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The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multidisciplinary peer-reviewed journal intended to promote animal health and production of national and regional/international importance. The journal publishes review articles, original research articles, short communication as well as technical notes in English. Under special circumstances, articles in Amharic may be considered for publication.

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Giardiasis of domestic animals and its zoonotic significance: A review

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Abstract

Giardiasis is the most common waterborne parasitic intestinal infection of both animals and humans worldwide, and it leads to significant morbidity and mortality in animals, particularly in young aged group like calves in the developing and developed world. It is a zoonotic infectious disease where animals are considered as sources of infection for humans through consumption of water and food contaminated with cysts of *Giardia*. *Giardia* species differ significantly in host range, with *G. duodenalis* having the broadest host range and greatest public health significance. *Giardia duodenalis* has eight different assemblages A through H. The disease Causes gastro intestinal disturbance, gall bladder colic and jaundice. Malabsorption and maldigestion mainly result from a diffuse shortening of epithelial microvillus. *Giardia duodenalis* (synonyms *intestinalis, lamblia*) is a flagellate binucleated protozoan, and it has two morphological forms: cysts and trophozoites. The life cycle of *Giardia* is direct, and the infective stage of the parasite, the cyst, is encysted when released into the feces and is immediately infectious. Trophozoites are pear-shaped, binucleate, multi-flagellated parasite forms and are the disease causing stage and colonize the upper small intestine, where they adhere to the epithelial surface and proliferate by binary fission. Infected animals, contaminated water and feed are main sources of infection. Age of animals, season of the year, area, housing, feeding, management practices are usually considered as risk factors for the occurrence of giardiasis. The infection can be transmitted by the fecal-oral route, or through contaminated food and water. To survive within the intestine, *Giardia* undergoes antigenic variation and few virulent factors have been identified to be responsible for the infection. These include ventral adhesive disc proteins and surface lectins, the four pairs of flagella which allow for movement and variant surface proteins. The clearance of *Giardia* from a host requires both innate and adaptive immunity effector mechanisms. The infection can be diagnosed by examining fecal specimens using different coproscopic techniques, serological tests and by molecular analysis. Restricting

animals from surface water during periods of high cyst shedding may reduce watershed contamination. Health education on personal and environmental hygiene would help in reducing the occurrence of the infection. This review work tries to gather information on giardiasis of domestic animals with respect to its epidemiology, host immune responses, pathophysiology, distribution and its public health importance.

Keywords: Giardiasis; *Giardia* assemblages; distribution; immune response; public health.

Introduction

Giardiasis is a diarrheal illness distributed throughout the world. It is caused by a flagellate protozoan parasite, *Giardia intestinalis* also known as *G. lamblia* and *G. duodenalis*. *Giardia* is a common cause of gastrointestinal disturbance in both high- and low-income countries (Hill and Nash, 2006). The synonyms of Giardiasis are Giardia enteritis, Lambliasis, lamblia intestinalis, beaver fever (Huang and White, 2006). *Giardia* is a ubiquitously distributed flagellate protozoon that inhabits the small intestine of its host. *Giardia* infection also affects the gall bladder of an adult cow (Degerli and Ozcelik, 2003). *G. bovis* (*G. duodenalis* assemblage E), *G. duodenalis* assemblage A and mixed infections of *G. duodenalis* assemblage A and *G. bovis* (*G. duodenalis* assemblage E) affect cattle especially calves (Geurden *et al*., 2012). *G. duodenalis* in livestock, particularly in cattle, is very common in this population and tends to infect younger calves leading to high prevalence of infection within herds.

Giardia infections are common in cattle and pet animals (Dogs and cats) (Armson *et al*., 2009). Dairy calves can excrete high number of the cyst of *Giardia*, and the disease in cattle is clinically important and can reduce the growth performance of ruminants. *Giardia duodenalis* assemblage E (*G.bovis)* is most prevalent in calves (Ralston *et al.,* 2003). Clinically, it is characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption. However, asymptomatic giardiasis occurs frequently, especially in developing countries (Hellard *et al*., 2000).

Direct transmission of *Giardia* between cattle and their handlers and indirect transmission through water ponds is possible. Giardiasis is zoonotic and zooanthroponosis. The major significance of giardiasis in veterinary medicine probably rests with its zoonotic potential although it has also been proposed as

a zooanthroponosis (Ehsan *et al*., 2015). Studies have reported between 45%-73% of calves 0-24 weeks of age having infections as well as infection rates as high as 100% (Winkworth *et al*., 2008). *Giardia*-infection in cattle is often subclinical or asymptomatic (Gillhuber *et al*., 2013); but when clinically occurs, the symptoms of giardial infection could include diarrhea, foul smelling stool, flatulence, loss of appetite, and emaciation in different proportions (Monis *et al*., 2009).

Giardiasis is considered to be an important zoonotic disease and animal reservoirs are believed to be cats, dogs, ruminants, and a variety of wild animals. Infections in domestic ruminants are of special concern because of the potential contamination of surface and ground waters through pasture run-offs and use of manure as a spray on fields. Outbreaks from waterborne giardiasis in humans have been attributed to pasture runoff leading to drinking water contamination. Morphological, protein, and DNA similarities among *Giardia* isolated from animals and humans have been demonstrated. The number of clinical reports and transmission studies suggest the possible zoonotic transmission of the *Giardia* parasite (Wolfe, 1992). The objective of this review work is therefore, to compile current information on giardiasis of domestic animals with regards to its epidemiology, host immune responses, pathophysiology, distribution and its public health importance

Etiology

Taxonomy of *Giardia*

Giardia is grouped under kingdom of protista, phylum of protozoa, subphylum of sarcomastigophora, class of mastigophora, order of Giardia, family of Giardia lamblia (Urquhart *et al.*, 1996). Currently, six *Giardia* species are recognized by most researchers. Among them, *G. agilis*, *G. ardeae*, *G. muris*, *G. microti*, and *G. psittaci* infect various animals, whereas *G. duodenalis* infects humans and many mammals (Upton and Zien, 1997). Thus, *Giardia* species differ significantly in host range, with *G. duodenalis* having the broadest host range and greatest public health significance (Yang *et al*., 2009). Cattle may be infected with *G. duodenalis*, which has synonyms of *G. intestinalis* and *G. lamblia*. *Giardia duodenalis* infects nearly all cattle at one time or another, and may cause diarrhea in calves older than 1 month. Although *G. duodenalis* is an important cause of diarrhea in people, and transmission from livestock has long been suspected, current genetic evidence suggests that cattle and people are infected by different subtypes (Olson *et al*., 2004).

The species names *G. duodenalis, G. intestinalis* and *G. lamblia* are used interchangeably in literature referring to the same organism. Both *G. duodenalis* and *G. intestinalis* are used in equal frequency in referring to the *Giradia* species infecting most mammals, including humans, their companion animals and livestock (Feng and Xiao, 2011).

Zoonotic (assemblages A and B) and Assemblage E are genotypes of *G. duodenalis* and *G. microti* (Yang *et al*., 2009). Assemblage A is frequently found in livestock (cattle, water buffalo, sheep, goats, alpacas, and pigs) and companion animals (dogs, cats, and horses). In comparison, assemblage B is less frequently reported from livestock and companion animals, with only a few reports of infection of cattle, sheep, horses, dogs, cats, and rabbits (Thompson *et al*., 2008). Assemblage A and to a lesser extent, assemblage B are commonly found in wild animals, with the exception of beavers and muskrats, which seemingly have a high occurrence of assemblage B.

The livestock-specific species *Giardia bovis* is the most frequently observed in cattle, however, the two zoonotic species *Giardia duodenalis* and *Giardia enterica* have also been found. Therefore, calves are thought to be of public health significance. *G. bovis*only affects cattle specially, calves (Gillhuber *et al.,* 2013). *G intestinG. duodenalis, G. lamblia)* can be subdivided based on molecular analysis into what are known as different genetic assemblages (A, B, C, D, E, F, G and H). Some of these assemblages can be classified even further into subtypes or genetic clusters (Groups I to IV) for example A-I, A-II, A-III, and A-IV. Each assemblage is capable of infecting certain species, and some assemblages are more commonly seen than others (Table1) (Feng and Xiao, 2011).

Phylogenetic analysis of a large set of nucleotide sequence data from the small subunit (SSU) rRNA gene and several housekeeping genes coding for glutamate dehydrogenase (gdh), ß-giardin (bg), elongation factor 1 alpha (ef1α) and triphosphate isomerase confirmed the genetic uniqueness of the assemblages A and B. Additional linages of *G. duodenalis* from animals were identified: assemblages C and D from dogs, assemblage E from artiodactyls, assemblage F from cats and assemblage G from rodents (Table 1).

There is sub structuring within assemblage A, which consists of mostly two subgroups or sub assebmblages AI and AII. The separation of subgroups AI and AII was initially made by allozyme analysis and is supported by phylogenetic analysis of assemblage A sequences at the *gdh* locus. The existence

of numerous subtypes related to subgroups AI and AII, however, made the separation of subgroup AI and AII less obvious at some other loci, such as *tpi* and *bg*. Recently, a third sub group within assemblage A, subgroup AIII, was identified and appears to be associated with wild hoofed animals. It has significant sequence difference from sub groups AI and AII at all loci examined so far.

Table *1.* Different genetic assemblages of *G. intestinalis*

Assemblages	Major species commonly infected
A-I	Humans and animals(Cats, dogs, livestock, Deer, Muskrats, Voles, Guinea pigs, Ferrets)
A-II	Humans (More common than A-I)
A-III and A-IV	Exclusively animals
B	Humans and animals (Livestock, chinchillas. Beavers, Marmosets, Rodents)
C and D	Dogs
E	Alpacas, cattle, goats, pigs, sheep
F	Cats
G	Rodents
H	Marine mammals

Source: (Feng *et al*., 2011)

Morphology

G. lamblia is bilaterally symmetrical like hexamita and possess eight flagella, six of which emerge as free flagella at intervals around the body. It is unique in possessing a large adhesive disc on the flat ventral surface of the body, which facilitates attachment to the epithelial cells of intestinal mucosa (Urquhart *et al.,* 1996) (Fig. 1). *G. duodenalis* (synonyms *intestinalis, lambilia*) is a flagellate binucleated protozoan. *G. lamblia* has two morphological forms: cysts and trophozoites. Trophozoites are pear-shaped, binucleate, multi-flagellated parasite having an adhesive disk on the ventral surface of the trophozoite (Buret *et al.,* 2005). Trophozoites resemble a pear or tear drop and measure 9-21 µm in length, 5-15 µm in width and 1-2 µm thickness (John *et al*., 2006). The trophozoite has two anteriorly placed nuclei and has four pairs of posteriorly directed flagella that aid in locomotion. Cysts are oval-shaped, thin-walled cyst that is 10 to 20 µm in length, 7-10 µm in width and 0.3-0.5 µm thickness (Huang and White, 2006).

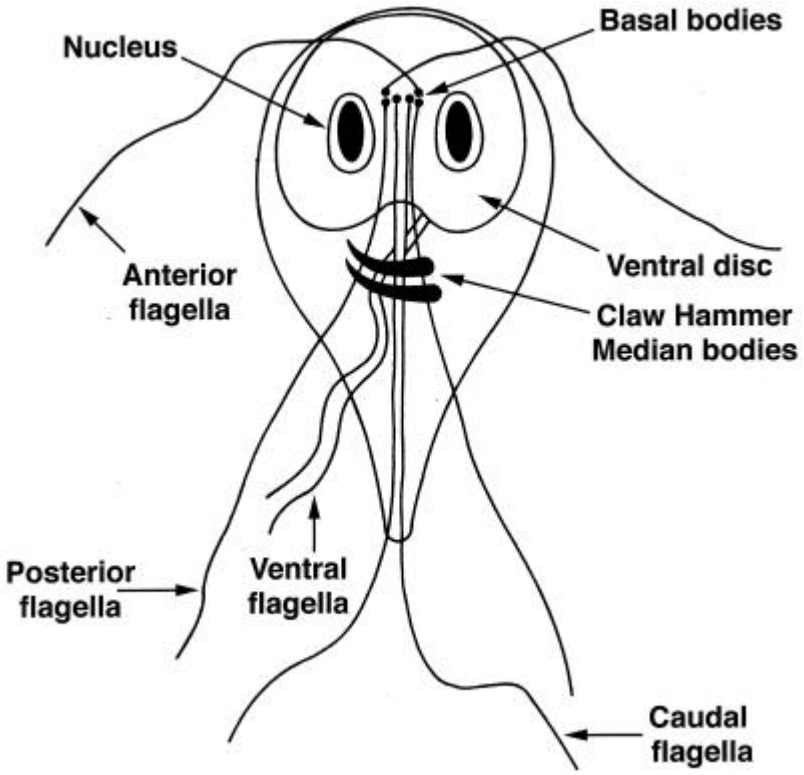


Figure 1. *Giardia lamblia* **trophozoite (Source: Faubert, 2000)**

Life cycle

G .duodenalis has a simple life cycle, which includes the dormant infective cyst released in the feces, each producing, upon exposure to gastric juices during ingestion, one tetranucleated short-lived excyzoite, which will divide twice to give rise to four diploid trophozoites. Trophozoites are the disease causing stage and colonize the upper small intestine, where they adhere to the epithelial surface and proliferate by binary fission (Muller *et al.,* 2008).

An adhesive disk on the ventral surface of the trophozoite facilitates attachment to the mucosal surface of the duodenum and jejunum, although the trophozoite does not invade the mucosal epithelium. Trophozoites that do not adhere to the small bowel move forward to the large intestine where they revert

to the infectious cyst form; conjugated bile salts appear to foster encystations (Buret, 2009).

The life cycle of *Giardia* is direct, and the infective stage of the parasite, the cyst, is encysted when released into the feces and is immediately infectious (Huang and White, 2006). Cysts remain infectious for months in cool, damp areas and rapidly accumulate in the environment. In soil, cyst infectivity is reduced by only 11% after 49 days at 4°C and is non-infective after 7 days at 25°C. In tap water, *Giardia* cysts are infectious for 56 days at 0°C to 4°C and 14 days at 20°C to 28°C. Similar results were obtained in lake water, with 56 days of survival at 0°C to 4°C or 6°C to 7°C and 28 days at 17°C to 20°C (Huang and White, 2006). Longer survival is noticed in river water, with 84 days of survival at 0°C to 4°C and 28 days at 20°C to 28°C (Huang and White, 2006). Cysts are the infectious form of the parasite; these can survive in moist environments for prolonged periods (Rendtorff, 1954). In seawater, *Giardia* cysts can survive for over 65 days at 4°C. Ingestion of 10 to 25 cysts can lead to giardiasis. When ingested by the host, cysts excyst in the duodenum, releasing the trophozoites (Erickson and Ortega, 2006). The latter trophozoites undergo repeated mitotic division and form environmentally resistant cysts in response to the stimulation of bile salts and other conditions. Cysts pass through the intestine in feces and are spread by contaminated water, food, fomites, and by direct physical contact (Erickson and Ortega, 2006) (Fig. 2).

Cysts are passed back into the environment in excreted feces; in the setting of diarrhea, trophozoites can also be found in the feces (Buret, 2009). A motile vegetative form (trophozoite) which resides in the small intestine and is responsible for disease manifestations and an infective resistant form (cyst) is responsible for transmission. The trophozoite is an aero tolerant anaerobe that requires glucose as a source of carbohydrate energy, and divides by longitudinal binary fission every 9 to 12 hours (Huang and White, 2006). The incubation period, which is the time from infection and manifestation of clinical signs is 7 to 14 days; however, the time from ingestion of the cysts to detection of the cysts may be longer than the incubation period (Leder and Weller, 2011).

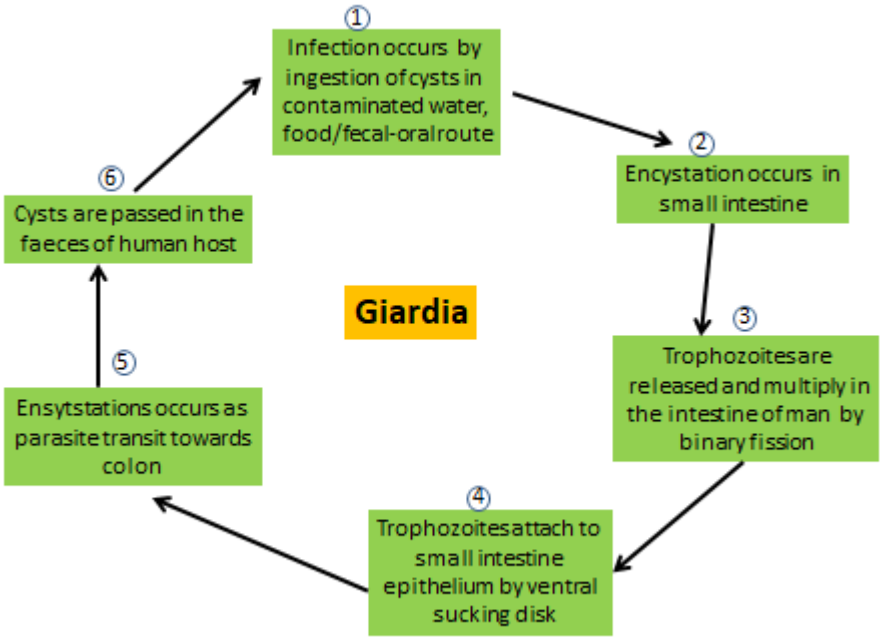


Figure 2. Life cycle of *Giardia intestinalis* (Muller *et al.,* 2008)

Epidemiology

An important aspect of the epidemiology of giardiasis is to understand the host range of different *Giardia* species and strains/genotypes, the potential for cross – species transmission and risk and environmental factors involved in the exposure of the pathogen. This is particularly important in determining the zoonotic potential of *Giardia* infections in domestic animals and in determining the human disease burden attributable to parasites of animal origin (Feng and Xiao, 2011).

