996		
	31 – 45	1111	147	964		
	>45	354	43	311		
Residence	Urban	2226	305	1921	1.20	0.54
	Rural	703	85	618		
Nutritional status	Normal	1766	162	1604	89.4	<0.001
	Moderate	978	187	791		
	Severe	143	41	102		
ART adherence	Good	1848	176	1672	92.31	<0.001
	Fair	965	175	790		
	Poor	103	38	65		
WHO clinical stage	Stage 1	911	43	868	119.3	<0.001
	Stage 2	1050	101	949		
	Stage 3	929	216	713		
	Stage 4	47	31	9		
CD4cell count	< 200 cell/mm^3	1067	200	867	58.22	<0.001
	200 – 500 cell/ mm^3	1108	142	966		
	Above 500 cell/ mm^3	762	49	712		
Educational status	Illiterate	689	122	567	20.22	0.062
	Primary education	1143	155	988		
	Secondary education	873	91	782		
	Tertiary	232	23	209		
Marital Status	Single	1112	211	901	74.8	0.10
	Married	1043	85	958		
	Separated/Divorced	325	37	288		
	Widowed/widower	457	58	399		
Occupation	Government Employee	209	13	196	54.10	0.35
	Farm and Industry	957	139	818		
	Students	213	12	201		
	Unemployed	1031	201	830		
	Others	229	18	211		

*Others (Wife, farmer, merchant, driver)

TB-HIV co-infection was significantly associated with $CD4^+$ cell count <200 cells/mm^3, studies conducted in other places also obtained similar results (Bekele et al., 2018). The association between TB/HIV co-infection and WHO clinical stages of HIV patients was statistically significant, studies in

other place in Ethiopia, and Gambia (Aweke et al., 2016;Hill et al., 2006) also documented congruent findings. The nutritional status of PLWHIVA was also significantly associated with TB-HIV co-infection, the finding was in line with that of (Aweke et al., 2016; Sudre et al., 1996). Cases with poor adherence were more affected and the association was statistically significant, which is in agreement with other findings (Sudre et al., 1996).

The finding of this study was able to determine the prevalence of TB-HIV co-infection and identified the possible TB- HIV associated factors in the study areas. The finding could be used as baseline data for professionals that involved in the prevention and control of TB and HIV and policymakers to design strategies that improve referral pathways between the TB and HIV clinics and improve TB screening among HIV patients and allocate resource accordingly. Further investigation should be carried on the incidence of TB-HIV co-infection and the trend and level of interaction of TB infection in HIV patients.

CONCLUSION

In the present study, the prevalence of TB-HIV co-infection was moderately high. The co-infection was associated with $CD4^+$ cell count less than 200/µl, WHO clinical stage III, poor nutritional status and antiretroviral drug adherence. Therefore, compulsory TB screening among HIV-positive patients is required for early detection and treatment, and thus reduces associated morbidity and mortality. Moreover, intervention strategies on the reduction of TB-HIV co-infection should focus on improving the $CD4^+$ cell count, nutritional status and antiretroviral drug adherence of the clients.

LIMITATION OF THE STUDY

The study was on the prevalence and associated risk factors of TB-HIV co-infection among PLWHIV in Burayu and Holletea, Ethiopia. It was facility-based study and therefore difficult to make generalizations. Community-based studies are required to fully understand the extent of the problem and identify feasible intervention measures. Moreover, since the study design was retrospective, the data source relies on records where some of the case records are incomplete or absent and consequently have a negative impact on the final finding.

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