Occurrence of giardiasis

Giardia infections occur worldwide (Krauss *et al*., 2003). Giardial infection is found in most continents and is identified in all of the common agricultural animals. A longitudinal studies of excretion patterns in grazing beef cattle,

feedlots cattle, dairy cattle, calves and foals show infection rates approaching 100% (Radostits *et al*., 2009). *G. lamblia* is one of the most commonly identified intestinal pathogens in humans and animals in the world (Xiao, 1994). Recent studies have identified domestic livestock (e.g., cattle, sheep, pigs, horses) as major hosts for this parasite. Calves become infected with *Giardia* at around 4 weeks of age and infections persist for over 120 days (Olson *et al*., 1997). In cattle, the occurrence of infection with *G. duodenalis* is higher in immature cattle than in adult animals. The highest risk of infection is seen in the pre-weaned age group (Minetti *et al*., 2012).

Source of infection of giardiasis

Young animals are the primary source of infection; infection being transmitted through the oral-fecal route. High excretion rates and excretion intensities in young animals result in the contamination of the environment and infection via fomites. The dam is also a source of infection for the young. Cross-infection from other species and infection from contaminated water and feed are other sources of infection. Epidemiological evidence suggests that domestic animals act as a reservoir for the parasite and serve as source of infection for humans (Radostits *et al*., 2009**)**.

Risk factors of Giardiasis

The parasite risk factors

The infectious dose of giardiasis is thought to be very small. *Giardia* is relatively resistant to environment and at 4 ^{o}C can survive for 11 weeks in water, 7 weeks in soil and 1 week in cattle feces. But, they do not survive in freezing. They are resistant to chlorination and extensive disinfection of the environment of calves does not prevent reinfection (Radostits *et al*., 2009).

Host risk factors

The age of the cattle probably contributed to the different infection rates observed (Feng and Xiao, 2011**)**. Age is a major determinant of infection; excretion rates are much higher in the young of all livestock species than in adults. Cysts excretion falls after weaning, but may persist intermittently into adulthood. Excretion rates in groups of calves are highest between 3 and 10 weeks of age with the number of cysts in feces highest at 1-6 weeks of age. The cyst

excretion levels varied considerably both within and between herds for all age of animals (Radostitis *et al.,* 2009). The prevalence of infection is varied according to the age group, with the highest prevalence of infection seen in preweaned animals followed by neonatal calves, post-weaned animals and adult cattle. The risk of *G. duodenalis* infection is significantly higher only in preweaned calves (Minetti *et al*., 2012).

Management risk factors

The prevalence of infection is higher in calves left with their dams to nurse colostrum than in calves removed from the dam at birth to individual housing and fed colostrums by nipple bottle (Radostitis *et al.,* 2009). There is a potential association between *G. duodenalis* infection and parameters relating to housing and production type. Prevalence of *G. duodenalis* varies according to production system; where an infection prevalence is high in suckler systems than infection prevalence in non-suckler systems (dairy, calf rearer or beef fattener). The prevalence of infection in housed animals is higher than that in cattle that are kept in outdoors or in mixed accommodation (Minetti *et al*., 2012). It is noted that the monthly distribution pattern of giardiasis in bovine is related to the temperature, humidity, the rainfall, season and area (Ayaz *et al*., 2008).

Mode of transmission of giardiasis

Giardia cysts are commonly excreted in the faeces. The organism is passed as multinucleated cysts in the faeces, where the flagella may be visible, and occasionally as trophozoites (Urquhart *et al*., 1996). Infection is acquired via the faecal-oral route**,** often through the ingestion of *Giardia* cysts from faecally-contaminated water. *G. lamblia* can be transmitted from person-to-person, animal-to-person, food borne or waterborne. Ingestion of as few as 10–25 cysts can cause illness (Hill and Nash, 2006).

Sexual behaviors that aid in transmission of *G. lamblia* include oral-anal, genital-anal, and digital anal contact. Contaminated soil and fomites can also contain infective cysts (Berger and Marr, 2006). It can be transmitted from person-to-person, especially persons with poor fecal-oral hygiene, causing epidemics (Huang and White, 2006).

The most common method of transmission in animals and humans is the fecal-oral route (Xiao, 1994). Disease outbreaks have most often been attributed to the waterborne method of transmission. It is believed that human effluent is the major source of water contamination, but certainly contamination of water with infected animal feces can lead to widespread infections in human and animals (Wallis *et al*., 1996). *Giardia* cysts are infectious at the time they are passed in feces (Olson *et al.,* 2004).

The high prevalence of infection in cattle reports that cattle shed cysts of zoonotic types that infect humans (Handley *et al*., 2000; Keulen *et al*., 2002). *Giardia* from cattle is potential zoonotic pathogen, and contact with their manure is believed to be the major risk factor for infection in humans (Olson *et al*., 2004). In cattle, direct transmission through the contamination of barns and/or pasture appears to be the principal mode of infection (Ruest *et al*., 1998).

Status of giardiasis

Global status of giardiasis

Giardia is the most common causes of protozoan diarrhea that lead to significant morbidity and mortality in the developing and developed world. Outbreaks of giardiasis are well documented worldwide. High infection rates for giardiasis are found in developing countries. The rates of infection are high in various Asian countries (Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Nepal, Philippines, Thailand, Turkey, Saudi Arabia, and Vietnam), South America Cuba, Mexico, and Nicaragua, Argentina, Brazil, Colombia, and Peru, and Africa (Northern Africa, West Africa, and South Africa) (Wegayehu *et al*., 2013). *G. lamblia* is the most commonly identified intestinal parasite in the US as well as in Canada (Hill and Nash, 2009). The prevalence of cumulative infection is 73 to 100% in cattle in the world (Feng and Xiao, 2011) (Table 2).

Table 2. Global distribution of giardiasis in cattle

Country	Prevalence (%)	Reference
Turkey	3.7	Degerli and Ozcelik, 2003
Britain	36	McAllister *et al.*, 2005
Bangladesh	22	Ehsan *et al.*, 2015
Denmark	43.6	Feng and Xiao, 2011
Germany	38	Feng and Xiao, 2011
Iraq	30.6	Monis *et al.*, 2009
Italy	30.0	Feng and Xiao, 2011
Norway	49.0	Feng and Xiao, 2011
Ontario	64	McAllister *et al.*, 2005
Spain	26.6 to 30.1	Feng and Xiao, 2011
Canada	8.7 to 57.0	Feng and Xiao, 2011
United States	19.1 to 52.0	Feng and Xiao, 2011
Sweden	23	Bjorkman *et al.*, 2003
New Zealand	4.5 to 40.6	Feng and Xiao, 2011
Australia	58.0	Feng and Xiao, 2011

Status of giardiasis in Africa

In Africa, *Giardia* has been reported in several domesticated animal species including cattle, sheep, goats, farmed buffalo, horses, poultry (chicken and turkey), pigs, cultured tilapia (fish) and dogs (Di cristanziano *et al*., 2014). However, the majority of research has been conducted in cattle.

Giardia duodenalis Assemblage E is the dominant species in ruminant livestock (cattle, farmed buffalo and goats) from the Central African Republic, Egypt, Rwanda, Tanzania and Uganda (Helmy *et al*., 2014; Abdel-Moein and Saeed, 2016; Sabry *et al*., 2009). Assemblage A (subtypes AI and AII) has been reported in goats, cattle, buffalos, ducks and chickens from Cote d'Ivoire, Egypt, Tanzania and Uganda and Assemblage B (BIV) and/or Assemblage A and B have been reported in goats, ducks and cattle from Cote d'Ivoire, and Tanzania (Sabry *et al*., 2009; Helmy *et al*., 2014; Abdel-Moein and Saeed, 2016) (Table 3).

Table 3. Prevalence and *Giardia duodenalis* assemblages reported in animals from Africa

Country	Animal spp	Prevalence (%)	Assemblage	Reference
Central Africa republic	Goat	11.1	E	Ignatius *et al.* (2012)
Cote d'voire	Dog	54.5	A	Berrilli *et al.* (2012)
	Goat	50	A, B	Berrilli *et al.* (2012)
	Chicken	58.1	A	Berrilli *et al.* (2012)
Egypt	Cattle	6.7	A	Helmy *et al.* (2014)
	Cattle	8.7	E	Abdel-Moein & Saeed, 2016
	Calves	30.8	A	Sabry *et al.* (2009)
Rwanda	Cattle	5.9	E	Hogan *et al.* (2014)
Tanzania	Zebu Cattle	21.1	A	Di Critanziano *et al.* (2014)
	Goats	22	E	Di Critanziano *et al.* (2014)
Uganda	Cattle(*Bos Taurus*)	10	A	Graczyk *et al.* (2002)

The status in Ethiopia

There are very few reports on the occurrence of *G. duodenalis* infection (Giardiasis) are available in domestic animals in Ethiopia. Outbreak of giardiasis was reported from cattle and human in Girar Jarso and Dera Districts of North Shewa Zone, Oromia Region, Ethiopia. (Wegayehu *et al*., 2013). The outbreak of *Giardia lamblia was also recorded in* Lante, Kolla Shelle, Dorze and Geressie kebeles of Gamo Gofa Zone, South Ethiopia. *Giardia lamblia (Giardia duodenalis)* infections have been reported from the lowland and highland areas of Gamo area **(**Wegayehu *et al*., 2013**).** Hailu *et al.* **(**2020) has reported 9.7 % prevalence of Giardia from dairy calves from southern Ethiopia (Arsi Negele, Shashemene and Hawassa dairy farms)**.** *Giardia lamblia (Giardia duodenalis)* infection has also been reported from Benishangul-Gumuz Region and South west Ethiopia (Jimma) (Eyasu *et al*., 2010; Mengistu *et al*., 2007) (Table 4).

Table 4. Prevalence of G. duodenalis species among cattle and human in Ethiopia

Study subjects	Study sites	Prevalence (%)	References
Cattle	Girar Jarso and Dera	2.3	Wegayehu *et al*., 2013
	Gamo Gofa zone	10.6	Teklu *et al*., 2013
Human	Girar Jarso and Dera	50	Wegayehu *et al*., 2013
	Benishangul Gumuz Region	26.6	Eyasu *et al*., 2010
	Jimma	47.7	Mengistu *et al*., 2007
Dairy calves	South Ethiopia	9.7	Hailu *et al*, 2020
Dairy calves	Central Ethiopia	9.6	Teklu *et al*., 2016

Pathology, virulence factors and host defense to giardiasis

Pathology of giardiasis

Animals and humans infected with *G. duodenalis* develop a broad range of clinical manifestations, ranging from an absence of symptoms to acute or chronic diarrheal disease with abdominal pain and nausea. Diarrheal symptoms mostly occur during the acute phase of the infection. The majority of *Giardia* infections are self-limiting, although reinfection and chronic infection can occur (Buret *et al*., 2015).

The pathophysiology of giardiasis is multifactorial and involves parasitic, host, dietary, and environmental factors, as well as immunological and non-immunological processes. The pathophysiology of acute diarrhea in giardiasis implicates increased rates of enterocyte apoptosis, a disruption of the intestinal barrier function, activation of host lymphocytes, CD8+ lymphocyte -mediated shortening of brush boarder microvilli with or without coinciding villous atrophy, disaccharidase deficiencies, small intestinal malabsorption, anion hypersecretion, and increased intestinal transit rates (Buret *et al*., 2015).

The small intestine is the site of the major structural and functional abnormalities associated with giardiasis. Mild or moderate partial villous atrophy or subtotal villous atrophy occurs in severe cases. An increase in crypt depth may be seen, and microvillus shortening or disruption may occur. Deficiencies in epithelial brush border enzymes, such as lactase, may develop (Buret, 2009).

Loss of epithelial barrier function is a result of *Giardia*-induced enterocyte apoptosis. These effects may facilitate the development of chronic enteric disorders, including inflammatory bowel disease, irritable bowel syndrome, and

allergies (Buret *et al.,* 2008). Reported changes are an increase in intraepithelial lymphocytes in the jejunum, with moderate to severe diffuse inflammation, crypt distortion and a reduction in villus to crypt ratio (Radostits *et al*., 2009).

In addition to duodenal involvement, the gall bladder may occasionally become parasitized by G. intestinalis. In such individuals, there may be associated gall bladder colic and jaundice, due to obstruction of the bile passages or irritation and edema (Buret *et al.,* 2015).

Pathogenic effects and virulence factors

Giardia lamblia causes infection of the small intestine, which leads to malabsorption and chronic diarrhea. In giardiasis, mucosal surface area per unit serosa area is decreased to 75% of control, as a result of which, epithelial resistance of giardiasis biopsy specimens is decreased. *G. lamblia* infection causes epithelial barrier dysfunction and increased epithelial apoptosis (Troeger *et al*., 2007).

Malabsorption and maldigestion mainly result from a diffuse shortening of epithelial microvillus and a loss of total epithelial brush border surface area, and diffuse microvillus shortening is mediated by activated host T- lymphocytes. This activation is secondary to *Giardia*- induced disruption of epithelial tight junctions, which in turn increases intestinal permeability. This parasite may breach the epithelial barrier by inducing enterocyte apoptosis. These effects may facilitate the development of other enteric disorders in infected patients. Recent observations indicate that host epithelial nitric oxide responses, as well as a newly discovered glucose-mediated cytoprotective mechanism, may represent effective modulators of the epithelial apoptosis induced by these parasites. Infection appears to cause diarrhoea via a combination of intestinal malabsorption and hyper secretion (Buret *et al*., 2008; Buret, 2009).

Clinical manifestations of giardiasis are quite variable, ranging from the absence of symptoms to acute or chronic diarrhea, dehydration, abdominal pain, nausea, vomiting, and weight loss (Robertson *et al*, 2010)). The severity of disease is probably determined by the interplay between the virulence of the parasite, the developmental, nutritional, and immunological status of the host, the nature of intestinal microflora, and the presence or absence of other co-pathogens. Although different *G. duodenalis* assemblages may produce different toxins or metabolic products that may contribute to their pathogenic-

ity, studies of the possible association between *G.duodenalis* assemblages and virulence (indicated by the likelihood of causing diarrhea and other clinical symptoms, as many *G. duodenalis* infections are asymptomatic) have thus far produced inconsistent results. Overall, *Giardia*-induced diffuse shortening of brush border microvilli causes small intestinal malabsorption due to impaired absorption of water, glucose, and electrolytes.

Giardia trophozoites undergo fundamental changes to survive outside the intestine of their host by differentiating into infective cysts. Encystation entails the synthesis, processing, transport, secretion and extracellular assembly of cyst wall components. To survive within the intestine, *Giardia* undergoes antigenic variation, a process by which the parasite continuously switches its major surface molecules, allowing the parasite to evade the host's immune response and produce chronic and recurrent infections (Lujan, 2011).

A few virulence factors have been suggested on the basis of their targeted effects (Ankarlev *et al*., 2010). For example, ventral adhesive disc proteins and surface lectins ensure appropriate attachment to enterocytes of the upper small intestine, while the four pairs of flagella allow for movement, and variant surface proteins help evade host IgA-directed clearance (Ankarlev *et al*., 2010).

Variant surface protein expression may also confer zoonotic infectivity to *Giardia* isolates. The arginine deiminase produced by the parasite deprives intestinal epithelial cells of arginine, thereby impairing intestinal epithelial proliferation and nitric oxide production (Stadelmann *et al*., 2013). Alpha-2 giardin appears to be an assemblage A-specific protein, but its role in host– parasite interactions remains obscure.

An unknown 58-kDa product released by *Giardia* trophozoites has been reported to induce anion hypersecretion and intestinal fluid accumulation by activating signal transduction pathways in host enterocytes (Stadelmann *et al*., 2013). However, parasite products directly involved in the pathogenesis of giardiasis remain largely unknown.

Host defense to *Giardia* infection

Although current knowledge regarding the mechanisms of immunity to *Giardia* is limited, several studies have produced significant advances in the

understanding of innate and adaptive host responses against the parasite (Faubert, 2000).

Innate and adaptive immune systems act in synchrony to control *Giardia* infection. Innate immune mechanisms are the first line of defense against *Giardia* colonization. The mucus layer on the intestine surface and peristaltic movements constitute mechanical barriers to *Giardia* attachment. Antimicrobial peptides (AMP) released by paneth and other cells can kill trophozoites (Faubert, 2000).

Gut microbiota have an anti-*Giardia* effect by competition, direct toxicity or modulating the immune response. Additionally, microbiota contributes to preserve gut integrity. Mast cells release pro-inflammatory cytokines such as IL-6; mast cell degranulation promotes peristalsis. Gut epithelial and immune cells produce nitric oxide (NO), which has a cytostatic effect on *Giardia* trophozoites, inhibits excystation/encystation processes and contributes to peristalsis (Faubert, 2000).

Mast cells take up antigens from the lumen of the gut by endocytosis into peyer patches (PP) to induce immune responses. Dendritic cells (DC) play a role as a 'connector' between innate and adaptive immunity. Dendritic cells are localized in lamina propria and peyer patches where they can recognize antigens. Dendritic cells also can expand their dendrites into the intestinal lumen to take up antigens (Faubert, 2000).

Dendritic cells ingest and process *Giardia* antigens for further presentation to naive T cells by MHC class II molecules (MHC-II). Activated T cells release a panel of cytokines, which modulate the anti-*Giardia* response. IL-6 released by mast cell, DC or T cells is an important modulator of B-cell maturation, and it induces antibody class switching to produce IgA. Plasma cells migrate to lamina propria to release IgA, which can inhibit *Giardia* attachment to intestinal epithelial cells. Th17 $CD4^+$ T cells are activated during early adaptive immunity against *Giardia,* and release cytokines such as IL-17, IL-21, IL-22, which play a pro-inflammatory role. Intra-epithelial lymphocytes (IEL) are mainly $CD8^+$ T cells and play a role in pathological damage of intestine during giardiasis (Faubert, 2000).

Public health significance of giardiasis

Giardiasis exerts a significant public health impact because of the high prevalence and disease burden of the infection, its propensity/inclination in causing major outbreaks and emergency responses, and its effects on growth and cognitive functions of infected children. Giardiasis is also a common disease in livestock and companion animals; thus, it is of veterinary health importance. In General, giardiasis is highly under reported for various reasons.

Infection rates for giardiasis in humans are generally lower in developed countries. Previous studies reported infection rates of 4.0% in Belgium (Geurden *et al.,* 2009), 1.5% in Germany (Sagebiel *et al*., 2009), 0.4% to 6.2% in Italy (Crotti *et al*., 2005, Giangaspero *et al*., 2007), 3.7% in Portugal (Almeida *et al*., 2006), 5.4% in Spain (Manzardo *et al*., 2008), 1.3% in the United Kingdom (Davies *et al*., 2009), 1.4% in the United States (Church *et al.,* 2010), 2.5% in South Korea (Huh *et al*., 2009), 1.6% to 7.6% in Australia, and 7.6% in New Zealand (Hellard *et al*., 2000).

Most of the surveys were conducted with asymptomatic children. The occurrence of giardiasis is probably higher in children with diarrhea. Thus, in the Nordic countries of Denmark, Finland, Norway, and Sweden, the infection rate of giardiasis was estimated to be 2.9% and 5.8% for asymptomatic and symptomatic persons, respectively (Horman *et al*., 2004).

A similar trend was seen in the Netherlands, with infection rates of 14.0% in patients with persistent diarrhea and 2.0% in asymptomatic subjects (Homan and Mank, 2001). Although *Giardia* is not considered an opportunistic pathogen in immunocompromised patients, the infection rates of giardiasis in HI infected people ranged from 3.5% to 6.2% in Italy before the introduction of highly active antiretroviral treatment (Giangaspero *et al*., 2007).

Relatively few *Giardia* genotyping studies have been conducted in Africa, however available reports reveal that five *G. duodenalis* assemblages (A, B, C, E and F) have been identified in humans. In Africa, Assemblage B was the most prevalent among typed samples (19.5– 100%) in 18 out of 28 studies reviewed (Ryan, 2017). Although many studies have reported that *Giardia* is not associated with severe diarrhea, one study reported that the prevalence of *G. duodenalis* Assemblage A was higher among children with vomiting and abdominal pain (Ignatius *et al*, 2012).

Assemblage C was detected in an adult immunocompromised male suffering from bladder cancer and diarrhoea in Egypt (Soliman *et al.* 2011) and Assemblage F was reported in six diarrhoeal and one asymptomatic individual in Ethiopia (Gelanew *et al.,* 2007). In that study, four of the identified Assemblage F isolates were mixed infections with Assemblage A. Assemblage E has been reported in humans in three separate studies in Egypt with a prevalence of up to 62.5% in one study population (Helmy *et al.,* 2014). Subtyping studies in Africa have identified sub assemblages AI, AII, BIII, BIV and various novel sub assemblages (Ryan, 2017).

Outbreaks of Giardiasis in humans

Although most cases occur sporadically, outbreaks of giardiasis are well documented. A review by Karanis *et al.,* 2007, indicated that there have been at least 132 reported waterborne outbreaks of giardiasis worldwide since 1954. Among them 104 were related to drinking water, 18 were related to recreational water and ten were related to foreign travel. The majority of the outbreaks were reported in North America and Europe because of better surveillance and reporting systems.

In addition, food-borne outbreaks of giardiasis linked to infected food handlers and food handlers who changed diapers of infected children prior to handling food have been reported (Hoffmann *et al*., 2007). Outbreaks resulting from person to person transmission in child care centers are also common (Ang, 2000). Few direct animals to human outbreaks have been documented.

Sources of infection for humans

Contaminated water

A review of waterborne disease outbreaks, between the years of 2004 and 2010, stated that *G. duodenalis* accounted for 70/199 (~35%) reported outbreaks of human disease due to waterborne protozoa within high-income countries (Baldursson and Karanis, 2011). It was however noted that the majority of the data in these studies was taken from higher income countries, as low-income countries often lack the necessary systems allowing surveillance capabilities. The real-world burden of waterborne disease outbreaks with *G. duodenalis* will remain unknown until global surveillance is implemented. Young children are often at the forefront of the effects of diarrhoeal-causing disease organisms, such as *G. duodenalis*, which can have a large impact on development.

Effective filtration and identification of the parasite from water samples is becoming significantly important to minimize the risk of water-borne outbreaks (Baldursson and Karanis, 2011).

Contaminated food

Salad items and vegetables have been often highlighted as a foodborne risk for giardiasis. Salad and vegetable items which are (a) improperly washed to remove cysts or (b) washed, but in contaminated water, and (c) poor food handler hygiene can lead to human giardiasis (De Lalla *et al.*, 1992).

Additional methods of foodborne giardiasis include infection via cold drinks (contaminated water frozen to make ice) (De Lalla *et al.,* 1992), shellfish which are contaminated with cysts (filter feeding shellfish can accumulate cysts within them) (Giangaspero *et al*., 2014).

Infected persons

Giardia duodenalis prevalence within the global human population varies between high- and low-income countries throughout the world, with infection estimates of between ~5 and ~20% respectively (Roxstrom-Lindquist *et al.,* 2006). Within the high-income countries, strict control and filtration of drinking water, as well as high-quality sanitation systems, prevent the disease being as prevalent as it is in poorer areas of the world (Roxstrom-Lindquist *et al.,* 2006).

Due to the cysts being infectious when excreted in feces, humans have the potential to pass on the pathogen to other humans either from direct contact or via transmission from contaminated sources, e.g. foods, most likely due to poor hygiene (Roxstrom-Lindquist *et al*., 2006).

Control and prevention

Treatment

Supportive treatment should be done; dehydration and electrolyte abnormalities should be treated symptomatically in humans (Handley, 1997).

Metronidazole, tinidazole, and furazolidone are effective drugs. Benzimidazoles (Fenbendazole, Mebendazole, Albendazole) have also been shown to be

effective in elimination of *Giardia lamblia (G. duodenalis)* in cattle (Handley, 1997). *Giardia* infections in agricultural animals have been successfully treated with Dimetridazole at a dose of 50mg/KgBW daily for 5 days (Radostits *et al*., 2009).

Preventive approach

Restricting cattle from surface water during periods of high shedding may reduce water shed contamination (McAllister *et al*., 2005). Cysts are relatively resistant to chlorination, but *Giardia* cysts in water can be inactivated with 4 mg/L of chlorine after 60 min at 5°C, at pH levels 6, 7 and 8. A chlorine concentration of 8 mg/L will inactivate *Giardia* cysts at pH 6 and 7 after contact for 10 min, and at pH 8 after 30 min. Also, at 25°C, *Giardia* cysts will be killed when exposed to 1.5 mg/L of chlorine for 10 minutes at pH 6. 6% H_2O_2 can be used as a surface disinfectant or in disinfection of spills. *Giardia* is inactivated by exposure to UV light (10 JM^{-2}). Cysts are also susceptible to boiling and freezing (Linden *et al*., 2002).

We can also decontaminate all wastes that contain or have come in contact with the infectious organism before disposing by autoclave, chemical disinfection, gamma irradiation, or incineration. The infectious agent should be stored in leak-proof containers that are appropriately labeled. Lab coat, gloves and eye protection must be used where there is a known or potential risk of exposure to splashes (Burnett *et al.,* 2009).

A *Giardia* vaccine (*Giardia*Vax®) is commercially available for dogs and cats but vaccine is not efficacious in preventing giardiasis or reducing cyst shedding in calves (Uehlinger *et al*., 2007).

G. duodenalis infection is widespread in varying magnitude among children and cattle. Significantly, higher prevalence of *G. duodenalis* is detected among children who have close contact with cattle than those who have no contact. This suggests that contact with cattle and their manure will be a risk factor for infection with this protozoan parasite. Therefore, molecular techniques are necessary to clarify the source of infection and transmission pattern of the parasites. Moreover, measures including health education on personal and environmental hygiene would help in reducing the prevalence of these parasites (Wegayehu *et al*., 2013).

Conclusions

Giardiasis is a protozoan infection affecting various domestic animals, wild animals and humans throughout the world. It has both zoonotic and veterinary importance. The causative agent, *Giardia doudenalis* has various assemblages with various host ranges. Transmission of the parasites usually occurs through fecal oral route from infected animals through cyst contaminated water and feed. The disease is very important in young animals, which are kept in confinements for instance in dairy farms, feedlots particularly in areas with poor hygiene. Various reports on the occurrence of giardiasis are available from developed countries however very few ones are reported from Ethiopia requiring attention to study its occurrence, epidemiology, transmission patterns of the parasite and its public significance. Giardiasis has both public health and veterinary significance being transmitted from infected animals to humans through drinking water, consumption of uncooked feed and through direct contact with infected animals. Detailed studies on the distribution and occurrence of giardiasis, pathogenic significance of *Giardia* and identification of circulating species/ genotypes of *Giardia* in domestic animals and humans in Ethiopia should be initiated.

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Assessment of bovine fasciolosis and the analytical test evaluation at Yirgalem municipal abattoir, Ethiopia

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Abstract

A cross-sectional study was conducted from November 2021 to April 2022 to estimate the prevalence of bovine fasciolosis, assess the risk factors, and estimate the direct financial loss due to liver condemnation in cattle slaughtered at Yirgalem municipal abattoir. Furthermore, the comparison of the diagnostic efficiency of fecal and postmortem examination was evaluated. The study was based on both postmortem inspection of the liver for liver fluke infection and coprological examination using the sedimentation technique. Thus, a total of 400 cattle were sampled using systemic random sampling techniques and the prevalence was 27.0% (108/400) and 20.8% (83/400), by postmortem and coprological examination, respectively. The prevalence of *F. hepatica, F. gigantica*, mixed infections, and unidentified species were 56.5%, 19.4%, 16.7%, and 7.4%, respectively. The difference between age groups was statically significant ($p<0.05$). Coprological examination revealed the highest prevalence was recorded in the age group of > 7 years old (34%, adjusted odds ratio [AOR]= 4.2) followed by 4-6 years old adults (26.8%, AOR=4.2) and in young cattle of <3 years old (6.7%). Prevalence by postmortem examination was 43.0% (AOR= 4 0), 27.8% (AOR= 2.4), and 8.3%, for age groups > 7 years old, 4-6 years old adults and in young cattle of <3 years old, respectively. The body condition score result revealed a significant difference ($p<0.05$) as a greater magnitude of infections were detected in poor body condition animals (AOR=7.7) than in medium and good body condition ($p<0.05$). Considering the liver examination as a gold standard for diagnosis of fasciolosis, the sensitivity of the sedimentation technique was 76.8%, and the specificity of 100% with a very good agreement (kappa = 0.94) between the two methods. The total financial loss per annum due to the condemnation of infected liver was 945, 999 ETB (18,192.288 USD). Therefore, strategic control and prevention of the snail intermediate host should be implemented. Furthermore, upon diagnosis of fasciolosis, epide-

miological information about the disease with suggestive clinical examination should be considered even in the absence of *Fasciola* eggs during coprological examinations and also other parasitological techniques such as the FLOTAC and FLUKE FINDER methods with high sensitivity and specificity should be employed.

Keywords: Abattoir; Cattle; Economic loss; Fasciolosis; Prevalence; Yirgalem.

Introduction

Fasciola species infect mainly ruminants (especially sheep and cattle), but humans can also become infected (Mas-Coma *et al*., 2009; Tadesse *et al*., 2019a, b). The effects of fasciola infection in livestock result in profound consequences of decreased production outcomes such as reduced weight gain and body condition scores, poor carcass quality and organ condemnation, decrease in reproductive performance, and, in acute cases, sudden death in sheep has been well-documented (Dalton, 1999; Taylor *et al*., 2007; Mas-Coma *et al*., 2014; Tadesse *et al*., 2019b). Despite the significant human and animal health impacts, it remains a neglected disease, especially in developing countries (Mas-Coma *et al*., 2009; Cwiklinski *et al*., 2016).

F. hepatica infects more than 300 million cattle and 250 million sheep worldwide and, together with *F. gigantica* causes significant economic losses to global agriculture estimated at more than US$3 billion annually (Keiser and Utzinger, 2005; Torgerson and Macpherson, 2011). The study on Ethiopian ruminant livestock in five abattoirs indicated an estimated annual loss of over 7, million ETB or more than 335, 697.1 USD due to liver condemnation alone (Tadesse *et al*., 2019b).

The total cost of bovine fasciolosis in Turkey was estimated as 7.4 million US$ (6.1–8.8) for beef cattle and 35.4 million US$ (28.9–42.6) for dairy cows (Sariozkan and Yalc, 2011). This cost was estimated based on a 1.9% mean prevalence of fasciolosis to calculate the total cost of a disease. The cost will be much higher if it was estimated by the current 20.8% and 27% prevalence that were obtained in the present finding through coprological and postmortem examination at Yirgalem, respectively.

Infection with *F. hepatica* has been shown to modulate the immune system of infected hosts by causing a shift towards a T helper 2 (Th2) cell response,

leaving hosts more susceptible to infection with other bacterial pathogens such as *Mycobacterium bovis* and *Bordetella pertussis* (Brady *et al*., 1999; O'Neill *et al*., 2001). This immunomodulation has also been shown to confound the outcomes of diagnostic tests for diseases, including bovine tuberculosis, due to the suppression of an effective Th1 response, increasing the rate of false-negative results (Garza-Cuartero *et al*., 2016).

The prevalence of fasciolosis is global and increasing due to a changing climate, emerging anthelmintic resistance, and adaptations in agricultural practices (Coles *et al*., 2005; Mas-Coma *et al*., 2014; Cwiklinski *et al*., 2016; Fairweather, 2020). In Ethiopia, where the majority of production systems are grassland-based with an extensive type of production system, the cost of fasciolosis to the Ethiopian livestock sector will be estimated to increase annually. In fact, in the highlands of Ethiopia, communal grasslands are critical resources that provide livestock feed to farmers and ecosystem services to a substantial sector of society (FAO, 2018; Eba and Sircely, 2021). In rural settings of traditional extensive types of livestock keeping of the country, diagnosis, and monitoring of ruminant fasciolosis are challenging. In Ethiopia, an indication of the liver fluke presence on ruminants on farms often comes from liver condemnation reports (Tadesse *et al*., 2019 a, b; Tadesse and Usman, 2022), but these are based on visual evaluations and therefore not standardized between abattoirs.

The effective characteristics of diagnostic tests are commonly evaluated in terms of diagnostic sensitivity and specificity. Sensitivity reflects the ability of a test to correctly identify those individuals that are infected and specificity is the ability of a test to correctly identify those uninfected as negative (Thrusfield, 2007). There are several antemortem fluke diagnostic tests available, but no one test can be considered as having adequately high sensitivity and specificity in the field setting (Mazeri *et al*., 2017; Rapsch *et al*., 2006). The diagnosis of acute fasciolosis is implemented by the determination of serum hepatic enzyme activities which are released from the damaged hepatic cells (Matanovic *et al*., 2007). Clinical diagnosis and positive fecal egg count along with increasing levels of gamma-glutamyl transferase (GGT) confirm the presence of chronic fasciolosis. Confirmatory diagnosis for fasciolosis is based on the demonstration of characteristics of *Fasciola* eggs through standard examination of feces in the laboratory and the presence of immature and mature flukes in the liver through postmortem examination (Khan, 2005).

Even though several researchers (Tadesse *et al*., 2019 a, b; Tadesse and Usman, 2022) in the country have investigated many aspects of the parasite; specific studies are always needed at a particular site to update different aspects of fasciolosis; disease status and the most prevalent species on regular basis. Moreover, most of the available studies only calculated the cost of the condemned liver due to bovine fasciolosis and ignored the analytical test evaluation and important potential risk factors. To this end, the objectives of this study were to estimate the prevalence of bovine fasciolosis, assess the potential risk factors, and estimate the direct economic loss exerted by the disease at Yirgalem abattoir. Furthermore, the techniques used for the detection of *Fasciola* and the intensity of the disease such as fluke burden and liver lesions were evaluated.

Materials and methods

Study area

The study was conducted at Yirgalem municipal abattoir in Yirgalem. Yirgalem is the Dale woreda administrative town in Sidama Region and 320km far from Addis Ababa. Geographically the town lies between 6°27'00" - 6° 51'00" N latitude and 38°00'00" -38°37'00"E longitude. The altitude ranges from 1650-2800 m.a.s.l. The mean annual temperature ranges between 9.6°C and 29.2°C. The area has a bimodal rainfall pattern with the first peak from April to May and the second peak from August and October. The lowest rainfall was recorded between November and February. The mean annual rainfall at the Awada Research sub-center in Yirgalem is 1314 mm (Gelfeto, 2019). The study areas encompassed as the source of animals for slaughter were Bore, Arsi-Negele, Dimtu, and Wonshu. These are areas in close proximity to the abattoir and have variable climatic (rainfall and temperature) condition and geographical location (SRADO, 2021).

Study animals

The study animals comprised all cattle slaughtered at the Yirgalem municipality abattoir. The study population includes all cattle that were brought to the abattoir from different areas for slaughtering purposes and were managed under an extensive and semi-intensive production system. This population comprised different breeds, sex, age, body condition groups, and cattle originating in and around Yirgalem city. Sometimes it is difficult to trace the origin of the

animals as they usually pass a chain of markets and due to the lack of reliable animal identification methods to relate the findings to a particular locality.
Age determination was categorized based on dental characteristics into three such as young, adult, and old (Ensminger, 1992). The data were collected according to recording *preformat*: Young (1-3 years), adult (3-6 years), and old (above 6 years). Also during the survey, the sex and breed of animals were recorded.

The body condition of the study animals was scored based on the criteria set by Nicolson and Butterworth (1986). Accordingly, the body condition was grouped into three, poor, medium, and good.

Study design

A cross-sectional study was conducted from November 2021 to April 2022.

Sampling strategy

A total of 400 samples were collected during the study period from cattle slaughtered in the Yirgalem municipal abattoir by using a systematic random sampling technique.

Sample size

The animals were selected by using a systematic random sampling method where a sampling interval of 7 animals was used. To determine the sample size, an expected prevalence of 50% was taken since there was no prior research carried out in the area. The sample size was calculated using the formula given by Thrusfield (2007). The 95% confidence interval and 5% absolute precision were taken.

$n = (1.96)^2 \text{ pexp } (1- \text{ p exp})/d^2$

Where,

n= Number of samples

d= Desired absolute precision

p = expected prevalence

$n = (1.96) \text{ X } 50\% \ (1- 50\%)/ \ (5\%)^2$

$= 3.8416\text{X}0.5\text{X}0.5)/ \ (0.05)^2$

$0.9604/ \ 0.0025 = 384$ cattle

Therefore, using a 95% confidence interval, 5% precision, and 50% expected prevalence, the number of cattle needed was 384, but to increase the precision 400 cattle were sampled.

Sampling methods

Coprological examination

Before sampling, an identification number was given to each animal presented to the abattoir for slaughter. At the time of sampling, 15-20 animals were selected in the lairage where the date of sampling, the sex, the origin, the body condition, the management system (through the interview), and the breed of the cattle were recorded for each cattle on a recording format. Faecal samples were collected directly from the rectum of each study animal, using disposable plastic gloves, and placed in a clean universal bottle and each sample was clearly labeled. Faecal samples were preserved with 10% formalin solution to avoid the eggs developing and hatching. The samples were transported to Hawassa University Veterinary Parasitology laboratory on the same day of collection and examined for *Fasciola* eggs by using sedimentation as described (Hansen and Perry, 1994). And their specificity and sensitivity were interconnected with post-mortem findings. Samples that were not processed within 24 hours were stored in a refrigerator at 4°C.

Abattoir work

Antemortem and postmortem examinations were conducted by visiting the abattoir two days a week. At the time of antemortem examination, detailed records about the species, breeds, sexes, origins, fecal consistency, and body conditions of the animals were performed. In the postmortem examination, the previously identified animals and their livers were carefully supervised and examined, to avoid mixing up the organs to be inspected with the faecal samples and to correlate the coprology and postmortem examination of each animal. The fluke recovery and count were conducted following the approach of (Taylor *et al*., 2007), as follows: the gall bladder was removed and washed to screen out mature flukes. The liver was cut into slices about 1 cm thick and put in a metal trough of warm water to allow mature flukes lodged in smaller bile ducts to escape and then the heads of the flukes were counted. Identification of the species involved was carried out using the size parameters described by (Soulsby, 1982; FAO, 2003), whereas pathological lesion categorization of the

affected livers was undertaken based on the intensity of lesions. Hence, affected livers were grouped into three categories as per the criteria previously described by Ogunrinade and Adegoke (1982) as lightly, moderately, and heavily affected.

Species identification

The liver of each study animal was carefully examined for the presence of lesions suggestive of *Fasciola* infection externally followed by slicing for confirmation. Liver flukes were recovered for the differential count by cutting the infected liver into fine, approximately 1 cm slices with a sharp knife. The sample was placed in a universal bottle and transported to Hawassa University Veterinary Parasitology laboratory. Species identification and further investigation were conducted on recovered Fasciola based on morphological features of the agents and classified into *Fasciola hepatica*, *Fasciola gigantica*, mixed infection by both flukes and unidentified or immature forms of liver fluke (Urquhart *et al.,* 1996; FAO, 2003).

Assessment of direct financial loss

Several parameters were used to estimate the losses attributable to liver condemnations in slaughtered cattle to determine the direct annual financial loss. Such parameters were taken by considering the overall prevalence of the disease, the total number of animals slaughtered annually in the abattoir, and the retail market price of the liver. The annual number of animals slaughtered was estimated from retrospective abattoir records of the last three years, while the average selling price of cattle livers was established through a survey which was conducted in various meat shops in the town. The average current price of one liver in Yirgalem town was about 900 ETB. All affected livers were rejected since partial approval was not practiced in the abattoir. The collected information was subjected to mathematical computation using the formula set by (Ogunrinade and Ogunrinade, 1980).

ALC = MCS x MLC x P Where ALC=Annual loss from liver condemnation,
MCS= Mean annual cattle slaughtered at Yirgalem abattoir,
MLC= Mean cost of one liver in Yirgalem town,
P= Prevalence of the disease at the study abattoir.

Data management and data analysis

The raw data were entered and managed using a Microsoft Excel worksheet and summarized with descriptive statistics. STATA statistical software version 14 was used to determine the association between prevalence and hypothesized risk factors. Many attribute data that were imported to the database system, include information on the origin, age, sex, breed, body condition, detection of fluke eggs, and the liver fluke. In addition to these, the number of livers condemned, species of the flukes, and worm counts were entered for abattoir data analysis. Variations in infection prevalence between species, age, sex, breed, origin, and body condition were determined and evaluated by using logistic regression analysis. The output of the statistical analysis is considered significant when the p-value is less than 0.05 at a 95% confidence level.

Moreover, the sensitivity and specificity of the sedimentation method were assessed by taking a necropsy examination as a gold standard for the diagnosis of fasciolosis. Kappa statistic was used to determine the degree of agreement between the two diagnostic methods employed (fecal sedimentation technique and necropsy finding) in the study. The kappa value was interpreted as: no agreement ($k < 0.0$); slight agreement ($k = 0.1$-0.2); fair agreement ($k = 0.2$ to 0.4); moderate agreement ($k = 0.4$ to 0.6); substantial agreement ($k = 0.6$ to 0.8); and almost perfect agreement ($k > 0.8$) (Thrusfield, 2007).

The formula for Cohen's kappa is calculated as:
$k = (po - pe) / (1 - pe)$ = observed agreement–chance agreement / 1- chance agreement
where:
po: Relative observed agreement among the analytical tests
pe: Hypothetical probability of chance agreement

Results

Coprological examination

The coprological examination of 400 cattle slaughtered at the Yirgalem municipal abattoir during the six months study period detected 20.8% (83) of animals for *Fasciola* eggs. The highest prevalence of fasciolosis was observed during March (33.8%) followed by April (25.7%), November (23.2%), February (22.7%), December (14.9%), and January (9.6%). Similarly, the prevalence

among young, adult, and aged revealed the prevalence of 4 (6.7%), 60 (26.8%), and 19 (34%), respectively, and the difference was statistically significant ($p<0.05$) (Table 1).

Moreover, the assessment of the breed and management system revealed significant disparity ($p<0.05$) as a greater magnitude of infections were detected in local and animals reared extensively. However, there is no significant difference between sex, origin, and altitude ($p>0.05$). Variation in prevalence was observed among poor, medium, and good body condition scores with a prevalence of 48% (12/25), 25.2% (61/242), and 7.5% (10/133), respectively and the difference was significant ($p<0.05$) (Table 1).

Table 1. Prevalence of fasciolosis and associated risk factors using the coprological examination

Variable	Category	No of examined	Number +Ve (%)	AOR	95%CI	p-value
Sex	Male	297	57(19.2)	1.1	0.6-2	0.757
	Female	103	26(25.2)			
Age	<3	60	4(6.7)	2.7	0.9	0.089
	4-6	224	60(26.8)		8.7	0.038
	>7	56	19(34.0)	4.2	1.1-16	
Breed	Local	347	80(23.0)	1.5	0.9-12	0.683
	Cross	53	3(5.7)			
Management system	Extensive	326	79(24.2)	13	2-84.6	0.008
	Semi-intensive	74	4(5.4)			
BCS	Poor	25	12(48.0)	2.4	0.8-6.9	0.113
	Medium	242	61(25.2)	7.7	2-27.7	0.003
	Good	133	10(7.5)			
Month	November	43	10(23.2)	2.8	0.92-8.5	0.07
	December	74	11(14.9)	6.4	2-21.3	0.003
	January	83	8(9.6)	1.8	0.6-5	0.293
	February	97	22(22.7)	1.3	0.38-3.3	0.82
	March	68	23(33.8)	1.4	0.4-5	0.576
	April	35	9(25.7)			
Altitude	Midland	264	60(22.7)	1.9	0.9-5.9	0.093
	Lowland	94	10(10.6)	1.2	0.3-4	0.832
	Highland	42	13(31.0)			

Variable	Category	No of examined	Number +Ve (%)	AOR	95%CI	p-value
Origin	Tulla	66	14(21.2)	3.1	0.8-12.2	0.09
	Loka abay	68	6(8.8)	1.8	0.7-4	0.201
	Arsi	31	7(22.6)	1.12	0.4-2.7	0.848
	Wonsho	73	18(26.7)	1.1	0.42-3	0.788
	Dale	91	21(23.1)	1.1	0.3- 3.2	0.892
	Bore	42	13(31)			
	Dimtu	29	4(13.8)			

BCS=Body Condition Score, +ve= Positive; AOR=Adjusted Odd Ratio; CI= Confidence Interval; p-value= Probability value

Faecal egg count based on the severity

Among 83 coprological positive animals, faecal egg count-based severity was performed and 52(62.6%), 19(22.9%), and 5(6.0%) were affected lightly, moderately, and heavily, respectively (Table 2).

Table 2. Prevalence of fasciolosis and level of egg burden

EPG	No examined	Number of +Ve (%)	Severity	Min.	Max.
0	83	7(8.4)	None	-	-
1-5	83	52(62.6)	Light infestation	100	500
6-10	83	19(22.9)	Moderate infestation	500	1000
11-13	83	5(6.0)	Heavy infestation	1000	1500

Where, Min=Minimum, Max= Maximum

Postmortem examination

Out of 400 cattle slaughtered at the abattoir and examined for fasciolosis, 27.0% (n=108) of livers were positive for *Fasciola* species. Among 108 livers having fluke infection during postmortem inspection, 56.5% (61) *F. hepatica*, 19.4% (21) *F. gigantica*, 16.7% (18) mixed infections and 5% (5) contained unidentified immature fluke (Table 3).

The highest prevalence was seen in March (41.1%) and April (40%) while the lowest was in January (14.4%). "The comparison according to the body condition score showed higher prevalence in animals with poor body condition (60.0%) compared to animals with moderate (30.6%) and good body condition (14.3%). During postmortem examination, the prevalence of fasciolosis was proved to be higher in local cattle 103 (29.7%) than cross breed 9 (12.2%) animals and the difference was significant ($p< 0.05$) (Table 3). The prevalence between the

six different areas in the present study showed the presence of variability in the prevalence of bovine fasciolosis between them. The highest (42.9%) and the lowest (16.2%) prevalence of bovine fasciolosis were observed in the Bore and Loka Abay areas, respectively. Similarly, the prevalence among young, adult, and aged revealed the prevalence of 5(8.3%), 79(27.8%), and 24 (43%), respectively with significant differences ($p< 0.05$) (Table 3). Furthermore, the management system revealed a significant difference ($p< 0.05$) as a greater magnitude of infections were detected in animals reared extensively (30.3%) (Table 1). However, there was no statistically significant difference ($p> 0.05$) in the prevalence of fasciolosis between the sexes and the altitude differences (Table 3).

Table 3. Prevalence of fasciolosis in cattle using postmortem results and the associated risk factors

Variable	Category	No. examined	Number +Ve (%)	AOR	95% CI	p-value
Sex	Male	297	74(25.0)	1.05	0.6-1.9	0.855
	Female	103	34(33.0)			
Age	<3	60	5(8.3)	2.4	0.86-6.8	0.093
	4-6	284	60(27.8)	4.0	1.2-13.3	0.024
	>7	56	24(43.0)			
Breed	Local	347	103(29.7)	1.44	0.3-7.24	0.683
	Cross	53	5(9.4)			
Management system	Extensive	326	99(30.3)	4.4	1.1-20	0.36
	Semi-intensive	74	9(12.2)			
BCS	Poor	60	15(60)	3.2	1.14-9	0.027
	Medium	224	74(30.6)	6.6	1.96-25	0.002
	Good	56	19(14.3)			
Month	November	43	15(34.9)	2.5	0.91-7.14	0.075
	December	74	16(21.6)	5.0	1.7-16.6	0.003
	January	83	12(14.4)	2.0	0.76-5.2	0.157
	February	97	23(23.7)		0.41-2.93	0.845
	March	68	28(41.1)		0.4-3.6	0.780
	April	35	14(40)			
Altitude	Midland	264	71(26.9)	1.12	0.42-2.32	0.92
	Lowland	94	19(20)	1.2	0.45-2.7	0.867
	Highland	42	18(42.9)			

Origin	Tulla	66	15(22.7)	2.37	0.69-8.1	0.168
	Loka abay	68	11(16.2)	1.4	0.6-3.2	0.477
	Arsi	31	8(25.8)	1.06	0.45-2.5	0.878
	Wonsho	73	22(30.1)	1.22	0.46-3.21	0.68
	Dale	91	26(28.6)			
	Bore	42	18(42.9)			
	Dimtu	29	8(27.6)			

AOR= Adjusted Odds Ratio; +Ve = Positive; CI= Confidence Interval; p-value= probability value

Liver fluke burden and degree of pathological lesions

A total of 2414 flukes were recovered from the infected bovine livers examined. Of these, 57 (52.8%), 46 (42.6%), and 5 (4.6%) were affected lightly, moderately, and severely, respectively (Table 4).

Table 4. Categorization of livers according to the severity of lesions and their fluke burdens

Fluke count Interval	Severity of lesions	No of livers	Relative proportion (%)	Min	Max
4-20	Lightly affected	57	52.8	4	20
21-49	Moderately affected	46	42.6	21	49
50-57	Severely affected	5	4.6	50	57
	Total	108	100		

Where: Min= Minimum, Max= Maximum

Fasciola species identification

From the total of 108 infected livers, infections with *F. hepatica* and *F. gigantica* occurred with a prevalence of 56.5% (n =61) and 19.4% (n = 21), respectively, while mixed and unidentified species or immature fluke were 16.7% (n = 18) and 7.4% (n =8) (Figure 1).

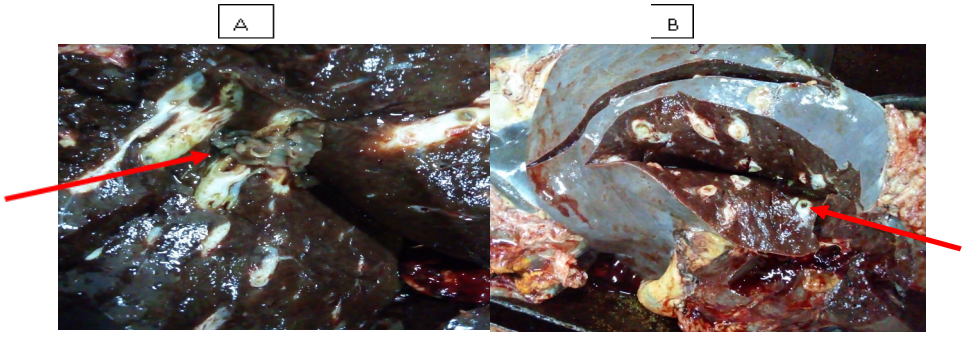


Figure 1: Infected bovine liver samples. (A) Adult parasite (*arrow*) (B) Thickening of the bile duct (*arrow*)

Table 5. Prevalence of *Fasciola* species in cattle slaughtered from the total Positives

Fasciola species	No. of infected liver	Prevalence (%)
Fasciola hepatica	61	56.5
Fasciola gigantica	21	19.4
Mixed	18	16.7
Immature	8	7.4
Total	108	100

The diagnostic test evaluation

From the total of 400 cattle examined for the presence of *Fasciola*, postmortem findings revealed better results (27.0%) than coprological examination (20.8%), which showed us that the sedimentation technique used for *Fasciola* egg assessment failed to detect eggs from some faecal samples (Table 6)

Table 6. Measuring the sensitivity, specificity, and agreement level of the two tests

Fecal examination	Postmortem examination		Total	Kappa
	Positive	Negative		
Positive	83(a)	0(b)	83	0.84
Negative	25(c)	292(d)	317	
Total	108	292	400	

Sensitivity= [a/ (a+c)] ×100 = 83/108= 76.8%

Specificity= [d/ (b+d)] ×100 = 292/292= 100%

Economic loss analysis

The total economic loss due to fasciolosis in Yirgalem municipal abattoir was estimated from the summation of annual liver condemnation. Partial condemnations of the liver were not a common practice in this abattoir. The average retail price of one liver (one liver=3kg) in town was taken as 900 ETB. The average numbers of cattle slaughtered in the Yirgalem municipal abattoir were 3893 cattle per year based on 3 years of recorded data. Accordingly, the annual loss from liver condemnation at Yirgalem municipal abattoir was found to be 945,999 ETB (18,192.288 USD).

Discussion

The present result disclosed fasciolosis as an economically important disease in the study area. Therefore, the findings of this study have relevance and significance to cattle owners, veterinary personnel in the area, and livestock authorities. Accordingly, the coprological and postmortem examination revealed a prevalence of 20.8% and 27.0, respectively. Comparable results of 19.4% and 26.8% prevalence were disclosed at Wolaita Sodo municipal abattoir through coprological and postmortem studies, respectively (Assefa *et al*., 2015). Similar findings unveiled by Alemu and Mekonen (2014) at Dangila municipal abattoir revealed a prevalence of 22.1% and 30.2%, whereas a different record by Mulat *et al*. (2012) at the Gonder ELFORA abattoir revealed a similar prevalence of 19.5% and 29.8% both by coprological and postmortem examination, respectively.

This could be related to the fact that the development of *Fasciola* eggs in the feces takes eight to fifteen weeks after infection, uneven egg discharge to the duodenum, and specific groups of animals that may have had pharmacological therapy before being transported to the abattoir. Furthermore, because eggs are released intermittently depending on the evacuation of the gall bladder and the life cycle of *Fasciola*, the detection of *Fasciola* eggs and the emergence of the disease in some locations was difficult to detect during the prepatent period (Radostits *et al*., 2007; Tadesse *et al*., 2021).

A comparison of the current findings disclosed that there was a significant difference in the prevalence of fasciolosis found through coprological (20.8%) and postmortem examinations (27.8%). The current result was significantly lower than that of 50.8% coprological prevalence previously reported by Tadesse *et al*., (2019b). Tadesse *et al*. (2019a) reported significantly higher overall coprology, and abattoir prevalence of 59.2%, and 81.0% in cattle in Debre Berhan. In the present study, the overall prevalence of fasciolosis (27.8%) observed in post-mortem examination was relatively similar to 26.8% in Wolaita (Asefa and Belay, 2015), 28.63% in Hawassa (Rahmeto *et al.,* 2010) and 28% in Kombolcha (Nuraddis *et al.,* 2009). But the prevalence in this study was lower when compared to 35% in Kombolcha by George Opio (2021), 54.5% in Jimma by Abie *et al*., (2012), 45.3% in Bahir dar by Ayalew *et al*., (2013) and 56.2% in and around Chora woreda by Tadelle and Behablom (2018). On the other hand low prevalence (14.4%) has been reported from Wolaita Soddo municipal abattoir

(Adane, 2019), 20.3% in Addis Ababa (Kassaye *et al.,* 2012) and 21.3% in Bonga municipal abattoir by (Simegnew *et al*., 2016).

Variations in the origin of the samples, ecological and climatic conditions such as altitude, rainfall, and temperature; animal management systems, sample size, and the inspector's ability to detect the infection may all play a role in the difference of the prevalence of the current study among other researchers' findings across the country. The availability of suitable habitat for the snail intermediate hosts, which is necessary for the development of fluke eggs, miracidia hunting for snails, and cercariae dissemination, is one of the most important elements that influence the occurrence of fasciolosis in a given location (Urquhart *et al.,* 1996; Dalton *et al*., 1999; Taylor 2007).

The species identification on 108 *Fasciola* infected livers disclosed that 56.5% of them were positive for *F. hepatica,* whereas, *F. gigantica*, mixed infection and immature or undifferentiated were recorded to be 19.4%, 16.7%, and 7.4%, respectively. These findings are in agreement with the reported prevalence in cattle with *F. hepatica* (46.1%), *F. gigantica* (27.3%), mixed infection (18.2%), and immature (9.1%) by Alemu and Mekonen (2014), *F. hepatica* (42%), *F. gigantica* (27.6%), mixed infection (19.0%) and immature (11.2%) by Asefa and Belay (2015), *F. hepatica* (42%), *F. gigantica* (27.6%), mixed infection (19.0%) by Paulos (2021) at Addis Abeba abattoir.

However, concerning the proportion of *Fasciola* species identified, the present finding was lower than the findings of Tadele and Worku (2007) and Nuraddis *et al*. (2009). These authors demonstrated the predominant species of bovine fasciolosis in Jimma municipality and Kombolcha Industrial abattoirs as *F. hepatica* (63.89%; 24.8%) followed by *F. gigantica* (63.6%; 24.3%), respectively. The prevalence of fasciolosis and the *Fasciola* species showed variability with the locality.

In Ethiopia *F. hepatica* and *F. gigantica* infections occur in areas above 1800 m.a.s.l. and below 1200 m.a.s.l., respectively which has been attributed to variations in the climatic and ecological conditions such as altitude, rainfall, temperature, and livestock management system. The high prevalence of *F. hepatica* may be associated with the existence of favorable ecological biotopes for *L. truncatula*, the recognized intermediate host of *F. hepatica* in Ethiopia. Asefa and Belay (2015) revealed a relatively small proportion of cattle infected with *F. gigantica* alone or mixed infections by both species. This may be ex-

plained by the fact that most cattle for slaughter came from high land and middle altitude zones and therefore drainage ditches are favorable habitats to the intermediate hosts (Tadesse *et al*., 2019a; Taylor, 2007; Urquhart *et al*., 1996). Studies in other countries of Africa showed that *F. gigantica* was the predominant species encountered (Phiri *et al*., 2006; Yabe *et al*., 2008) whereas, in Europe, the Americas, and Oceania only *F. hepatica* is concerned (Mas-Coma *et al*., 2005).

The current study indicated that animals with good body conditions had less prevalence of *Fasciola* infection. There was a statistically significant association between *Fasciola* infection and the body condition of the animals. Good body-conditioned animals were 6.6 times less likely to be infected as compared to poor body conditions. Concerning the body condition of the animals, the abattoir prevalence was higher in those animals with poor body conditions (60%) than in those with medium (30.6%) and good body conditions (14.3%). In support of this finding, a study conducted in five abattoirs in Ethiopia (Tadesse *et al*., 2019a) and another study in Bahir Dar, Ethiopia (Ayalew *et al*., 2013) indicated a significant association between the prevalence of fasciolosis and body condition of the animals. Reports of Mojo *et al*.(2014) were also in line with the current finding.

The reason for this could be because *Fasciola* worms drain blood and tissue fluid and damage the parenchyma of the liver due to migrating immature worms, and decreases body condition. It indicates the significance of fasciolosis in producing weight loss, which is a symptom of the disease (Radostitis *et al*., 2007; Asefa and Belay, 2015).

The monthly variation in the prevalence of fasciolosis has been studied for six dry months in the study area. It was difficult to indicate the effect of seasonal variation on the prevalence of bovine fasciolosis since the study period was too short without incorporating wet months of the season. An accurate description of seasonal occurrence requires long-term epidemiological investigation over several years. Even though the study period is short and in the dry season, the highest prevalence was seen in March (33.8%, 41.1%) and April (25.7%, 40%), and November (23.2%, 34.9%) while the lowest was in January (9.6%, 14.4%) both in coprology and postmortem examination. There was a statistically significant variation in the prevalence of fasciolosis in the study month of January and other months.

The significant variation in these months may be due to the area has been receiving additional rainfall in October and November, and short rain in March and April, This provides a favorable environment for the snail population. Moreover, the infection was reported throughout the year due to resistance of metacercariae to desiccation, especially during the dry season, and the continued presence of shallow water, enough vegetation, and humidity for continued exposure of the animals to encysted metacercariae and no restriction on cattle grazing habits and movement between the infected and treated localities (Nazima *et al*., 2016).

In this study, a significant variation was revealed in the prevalence of *Fasciola* between different age groups. The least infection in the age group <1-3 years might be due to the possibility of fewer chances of acquiring infection due to short exposure time as compared to older animals. This might be because adults were separated from their young's for grazing in the field whereas most young's were tethered at homesteads. Due to this reason, most adults might have a high chance of exposure to infective stages (encysted metacercariae). This finding agrees with the works of Fikirtemariam *et al.*(2013) and Teklu *et al*.(2015).

This result indicated a significantly lower prevalence of fasciolosis in cross-breed (9.4%) ruminants than the locals (29.7%). This might be due to the slaughter of a lower number of cross-breed ruminants. There was a statistically significant association between fasciolosis with breeds. Our results are in agreement with a study conducted by Teklu *et al*. (2015). This difference in prevalence based on breed might be due to the management of the animals as most of the indigenous animals were reared in an extensive system of management which makes them easily exposed to the parasites. Also, the same result was found in extensive and semi-intensive management systems, with a significant association in prevalence between extensive (30.3%) and semi-intensive (9.4%) cattle in necropsy findings. This might be because animals in extensive management systems were usually kept for grazing in the field whereas those animals in semi-intensive management systems were kept at homesteads. Due to this reason, most animals in extensive management systems might have a high chance of exposure to infective stages (encysted metacercariae). The maximum and minimum fluke burden observed in this study was 4 and 57 flukes per liver (range: 4-57). According to Soulsby (1982), the presence of more than 50 flukes per liver indicates a high pathogenicity. It has also been reported

that significant production losses occur in infections with 30 flukes and/or herd prevalence of 25% (Vercruysse and Claerebout, 2001).

In this study, the sensitivity of the fecal examination technique was found to be 76.8% about the results of the liver examination and a very good agreement (k = 0.84) was observed between the two tests. However, this test suggested that about 23.2% of infected animals might pass undetected with a single fecal examination technique. The present sensitivity value (76.8%) is comparable to the reports of 81.5% at Haramaya town municipal abattoir, East Harrarghe zone by Tadesse and Usman (2022) and 71.1% in Northern Ethiopia (Assefa *et al.,* 2015) and 69% in Switzerland (Rapsch *et al*., 2006). However, it is much higher than the 35% sensitivity reported by Abunna *et al*.(2010) at Welaita Sodo. The low sensitivity of the sedimentation method may be attributed partly to the fact that *Fasciola* eggs only appear in feces 8-15 weeks post-infection. Furthermore, the detection of *Fasciola* eggs is not reliable during the prepatent period as eggs are expelled intermittently, depending on the evacuation of the gall bladder (Tadesse *et al*., 2021).

In the present study, the economic significance of fasciolosis analyzed indicated the annual loss from liver condemnation to be 945,999 ETB (18,192.288 USD). The study by Tadesse *et al*. (2019b) indicated more than 7 million ETB losses in five Ethiopian abattoirs. This finding was also higher than the result reported by Asefa and Belay (2015) who revealed an economic loss of about 321,750.00 ETB per annum in cattle and by Adane *et al*. (2019) 1,505,856 ETB ($43,024.45) annual losses due to liver condemnation at Wolaita Sodo municipal abattoir. The variation of loss, due to liver condemnation in this study may be due to the current increment of the price of the liver as compared to the preceding reports.

Limitations of the study

The major limitation of the study is that it does not include other factors such as loss of carcass, milk yield, and fertility (calving intervals, additional service) to estimate the total economic loss of bovine fasciolosis. However, it relied on the economic loss estimation based on liver condemnation alone. It also lacks testing more sensitive methods such as fluke finder and flotac instead of the commonly used conventional sedimentation technique for the present analytical test evaluation.

Conclusions

Fasciolosis is an important parasitic disease of cattle caused by two liver fluke species: *Fasciola hepatica* and *F. gigantica*. The present study recorded a moderate prevalence of bovine fasciolosis in Yirgalem municipal abattoir. The dominant *Fasciola* species identified was *F. hepatica* which induces economic losses due to liver condemnation and was found economically important parasite of cattle at the Yirgalem municipal abattoir. Moreover, the current study reflected that the higher prevalence of fasciolosis was present in those animals with poor body conditions, extensively reared and aged. There was a very good agreement between coprological examination and liver inspection in the diagnosis of fasciolosis. However, the disparity still observed indicated that coprological examination for parasite eggs has significant limitations in detecting the presence or absence of fasciolosis, while examination of the liver of animals during postmortem is the most reliable method to detect fluke infection. The annual economic loss of 945,999 ETB obtained in this study, signified the severity of the problem and the need for effective control measures that should be supported through studies on the economic importance of the infection in bovine species and epidemiology of the disease. Therefore, strategic control of the snail intermediate host should be performed to interfere with the life cycle of *Fasciola* alongside strategic treatment of infected animals with Fasciolicides.

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Historical analysis of animal diseases: Nagana in Southwestern Ethiopia, Gambella.

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Abstract

Animals and humans have been intimate companions since antiquity. The history of animals discloses diverse episodes in human history. However, the history of animal disease via humans has been the least researched theme. Therefore, this study aimed to explore animal disease history with a special focus on Nagana in Gambella, Ethiopia. The study utilized content analysis of travelers' accounts, archival documents, reports, and secondary sources. It revealed that nagana was endemic in the region due to the presence of dense forests, abundant game resources, and climatic factors. Nagana had direct and indirect repercussions. It caused the loss of domestic animals and productivity. It also influenced the economic, political, and cultural life of the people in Gambella. Moreover, limited prevention efforts exacerbated the repercussions of the disease.

Keywords: Animal; Disease; Ethiopia; Gambella; History; Nagana.

Introduction

The diverse ecological zones bestowed Ethiopia with the copious resource of domestic and wild animals. Ethiopia has the largest livestock (cattle, sheep, goats, equines, camels, and poultry) resources in Africa (Mayen, 2006). Besides, Ethiopia has enormous wild and aquatic animal resources with endemic species, serving as tourist attractions (Zewudie, 2004). Animals have fundamental contributions to the national economy. The livestock in Ethiopia, for instance, has a significant share of the national gross domestic product (GDP). Livestock plays an enormous role in serving food and nutritional security, financial security, sustainable crop yields, employment prospects, social status, and soil fertility. In addition, food products such as meat, milk, and eggs are concentrated sources of high-quality protein, vitamins, and minerals (Abebe, 2004; Admassu, 2002).

Nevertheless, Ethiopia has been facing difficulties to take advantage of the resources at a significant level. Widespread diseases, lack of effective nutrition, problems in policy enforcement, limitations in genetic potential, and poor management practices were the major stumbling blocks (Abebe, 2004; Zewudie, 2004). In line with animal disease, the country has been suffering from noxious diseases caused by bacteria, viruses, protozoa, and parasites. These include Foot and Mouth Disease (FMD), Lumpy Skin Disease (LSD), Contagious Bovine Pleuropneumonia (CBPP), Bovine Tuberculosis (BTB), Peste des Petits Ruminants (PPR), and trypanosomiasis. Some of these animal diseases are zoonotic and parasitic which affect human health directly (FFILLS, 2016).

Nagana is among the endemic parasitic diseases in Ethiopia. It is caused by trypanosomes (blood protozoan parasites) and transmitted mainly by vectors known as Tsetse flies. Tsetse fly (*Glossina*) is the bloodsucking cyclical vector of trypanosomiasis which causes sleeping sickness in humans and nagana in livestock. The disease is transmitted through contact among the host, vector, and parasite. The disease affects animals more than people due to a couple of reasons. The first is the diversity of trypanosomes that can infect animals. Secondly, the fly preferentially bite animal hosts (Alsan, 2015). There are three subgroups of Tsetse flies: Palpalis (riverine), Morsitans (savannah), and Fusca (forest-dwelling). This division is made based on the ecological adaptation of the tsetse flies. The flies exist in more than 30 sub-Saharan African countries that cover around 10 million km^2 area (Lincoln and Dieter, 2017; Waldetensai *et al*., 2020).

When the vector feed on diseased cattle, sheep, goats, pigs, horses, donkeys, camels, and man, trypanosome parasite get into the host's blood. Later on, it develops into an infective stage inside the insect. During the infective stage of the parasite, metacyclic trypanosomes found in the saliva of the vector inject into animals in times of a fly bite (Waldetensai *et al*., 2020). Symptoms of Nagana include loss of weight, loss of power, failure to move equally with other members of herds, loss of milk, abortion, swelling of glands, anemia, pale mucus membranes, speedy heartbeat, and death (Puskur and Mulat, 2011).

Nagana is endemic mainly in sub-Saharan Africa for centuries. The region is suitable habitat for the tsetse fly vector (Kuzoe and Schofield, 2004). This vector is found in the western and southwestern lowland rainforest and wooded savannah regions of Ethiopia. It covers an area of more than 200,000 km^2 or 20,000,000,000 hectares of land which is fertile and vegetated, and suitable for

farming and animal rearing (Puskur and Mulat, 2011). This region includes the western part of Amhara and Oromia, Gambella, Benshangul Gumuz, and Southern Nations nationalities and peoples regions (Waldetensai *et al*., 2020). Animal diseases had several effects since ancient times. For example, Schistosomiasis affected farmers and irrigation laborers and caused the death of sheep and cattle in the ancient Egyptians and Mesopotamia. The effects of animal diseases were common during middle age. It decimated both human and animal populations. It also caused population movements, loss of agricultural production resulting in high mortality amongst livestock, and famine (Swabe, 1999).

Nagana hindered the economic development in African countries (Hamon *et al*., 1965). It instigated serious threats to the lives and livelihood of entire communities and constitutes the greatest constraint to livestock and crop production in sub-Saharan Africa (Dagnachew *et al*., 2011). Besides, it affected settlement patterns, land use, draught power use, animal husbandry, and farming (Waldetensai *et al*., 2020). As a result, nagana forced people to shift the area of settlement due to high livestock mortality. In turn, the secluded areas of the tsetse fly inhabited region have been densely populated (Puskur and Mulat, 2011). Therefore, the disease had a wide-ranging effect on the economic, social, and cultural affairs of people in the areas occupied by the vector.

Similarly, nagana caused devastating direct and indirect impacts in Ethiopia. It resulted in the reduction of fertility (calving rate), weight gain, milk, and meat off-take. The death of affected animals and a decrease in the potential opportunities for livestock and crop production are also common effects (Zeleke, 2011). Above all, it has begotten danger to human health and development. The disease posed challenges to animal production and agricultural livelihoods. Nagana hampered progress in crop and livestock production and triggered hunger, poverty, and the suffering of communities.

The rationale of the study and methodology

The history of humans is not an enclave in the ecosystem (Cannadine, 2002). Humans and Animals have had chummier interactions for many years. Animals have played paramount roles in the life of humans since time immemorial (Kalof, 2007). Accordingly, studying the history of animals has various significance. It can provide vital events to explore the different themes of history. In addition, it is crucial to get a deeper understanding of the ecological

challenges that humans face over time (Way *et al.,* 2020). However, the role of animals has been neglected in historiography. Animals were simply treated as one of the economic inputs in history (Fagan, 2015).

Likewise, the historiography of animals in general and animal diseases, in particular, is very scarce in Ethiopia (Brown, 2014). Animal diseases were mentioned in the historical research about the famine of 1889-92 in Ethiopia. In 1889 cattle plague or rinderpest occurred in Ethiopia due to the importation of diseased European oxen into the new Italian colony of Eritrea. Although the disease was not new, the catastrophe caused by the rinderpest was unprecedented. Only around 7 or 8% of the country's cattle had survived in the early years of the plague. The loss of cattle led to a shortage of grain and finally to starvation (Pankhurst, 1961).

Nevertheless, studies on the subject of animal diseases, including trypanosomiasis in Ethiopia, were mainly conducted by veterinary professionals. However, the priorities of these studies were not historical experiences. Rather, the assessment of disease prevalence was the focus of studies on trypanosomiasis (Waldetensai *et al*., 2020). Besides, the economic and social effects of the disease have won the attention of the researchers. Furthermore, sleeping sickness has been given better recognition than nagana (Endeshaw *et al*., 1997). There was also research conducted by animal health professionals to identify the prevalence and effects of trypanosomiasis in Gambella by the year 1970. However, it was limited in focus, time, and space. It only researched human sleeping sickness, the 1967-1970 phenomena, and only around the Gilo river (Connell *et al.,* 1970).

Hence, this study aimed at assessing the prevalence and effects of trypanosomiasis in the 20th-century southwestern Ethiopian region. It is a history of the animal disease (nagana) in Gambella. This research answered the following questions.

- Why Nagana was endemic in Gambella?
- What were the repercussions of Nagana?
- How the government and the people reacted to the disease?

The accounts of travelers who visited the region during the first half of the 20th century, archival sources from the Ethiopian National Archives and Library Agency (NALA), and reports were analyzed systematically. In addition, related content from books, journal articles, and scholarly web sources was analyzed

carefully. Therefore, the study used content analysis of both primary and secondary sources.

Nagana in Gambella

Description of the study area

Gambella is a region situated over 700 kilometers far away from the Ethiopian capital Addis Ababa. It has a size of 25,000 km2; a very hot climate with an annual average temperature reaching 37˚C; and average annual rainfall of 800 mm. In terms of vegetation, dense tropical forest covers the eastern edge of the region. Savannah forests, grasslands, and marshlands cover the lowland plain as far west as the border with Sudan (Markakis, 2011). The region has been rich in domestic and game animals including cattle, goats, sheep, hens, lions, elephants, tigers, *fiqo*, *qorqi*, *wore*, pigs, buffalo, monkeys, apes, etc. (A memo from Illubabour Province Land and resettlement office, 1977).

Five ethnic groups including Nuer, Anuak, Majang, Komo, Oppo, and various highlanders live in Gambella. The Nuer are among the predominantly pastoralist people of East Africa. Among the four sections, the Nuer in Gambella are Jikany, most of whom live in the districts of Akobo, Jikaw, Lare, and Itang. The Jikany Nuer has further divisions known as Gaajak, Gaaguang, and Gaajok (Markakis, 2011). The Eastern Jikany Nuer in the borderlands of Ethiopia and Sudan were once very rich in cattle (Pritchard, 1940). Livestock especially cattle have had various importance for the Nuer including social, economic, and cultural values (Seide, 2017). Economically, for instance, they used milk, meat, and blood for food. Horns and bones were important to furnish numerous household materials (Pritchard, 1940).

The Anuak are also among the ethnic group who live in Gambella (Zewudie, 1976). Based on oral sources from Udiek on the Akobo and Ulan on the Gila, Prichard (1940) wrote that the Anuak arrived at the Baro salient from the southwest of present-day Anuak land. The Anuak are believed to be pastoralists who moved northward with their herds of cattle from their southern homeland to their present location sometime in the 16th or 17th centuries (Wall, 1976). Although they are now primarily agricultural people with few cattle, there are indications of ancient pastoral interests in their elaborate vocabulary of cattle color names (Lienhardt, 1957). According to Lewis Wall (1976), the Anuak practiced pastoral livelihood in the past. The author took the presence

of pastoral elements in their vocabulary as evidence of a pastoral past. Their language contains many cattle-related words indicative of a nomadic past.

The Majang people are the third largest indigenous ethnic group in the Baro salient. They live in the hills and forests between the Baro salient adjacent to the highlands of Illubabour. Economically, they are dependent on hunting and gathering. Farming has been playing a supplementary role in their livelihoods. The Opo and Komo are also among the inhabitants of Gambella. They are dependent on agricultural livelihood (Ojod, 2013).

Historical evidence of Nagana in Gambella

Shreds of evidence from travelers' accounts, archival sources, and secondary documents indicated that Nagana was endemic in the region. The prevalence of Nagana extended to the adjoining areas of Gambella (Montandon, 1912). The vector tsetse fly was also prevalent in the areas to the north of Gambella. The presence of Tsetse flies in the areas adjoining the streams prohibits the Komo from keeping cattle although goats manage to survive for some time (Corfield, 1938).

The disease also stretched into the southern Sudanese lowlands bordering Gambella. The nagana-affected area of southern Sudan had a horseshoe shape around the Nile swamps with three gaps, the valley of the Bahr el Ghazal with the southern Nuba Hills, the arid country east of Kapoeta, and the Sobat area. There were sporadic outbreaks of nagana that stretches to a distance of some 300 km (about 180 miles) from the main vector inhabited areas. Other biting flies might serve as a vector for the disease. As a result, it kills tens of thousands of cattle in bad years (Lewis, 1949).

According to Jessen (1905), who accompanied McMillan's expedition into southwestern Ethiopia in 1904, only about 60 heads of equines survived from a total of 220 mules, ponies, and donkeys. This happened after three months of travel in the region. Equines were absent in the region due to Nagana. Besides, Jessen did not mention the death of equines throughout his journey over Ethiopian highlands. These two important facts associated the loss of equines with the incidence of nagana in the region.

In addition, the trouble that encountered Jessen was experienced by the other traveler George Montandon. Montandon traveled into southwestern Ethiopia

in 1910 and recorded the profuse existence of the nagana vector in the south-eastern edge of the region (Montandon, 1912) Montandon writes,

…On arriving at Badika we found the animals in a deplorable condition. The donkeys, so far from filling out their sides, were dying one after another, whether from the fatigues of the last few weeks or as a result of the bites of tsetse in the valley floor of the Aranga...(Montandon, 1912).

Moreover, in 1923, traveler Arnold Hodson lost around seven donkeys in the areas to the west of Pibor Post. In his second round travel of 1925, from Pibor post to the highlands of Ethiopia via Gambella, Hodson also lost twenty-five donkeys and a mule. The surviving equines are also deceased after he arrives at Gambella town. Hodson attested to the presence of tsetse in the area to the northwest of the Pibor River (Hodson, 1929). Therefore, the equines might be deceased because of nagana in the long journey from Maji-pibor post-Gambella route.

Evans-Pritchard, who explored western Gambella in 1935, confirmed that the region was difficult to herd cattle because of the tsetse fly. The line that connects Itang, Cenhthoaa, and Pocala from north to south is marked as the line that delimited tsetse areas. Pritchard (1947) states that cattle can be kept only westwards of the line Itang - Pokedi - Dier - Nyiwir - Ngela - Cenhthoaa - Pocala - and thence due southwards. Nagana was characteristically austere in the areas to the east of the line indicated by Pritchard (Figure 1). For example, in the dense forest areas of the Gog district, only dogs are observed around villages. Besides, in the thick riverside forest of lingo, the people could not even keep sheep, goats, or dogs (Pritchard, 1947). A memo from Lemma Gebremariyam (1961, Unpublished), who was the governor of Gambella *Awraja* in the late 1950s and early 1960s, to the Ethiopian Ministry of Interior, supported the testimonies of Pritchard. It revealed that half of Gambella *Awraja* (the current Gambella region) was disastrous to herding cattle and equine.

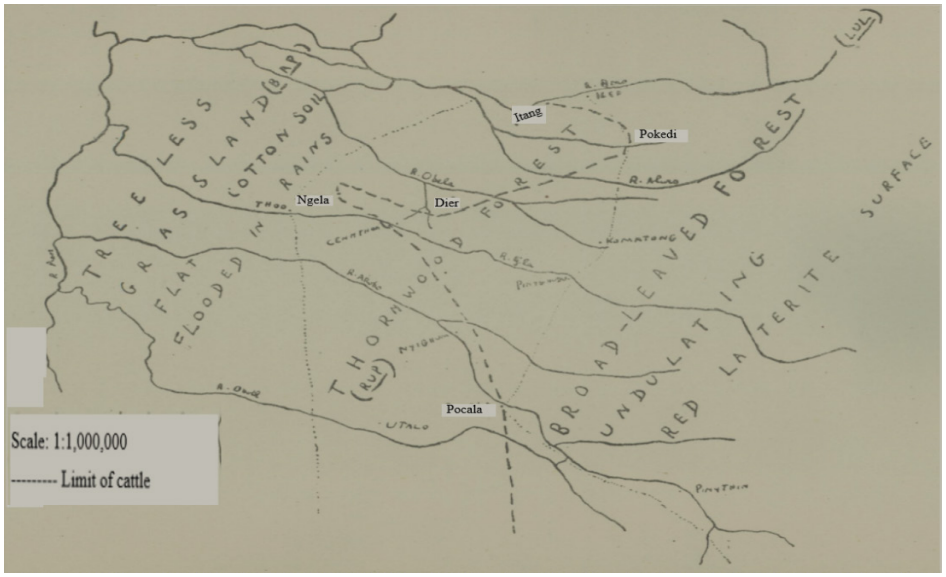


Figure 1. Map: pervasiveness line of Nagana in Gambella (Pritchard, 1947).

However, the vector was observed in the areas west of Pritchard's cattle limit. The line does not exactly correspond to the distribution of nagana in Gambella. For instance, the fly was prevalent on both banks of the Akobo near the village of Nyighum. Pritchard also wrote that a biting fly known as the Stomoxys was a threat to the cattle of Eastern Jikany around Jikau and Akobo. Probably this fly was the vector of nagana in parts of Nuerland, especially among the Eastern Jikany. It was evident throughout the year; however, conspicuous in the dry season and early rains (Pritchard, 1940). In addition, some villages keep cattle to the east of the line. Furthermore, all the livestock were not equally victims of the disease. Goats and sheep may have better resistance to trypanosomiasis or are less preferred blood sources for the vector than cattle and hence are less likely to get infected (Finelle, 1983). Thus, they were kept in all the Baro villages as far east as Gambella town (Pritchard, 1947).

Evidence of nagana for the second half of the 20th century was obtained from medical research. Medical research by Connell *et al*. (1970) indicated that there was an outbreak of sleeping sickness/human trypanosomiasis in the Gilo River region. The disease increased steadily from 4 confirmed cases in 1967 to 28 in 1968, 173 in 1969, and 27 in the first three months of 1970. The infection was

very high in the areas between the Gilo and Akobe rivers. Endemic sleeping sickness signaled the prevalence of the vector in the region. In those years, the occurrence of animal trypanosomiasis probably climaxed. In the years 1989-1992, there were human trypanosomiasis infections detected among the Anuak and southern Sudan refugees in Gambella (Endeshaw *et al*., 1997).

Causes for Pervasiveness of Nagana in Gambella

The abundance of dense forest, rich game resources, and climatic conditions were the major factors for the pervasiveness of Nagana in Gambella. The distribution of the tsetse fly follows the distribution of vegetation (Pritchard, 1947). As a result, the existing dense forest in Gambella created suitable habitat for the fly. The forest is denser in eastern Gambella than in the western part of the region. Similarly, nagana was more deadly in eastern Gambella. Whereas the absence of shady forests which, in its turn, is probably due in the main to flooding and partly to firing resulted in the non-occurrence of tsetse that carries trypanosomes pathogenic to cattle in the western part of the region (Pritchard, 1940). This signifies that the level of vegetation cover has had a direct effect on the pervasiveness of nagana.

Rich game resources in the region might have also contributed to the extensive transmission of the disease. Aronold Hudson (1929) wrote that there were substantial resources of game animals in Gambella to the northwest of the Pibor River. The other traveler B. H. Jessen also recorded the abundant presence of game animals in the area to the south of Pokum (A village between Itang and Gambella along the Baro River). These include elephants, giraffes, buffaloes; various antelopes and gazelles, wart hogs, wild pigs, lions, leopards, hyenas, jackals, wild cats, monkeys, etc. (Jessen, 1905).

Game animals serve as hosts and transmitters of trypanosomiasis in their movement. The movement of game animals was most commonly seasonal. The games move from southwestern and western parts of Gambella to the eastern and northeastern directions around the river basins of Akobo and Gila in the dry season starting from October. The western and southwestern grasslands are habitats of games in the rainy seasons (Lyth, 1947). The movement of game animals resulted in the spread of the disease beyond the horizon of highly affected areas and where the fly was less likely to appear probably through mechanical transmission by non-tsetse biting flies.

The climate is the third major factor for the Pervasiveness of nagana in Gambella. The hot and well-watered land of Gambella has been affluent for vegetation and animals (A memo from Illubabour Province Land and resettlement office, 1977). Besides, flooding, the common phenomenon in the Baro salient, had a significant effect on the spread of nagana. It spread as the game, fleeing the floods, came into closer contact with cattle (Johnson, 1989). In some parts of this region prevalence of the fly was more abundant in the less flooded riverine lands than in the interior. However, the danger of the disease reduces in the areas where the rivers top their banks and get flooded (Pritchard, 1947).

Effects of Nagana on the people of Gambella

Nagana influenced the economic, political, and cultural life of the people in Gambella. The disease caused death, and abortion, and affects the dairy yield of the cattle (Pritchard, 1940). Besides, it underdeveloped cattle rearing and caused the absence of equine (A memo from Illubabour Province Land and resettlement office, 1977). According to Tsehay Desta, who was the Governor of Gambella *Awuraja* in 1958, the Nuer in Gambella lost significant herds of cattle due to the disease. Moreover, it has been among the factors that made the region sparsely populated (A Note from Tsehay Desta (Governor of Gambella *Awuraja*) to Ministry of Interior, Gambella, 1958).

Nagana forced the Anuak and Majang to alter their livelihood. It was among the major factors that forced the Majang to change their livelihood. They became dependent on forest and game resources. Moreover, the Anuak were forced to shift their livelihood partly due to this disease. For instance, B. H. Jessen associated the obsession of Anuak with crop production due to the presence of Tsetse. Jessen records,

--*Their chief industry, as before mentioned, is agriculture, with comparatively few cattle, sheep, or goats being raised because a great death rate is caused among all domestic animals by a certain kind of fly* (Jessen, 1905).

People in nagana affected areas of Africa were less likely to use domesticated animals for the plow and had a lower population density. The disease hindered the ability of Africans to generate an agricultural surplus and transport goods overland easily (Alsan, 2015). Likewise, the people in Gambella were unable to use animal drown plows because of nagana. The absence of animal-drawn plow might partly be due to cultural factors (A Memo from Getahun Tesema

(minister of the Ministry of interior) to Tsehafe Tizaz Aklilu Habtewold (Prime Minister and Ministry of the Pen), Addis Ababa, 1973).

Nagana affected the Political life of the people in Gambella. It halted the Nuer expansion into eastern Gambella. The Nuer had been expanding eastward since the 19th century. They were able to expand as far east as the Ethiopian escarpment in the 1840s and 1850s (Stringham, 2016). However, this expansion was checked by the presence of a tsetse fly. The following record of Pritchard substantiates the political effects of nagana.

The prevalence of tsetse in the forest belt that stretches along the foothills of the Ethiopian escarpment has prevented the Nuer from expanding as far eastwards as they might have done, for it is clear that one of the reasons for their evacuation of Anuak land was the loss of stock (Pritchard, 1940).

Above all, nagana dictated changes in the culture of the people in Gambella. The loss of cattle, for instance, resulted in a reduction in the number of cattle to be paid for bride wealth. Thus, money (cash) was introduced as bride wealth-replacing cattle (Hutchinson, 1992). Agricultural livelihood after the loss of cattle because of Nagana and other diseases reduced interaction and cooperation among different villages of the Anuak. The only economic activities which require the cooperation of groups of people became occasional hunting forays, the erection of fishing dams at certain seasons of the year, and special services to the headman of the village (Lienhardt, 1957).

Attempts at disease prevention

Tsetse flies can be controlled by direct destruction of the fly using the net collection and glued, game destruction, bush clearing, insecticides, and others (Hamon *et al.,* 1965). The 19th-century African communities used to fight trypanosomiasis by avoiding tsetse belts and game destruction (Brown, 2014). Nevertheless, knowledge and understanding of the disease are significant in the delivery of veterinary services. The concept of disease in Ethiopia had different stages of understanding among the community. It ranges from a predator, evil spirit, and religious to biomedical chronologically (Tefera, 2012). Indigenous veterinary services in Ethiopia have been recorded since ancient times. Traditional healers used to treat diseased animals by drenching them with herbal drugs, incising and cauterizing abscesses and wounds using sharp

objects and hot metals, mending fractures, and rehabilitating dislocations (Admassu, 2002).

The efforts to cure and prevent nagana in the Gambella region in particular and Ethiopia, in general, were traditional methods until the second half of the 20th century. For example, spiritual leaders of the Nuer engage in the healing practice of diseased cattle. They used to put a curse on wild animals harmful to cattle (Markakis, 2011). Several regional and national factors contributed to the absence of modern veterinary services and dependence on only traditional healing practices.

The major factor that hindered the prevention of nagana disease was the absence of modern veterinary institutions and professionals. Veterinary professionals and animal health centers were limited or nonexistent in many parts of the country. Although Modern veterinary service was introduced during the first decade of the 20th century by the French Veterinary Mission in 1908, it had been creeping until the 1960s (Admassu, 2002). The coming of Ethiopians after acquiring high-level veterinary training abroad and the establishment and functioning of the National Veterinary Institute and the Animal Health Assistants School were major boosters for the practice of veterinary service in Ethiopia (Admassu, 2002). This slow development in veterinary service delivery limited the initiation of nagana disease prevention efforts in the Gambella region.

The lack of infrastructure was another hurdle to introducing and expanding modern veterinary services to Gambella. The region is geographically remote from the administrative capital of the country. In addition, transportation infrastructure was limited. There were no road infrastructures to distribute the service from the sub-provincial town of Gambella. It took around 28 days to travel by foot to visit Gambella from Akobo. The water transport system needs unavailable boats. The only option was to use air transport (Application letter of Anuak and Nuer *Balabats* of Akobo for Emperor Haile Selassie, 1963). Hence, the lack of infrastructure challenged veterinary services delivery in the region.

The memo written in 1958 indicated that there was no distribution of vaccines and deployment of veterinary service professionals in the region, although government appointees in the sub-province and the people requested continuously. They also requested the establishment of a veterinary service center in

the sub-province (A Note from Tsehay Desta (Governor of Gambella *Awuraja*) to Ministry of Interior, Gambella, 1958). In the year 1963, there was only one animal health service center in Gambella town (Application letter of Anuak and Nuer *Balabats* of Akobo for Emperor Haile Selassie, 1963). The *Balabats* of Anuak and Nuer in Akobo requested the establishment of health posts to reduce both human and animal diseases in the districts of the sub-province (A memo From *Dejazmach* Dereje Mekonen (regent of Illubabor Province) to Asefa Gebremariam (v/minister of the Ministry of Interior), Gore, 1964). The people in the borderlands used to cross the international boundary into Anglo-Egyptian Sudan for vaccination. However, the Ethiopians were forced to pay for the service while the Sudanese delivered it for free (A memo From Tezera Woldegiorgis (Illubabour Province general secretary) to *Qegnazmach* Feleke Ergetu (Assistant minister of the ministry of agriculture), Gore, 1961).

The fear of possible political discontent and development in the sector brought some changes in the struggle against nagana since the 1960s. Most of the measures employed to fight trypanosomiasis in Ethiopia were trypanocidal drug injection and eliminating the vector in various ways. The issue of medical injection has not shown full success due to the absence of domestic production of medicine, the higher cost of the medicine to purchase from abroad, and adaptation by trypanosomiasis parasite (Puskur and Mulat, 2011).

In early 1960, the government started applying DDT to eliminate the vectors. Thus, there was an improvement in cattle herding in the Gambella district and the surrounding areas, although it was not significant. There were also efforts to introduce trypanosomiasis-tolerant species of equine. The administrators in the sub-province tried to bring donkeys from Begi in Assossa *Awraja* on the assumption that the species is resistant to nagana. It also aimed to alleviate the problem of transportation services in the sub-province (A memo from Lemma Gebremariyam (governor of Gambella *Awraja*) to Dejazmach Kifle Erget (Ministry of Interior), Gambella, 1961).

There were efforts to disseminate vaccinations from the provincial capital of Gore in times of severe outbreaks. The efforts were patchy; however, animal diseases became worse and brought huge losses in 1961. In response, the administrative office of Illubabour Province sent vaccinators to the districts of Gambella (A memo from Admasu Mihretie, Ministry of Agriculture Director of Imperial Veterinary Service) to Imperial Veterinary Service Finance Section, 1961). Besides, there were endeavors to establish veterinary service centers in

all districts giving priority to Akobo and Gog, and Jor where there are large livestock resources (A memo From Tezera Woldegiorgis (Illubabour Province general secretary) to *Qegnazmach* Feleke Ergetu (Assistant minister of the ministry of agriculture), Gore, 1961).

Conclusions

Humans and animals have had intimate interactions since antiquity. Animals played a paramount role in the development of societies. However, historical accounts overlooked the history of animals. Some historical works of literature also mentioned animals for their economic importance. Nevertheless, analyzed evidence from archival sources, travelers' accounts, reports, and secondary literature confirm that animals have influenced the political and cultural life of societies beyond their economic values. The case of nagana in Gambella revealed the significance and the need to record the history of animals. Nagana has been endemic in the southwestern Ethiopian region, Gambella. The abundance of dense forest, rich game resources, and climate were the major factors for the pervasiveness of nagana in Gambella. Nagana influenced the economic, political, and cultural life of the people. It had a significant role in the settlement pattern, preference for livelihood, cultural changes, and political organization of the people in the region. However, the efforts to cure and prevent nagana in the region were limited due to the tardy development of infrastructure and veterinary professionals.

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Medicinal plants and non-plant remedies used in the treatment of livestock ailments in Dawuro Zone, Southwestern Ethiopia

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Abstract

Ethnoveterinary medicine is part of indigenous knowledge that has been used for many centuries in the world, particularly in developing countries. Owing to its history and wider utility in our society, a survey on ethnoveterinary practice was undertaken between October 2018 and December 2019, to assess and document this traditional practice in the treatment of livestock ailments in the Dawuro zone. Data on the type of herbs/shrubs and other non-plant consumables or materials were gathered from purposively selected 81 informants using a semi-structured questionnaire. Focus group discussions, participant observations, and field trips were also made. Data obtained from the questionnaire survey were analyzed using descriptive statistics and quantitative ethnobotanical methods. Samples of plants claimed to have medicinal value were collected and botanically identified. The study revealed that 92 plant species belonging to 44 families were in use. The family Asteraceae was the most frequently reported species (11.4%). Herbs (31.7%) were the main source of traditional prepared medicine followed by shrubs (31.5%). The leaves (40.5%) and roots (34.5%) were more commonly utilized parts of plants while pounding (78.8%) was the major formulation. Among the non-plant materials, bear faeces, sharp hot iron or wire/knife, and common salt showed the highest utility level (100%). *Azadirachta indica* was the most preferred species to treat blackleg and also with the highest use value (UVs=0.36). The study revealed that the local communities of the study areas were rich in indigenous knowledge of ethnoveterinary practices. Thus, a strong protective policy is needed to safeguard the plant species; and develop regulations in the applications of ethnoveterinary knowledge. Moreover, bioactive validation of plant chemical

content, along with testing for efficacy and safety would be part of indispensable technicalities for rational utility in the future.

Keywords: Dawuro zone; Ethnoveterinary Practice; Medicinal plant; Non-plant Remedies.

Introduction

Traditional people around the world possess unique knowledge of plant resources on which they depend for food, medicine, and general utility including tremendous botanical expertise. This implies that humans are dependent on other organisms for their life. Although various animal and mineral products contribute to human welfare, the plant kingdom is essential to human well-being, especially in supplying basic needs (Martin, 1995). The modern animal health care system is still at its lowest point in the country characterized by a lack of adequate clinics, workforce, and drug supplies. Furthermore, most conventional drugs are prohibitively expensive for the majority of Ethiopian farmers and pastoralists (Wabe *et al*., 2011; Kalayou *et al*., 2012; Melaku *et al*., 2014).

Therefore, the majority of Ethiopian farmers and pastoralists rely on traditional knowledge, practices, and plants to control livestock diseases (Giday and Teklehaymanot, 2013; Lulekal *et al*., 2014). On the other hand, non-plant materials are also reported to be used in ethnoveterinary management here in our country and elsewhere. In this regard, the Fulani pastoralist in Nigeria uses wood ash, honey, oils, kerosene, kaolin, potassium, local soap, and spent engine oil (Alhaji and Babalobi, 2015). The use of animals tissue (Ferreira *et al*., 2009; Volpato *et al*., 2015), salt, plant charcoal, alcohol, sugar, bee honey, and buffalo milk in eastern Amazonia of Brazil is not uncommon (Monteiro *et al*., 2011). The use of spent engine oil in the management of wounds, kerosene for foot rot, and local soap as a disinfectant in animals were also reported by McCorkle (1995). In light of its historical value and wider utility, Ethnoveterinary medicine should have been given special attention to its therapeutic potential development and low-cost alternative for allopathic medications (Matekaire and Bwakura, 2004), and could have contributed to the discovery of new drugs (Monteiro *et al*., 2011).

However, this indigenous knowledge, medicinal plants, and non-plant materials used in the treatment are not properly documented. Often handed down orally from generation to generation. The knowledge about plant use and the method of preparation is often kept secret (Gebrezgabiher *et al.*, 2013; Lulekal *et al.*, 2014). In addition, the loss of valuable medicinal plants due to population pressure for settlement, agricultural expansion, deforestation, overgrazing, and over-harvesting is alarmingly growing in Ethiopia (Fenetahun and Eshetu, 2017; Kebebew and Mohamed, 2017).

Despite the challenge, herbal and nonherbal materials used for animal disease treatment is a common traditional practice in Ethiopia. In this regard, Borena pastoralists and highlanders in Oromia consider ethnoveterinary medicine as one of the animal health service alternatives (Sori *et al.*, 2004; Amenu, 2007; Yigezu *et al.*, 2014). Likewise in Afar (Seifu *et al.*, 2006; Giday and Teklehaymanot, 2013; Teklehaymanot, 2017); in Tigray (Gebrezgabiher *et al.*, 2013; Teklay, 2015), in Amhara (Lulekal *et al.*, 2014); and some parts of southern region (Tekle, 2014; Eshetu *et al.*, 2015) evidence of its utility are available. To our knowledge, study on the ethnoveterinary practices of herbal and non-plant source medicine is uncommon in the Dawuro zone. Even though the practice is widely known among the traditional community. Therefore, the present study aimed to assess and document ethnoveterinary medicinal plants and non-plant materials used in the management of livestock ailments in the Dawuro zone, Southwestern region.

Materials and methods

Description of the study area

The present study was conducted in two selected districts of Dawuro zone, Southwestern region, Ethiopia: Tocha and Mareka districts representing similar agro-ecologic zone (highland, midland, and lowland) altitudes. The indigenous people inhabiting the area belong to the Omotic ethnic group. They speak *Dawurotsuwaa*, the local language of the area. Tocha Idiget is the administrative capital of the Tocha district lies between 6^0 97` to $7^0$ 25` North of latitude and 36^0 81` to $37^0$ 14` East of longitude with an elevation ranging from 501-3000 meters above sea level. The mean annual temperature ranged from 15.1-25 ^{0}C and the mean annual rainfall ranged from 1401-1800 mm. The total human population of the district is 124, 472; of which are males (68, 109) and females (56, 363). The livestock population reared in the district comprised cattle (154, 218), sheep (165, 571), goats (86, 243), donkeys (14, 472), horses

(9, 260), mules (10, 127), and poultry (188, 734) (Dawuro Zone Finance and Economic Development Department, 2017, unpublished).

Mareka district is one of the ten districts of the Dawuro zone. Waka is the administrative capital of the Mareka district and is located at 6^0 97` to $7^0$ 21` North of latitude and 37^0 01` to $37^0$ 26` East of longitude and its altitude ranges from 1401-2300 meters above sea level. It has a total human population of about 161,621. Of the total population, the males and females were 83,399 and 78,222, respectively. The mean annual temperature and rainfall are 15.1-25 ^{0}C and 1401-1800 mm, respectively. The district's livestock population comprised cattle 134,290 sheep 145,451, goats 44,568, horses 10,350, mules 10,926, donkeys 18,321, and poultry 185,418 (Dawuro Zone Finance and Economic Development Department, 2017, unpublished). The map of the study area is depicted in Figure 1**.**

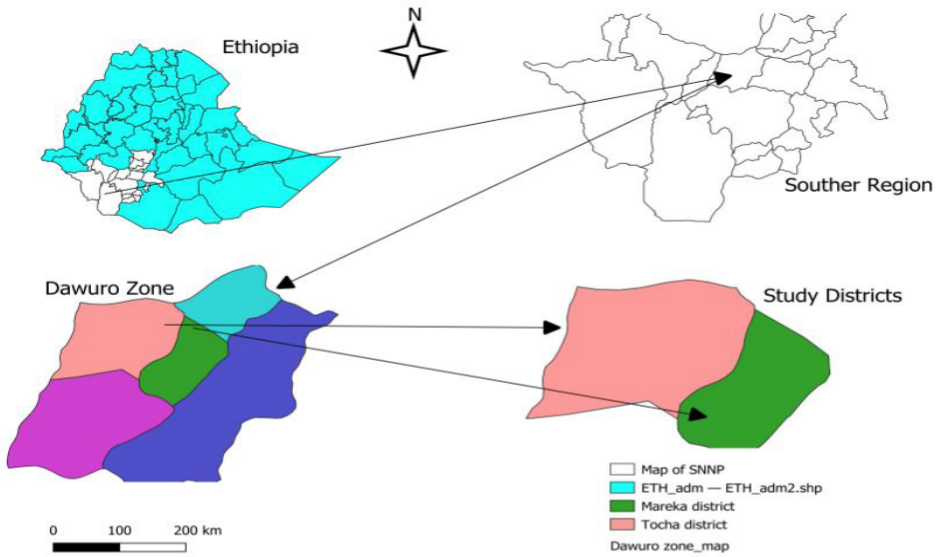


Figure 1. Map of the study area

Sampling method and specimen collection

A cross-sectional ethnoveterinary survey was conducted between October 2018 and December 2019, in ten *kebeles* (Peasant Associations) in Tocha and Mareka districts. A purposive sampling strategy was used to select informants

and study districts based on the wide use of ethnoveterinary practices and the availability of traditional healers. Ethnoveterinary data was collected from 81 informants (76 males and 5 females): 50 informants from Tocha and 31 informants from Mareka districts based on the availability of traditional practitioners. A face-to-face interview was made by using pre-tested semi-structured questionnaires prepared in English and translated into the *Dawurotsuwaa* local language. The interviews addressed questions regarding age (young [20-35 years], middle age [36-45 years], and adult/old [above 45 years]), gender (male, female), level of education (unable to read and write, able to read and write, elementary[1-8 grade]), secondary [9-12 grade]), above 12 grades]), occupation (government employee, farmer, trader), religion (orthodox, protestant, catholic), local names of medicinal plants, ailments treated, the habit of the species, sources (wild/cultivated or both), parts used, methods of preparation, dosage formulation, routes of administration, noticeable side effects of remedies, use of antidotes for side effects, source of knowledge, ways of indigenous knowledge transfer, and other uses of species mentioned were recorded. Each informant was separately interviewed in their local languages to keep the secrecy of their indigenous knowledge. In addition, 10 focus groups (6-10 participants) discussions were undertaken to gain further information on medicinal plants' knowledge of the community and prove the reliability of the data collected through semi-structured interviews (Martin, 1995; Cotton and Wilkie, 1996; Tefera and Kim, 2019).

Field trips were made with local herbalists for the collection of the reported medicinal plants. Voucher specimens of medicinal plants were collected on the field with the help of traditional healers and field assistants. Collected medicinal plants were dried, numbered, pressed, labeled, and brought to the Ethiopian Herbarium for further botanical identification. Specimen identification and confirmation were undertaken by using Flora of Ethiopia. Finally, the specimens were deposited at the National Herbarium (ETH) at Addis Ababa University.

Data management and analysis

Ethnoveterinary data collected were entered, coded using MS Excel, and transferred to the Statistical Package for Social Sciences Software (SPSS, 20). A descriptive statistical method such as percentage, frequency, and tables was employed to analyze the data. In addition, the fidelity level analytical ap-

proach was also used in evaluating the plants and non-plant remedies in the study area. The fidelity level is mathematically expressed as FL = Ip/Iu × 100, where FL is the fidelity level of each plant or non-plant material, and Ip is the number of informants who mentioned that a plant or non-plant material has specific ethnoveterinary uses against a particular disease condition, and Iu is the total number of key informants who independently suggested that the same plant or non-plant material has any therapeutic uses (Alhaji and Babalobi, 2015). Furthermore, the direct matrix ranking and use value methods were undertaken to evaluate multipurpose tree/shrub, and herb species and their relative importance to the local people, and the extent of the existing threats related to their use values (Phillips and Gentry, 1993).

Results

Socio-demographic profiles and indigenous knowledge of ethnoveterinary plants

Eighty-one (81) well-knowledgeable informants were interviewed; of which 76(93.8%) were males and 5(6.2%) were females (Table 1).

Table 1. Socio-demographic profiles of informants in the Dawuro zone

Profiles	Description	Number of respondents	(%)
Gender	Male	76	93.8
	Female	5	6.2
Age	20-35 years (young)	16	19.8
	36-45 years (middle age)	21	25.9
	Above 45 years (adults)	44	54.3
Marital status	Married	76	93.8
	Single	3	4.3
	Divorced	2	2.38
Occupation	Employee	5	6.2
	Farmer	73	90.12
	Trader	3	3.7
Education level	Unable to read and write	38	46.9
	Able to read and write	2	2.5
	Elementary (1-8 grade)	27	33.3
	Secondary (9-12 grade)	7	8.6
	Above 12 grades	7	8.6

Profiles	Description	Number of respondents	(%)
Religion	Orthodox	44	54.3
	Protestant	35	43.2
	Catholic	2	2.5

In this study, a total of 92 plant species reported to be used belonging to 44 families of medicinal plants were collected and identified for ethnoveterinary treatments of a wide range of livestock health problems. The best representation of ethnoveterinary species of the family Asteraceae with 10 species is the highest, followed by Fabaceae, Lamiaceae, and Euphorbiaceae, respectively. The reported medicinal plants and their respective indications, preparation, and parts used for preparation (additional file). Most (58.2%) of the traditional medicinal plants were harvested from a wild source. The area's most commonly used plant parts for herbal preparations were leaves (40.5%) followed by roots (34.5%).

Methods of preparation, routes of administration, and dosages variation and side effects

Different methods of preparation and application for different types of ailments were employed. As presented in Figure 2 herbal remedies were prepared mainly as pounding and homogenizing with water followed by crushing and grounding using either single or combining different plants.

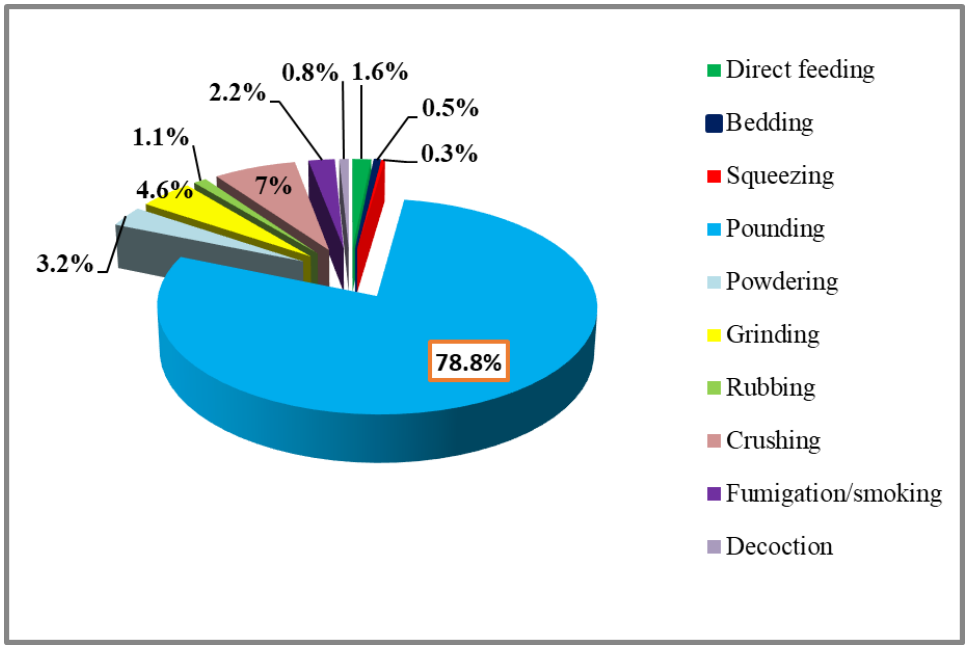


Figure 2. Modes of remedy preparations in the Dawuro zone

There was no standardized dosing; weight measurements and treatment duration were set by traditional healers on the number of herbal prescriptions for different livestock ailments. Due to the lack of standardized doses of herbal preparations, traditional herbalists observed some symptoms of side effects of herbal preparations on their animals in the study area such as shock and lacrimation. On the other hand, different utilizations of medicinal plants other than their ethnoveterinary roles were recorded. Of the total, 20.5% were for human disease treatment followed by firewood (7.1%) and construction (5.7%). About 93% of traditional practitioners reported that there are no taboos associated with the use of medicinal plants while few herbalists (7%) preferred days and times for plant collection like Sunday and Wednesday, and in the early morning without contact with any person.

Fidelity level, use value, and direct matrix ranking

Concerning fidelity level value, *Cyphostema* sp. and *Pentas schimperiana* showed the highest fidelity level value (100%) for diseases of the reproductive

system like mastitis and retained placenta, ectoparasites infestations (tick, lice, and leech), and fattening and bone fracture. Under the infectious therapeutic category, the highest fidelity level value was recorded for *Eucalyptus globulus* L. (91.67%) and *Croton macrostachyus* (90.9%) also showed a relatively high healing potential record under the wound and traumatic injuries disease category (Table 2).

Table 2. Fidelity level (FL) of medicinal plants commonly reported against a given veterinary ailments category in the Dawuro zone

No	Scientific name	Local name	Therapeutic category	IP	IU	FL%
1	*Azadirachta indica*	*Mimmiyaa mitsa*	Infectious/Blackleg	25	29	86.2
2	*Aloe otallensis* Baker	*Godare utsa*	Diseases of the respiratory system	9	11	81.8
3	*Cyphostema* sp.	*Higishsha d'aliya*	Diseases of the reproductive system/ Mastitis	22	22	100
4	*Croton macrostachyus*	*Anka*	Wound and traumatic injury	20	22	90.9
5	*Ageratum conyzoides*	*Kirkissa*	Diarrhea /dysentery	9	11	81.8
6	*Capsicum frutescens*	*Mis'imis'uwa*	GIT parasitism	14	16	87.5
7	*Echinops kebericho*	*Burssa*	Snake poising	11	14	78.6
8	*Pentas schimperiana*	*Dawuridama/ Dalbantsaa*	Fattening and bone broken	22	22	100
9	*Vernonia amygdalina*	*Gara*	Anti-trypanosomosis and tsetse fly control	14	21	66.7

IP =Number of informants who use a species for a specific ailment; IU = Total number of informants who mentioned the plant for any other use, FL=Fidelity Level

Among the 92 identified plants, 465 reports of ethnoveterinary use were recorded. The species with the highest use-value were "*Mimmiya mitsa*" (*Azadirachta indica*) (UVs= 0.36) followed by "*Botsa Barzaafiya*" (*Eucalphytus globules*), and "*Higishsha d'aliya/Ba'oo*" (*Cyphostema* spp.) (UVs = 0.29), and the plants with 0.025 were considered as the lowest use-value by informants. Direct matrix ranking was undertaken to evaluate the multipurpose tree species, their relative importance to the local people, and the extent of the existing threats related to their use values (Table 3). The results of direct matrix ranking showed that *Arundinaria alpine* and *Eucalyptus globulus* L. were ranked 1st and 2nd, and hence are the most preferred medicinal plants by local people for multipurpose uses and are the most threatened species as the informants

reported while *Maesa lanceolata* and *Prumnas africana* were the least ranked species in multi-purpose importance in the study area.

Table 3. Average scores for direct matrix ranking of eight medicinal plant species based on their general use values (5 = best, 4 = very good, 3 = good, 2 = less used, 1 = least used, and o= not used)

Plant species	Use categories									Total	Rank
	Medicine	Construction	Forage	Furniture	Food	Fencing	Charcoal	Shade	Firewood		
Azadirachta indica	5	4	0	0	0	3	0	5	5	22	5th
Croton macrostachyus	5	4	0	3	0	1	2	0	5	20	6th
Eucalyptus globulus	5	5	0	3	0	5	0	4	5	27	2nd
Juniperus procera	3	4	0	5	0	3	0	5	4	24	4th
Maesa lanceolata	4	2	0	0	0	0	4	0	5	15	8th
Prumnas Africana	2	4	2	1	0	1	2	2	4	18	7th
Syzygium guineense	3	4	0	2	4	0	4	4	5	26	3th
Arundinaria alpine	4	5	5	5	0	5	0	1	5	30	1st
Total	31	32	7	19	4	18	12	21	38	182	
Rank	3	2	8	5	9	6	7	4	1		

Non-plant remedies used in livestock health management

In this study, the community depended on traditional medicines for the primary livestock healthcare system. The traditional non-botanical ethnoveterinary practices are used in managing other cattle disease conditions as depicted in (Table 4).

Table 4. Fidelity level (FL) value of non-plant remedies used to treat livestock ailments in the Dawuro zone

S/N	Materials	Local name	Ethnovet erinary uses	IP	IU	FL (%)
1	Hyena faeces, Wood ash	*Babark'iya shi'aa*, *bidintsaa*	Listeriosis, evil eye	8	10	80
2	Kerosene	*Laambbaa*	Tick expulsion	6	8	75
3	Dear feaces	*Babantsaa shi'aa*	Blackleg	5	5	100
4	Hot wire/knife	*Ho'oo biraata/ Mashaa*)	Blackleg	10	10	100
5	Porcupine meat	*K'us's'ariya ashhuwa*	Blackleg	17	19	89.5
6	Milk	*Maatsaa*	Plant poising	6	6	100
7	Fermented kocho	*C'aalaa unc'c'a*	Stomatitis, Foot and Mouth Disease	5	10	50
8	Porridge and the end product of local kocho (*Ensete ventricosum (Welw.) Cheesman*)	*Shendeerannee Zaalima*	Antidotes, antitoxic for plant poising	8	9	89.5
9	Oil, soap	*Zaayttiya*	Blackleg, bloat	4	6	66.7
10	Honeydew	*Degeriya eesaa*	Blackleg	9	10	90
11	Common salt	*Mas's'inniya/ as'uriyaa*	Mineral deficiency, fattening, appetizer	6	6	100

Discussion

The present study showed the rich knowledge of ethnoveterinary medicinal plants in the Dawuro zone that was indicated by the number and diversity of plants reported. The number of plants reported by females was ranging from 2 to 8 and by males from 2 to 15 plants indicating both men and women members of the community have good knowledge of medicinal plant use. This finding did not agree with other studies conducted in the country (Giday *et al*., 2009; Lulekal *et al*., 2014; Teklehaymanot, 2017) reported that men had more knowledge of plant usage because they are naturally selected during childhood to be apprentices of ethnoveterinary practices.

In this study, the knowledge regarding the treatment of animal ailments was relatively comparable between different age groups. In other studies, however, it was observed that the number of medicinal plants reported increased with age, and the older informants reported more medicinal plants than younger individuals (Begossi *et al*., 2002; Teklehaymanot, 2009; Monteiro *et al*., 2011; Usmane *et al*., 2016). This could be related to the curiosity and knowledge of the elderly herbalists with curative plants than that of younger individuals in the community. Informants who could not read reported more medicinal plants than literate members did. This might be oral transfer, the influence of modernization and technological changes, and a lack of curiosity by educated generations to acquire indigenous knowledge like their fore parents (Monteiro *et al*., 2011; Lulekal *et al*., 2014). This finding is in agreement with the previous studies in Ethiopia (Seifu *et al*., 2006) and elsewhere in the world (Tariq *et al*., 2014; Randrianarivony *et al*., 2017) who reported as the majority of informants are illiterate while only a few of them reached the high school diploma level.

Oral transfer and secrecy on the acquisition of knowledge on ethnoveterinary practices within the study districts, most of the time-tested indigenous knowledge of the herbalists' passes away might also explain the decline of indigenous knowledge going down the generation ladder. This result agrees with other findings in Ethiopia (Lulekal *et al.,* 2008; Yineger *et al*., 2008; Giday *et al*., 2009; Teklehaymanot, 2009; Gebrezgabiher *et al*., 2013) and elsewhere in other countries (Begossi *et al*., 2002; Uniyal *et al*., 2006; Silva *et al*., 2011) share a similar concern on the knowledge gap down generations in different cultural groups.

The family Asteraceae was the best representation of plant species followed by Fabaceae and Solanaceae. The wider utilization of the Asteraceae family might be due to its plenty in the study area or high bioactivity (Tariq *et al*., 2014). This finding was, however, different from that of (Offiah *et al*., 2011; Tekle, 2014) who in an ethnoveterinary survey reported Solanaceae and Fabaceae families were the best use. The difference among studies might be related to the different dominant vegetation of the areas or might be associated with traditional beliefs of different cultures in using traditionally specific plants.

The present findings showed that the most commonly used medicinal plants in the study area are herbs followed by trees in the preparation of treatment. The present finding was in contrast with other investigators (Bekalo *et al*., 2009; Mesfin *et al*., 2009) who reported the dominance of shrub species for

ethnomedicinal preparation in Ethiopia. The variation might be related to different ethnolinguistic groups in the country and could be attributed to the wide agroecological diversity and specific indigenous knowledge of different communities.

The finding showed that leaves were the most harvested plant parts followed by roots in the treatment preparation. This finding is in line with the results of other ethnomedicinal studies (Amenu, 2007; Monteiro *et al*., 2011; Ugulu, 2012; Agize *et al*., 2022). The first line of choice for leaf might be associated with traditional beliefs in communities about no difficulty of collection, easy preparation, and effectiveness and efficacy against ailments. The dose administered often varied with the parts of the plant used and the methods of preparation. In this study, the majority of the treatments are taken orally as also reported earlier in other parts of Ethiopia (Lulekal *et al*., 2014; Kebede *et al*., 2017).

The physical manifestation of the diseased animal and visually confirmed degree of severity of illness are used to determine preparation doses to treat livestock ailments. Due to the lack of standardized doses of herbal preparations, traditional practitioners observed some side effects and symptoms of herbal preparations on their animals in the study area like shock and lacrimation. For example, the high amount of *Azadirachta indica* A. Juss. and *Solanum incanum* L. administered might cause noticeable side effects. However, no death cases were reported by the informants because they use different traditional antidotes like drinking and sprinkling cold water immediately on the body of animals especially the forehead region, milk, porridge, and the end product of traditional *kocho* fluid "*Zaalima*" to manage plant side effects.

In this study, traditional healers prepare ethnoveterinary recipes mostly in the forms of pounding and homogenizing with water followed by crushing. This finding is in agreement with those (Legesse; 2010; Lulekal *et al*., 2014; Eshetu *et al*., 2015) who reported pounding the remedial part and homogenizing it with water was found to be the major mode of remedy preparation. The present study showed that the claimed medicinal plants have values other than their medicinal roles. The community uses them for different purposes such as human disease treatment, firewood, construction, timber production, animal fodder, fence, edibles, spices, and other uses such as agricultural tools and soil conservation. This finding is similar to other studies from different parts of Ethiopia (Amenu, 2007; Tekle, 2014; Kebebew and Mohamed, 2017).

The results also revealed that few traditional herbalists preferred days and times for plant collection like on Sunday and Wednesday and in the early morning without contact with any person, which they believe, could make the treatment more efficacious to cure diseases. Nearly similar findings were reported from the Ejaji area (Chelya Woreda) in West Shoa. Amenu (2007) pointed out that medicinal plant collection and provision were restricted to and done on Sunday, Wednesday, and Friday. However, the majority of traditional healers reported that there is no taboo association with medicinal plant collection and uses in the study area. This result is in line with the finding from other parts of Ethiopia (Yineger *et al*., 2008).

Highest fidelity level values were obtained for *Cyphostema* sp. for diseases of the reproductive system, *Calpurnia aurea* for ectoparasites, and *Pentas schimperiana* for fattening and orthopedic disease category accounts followed by *Eucalphytus globules* for the infectious/blackleg disease category and *Croton macrostachyus* for the wound and trauma category. This indicates the relatively high healing potential of the species for treating ailments under the respective categories. The highest FL might be related to the cited plant species with more healing power due to the presence of bioactive compounds for the respective ailments.

According to Monteiro *et al.* (2011), the use value is a quantitative method that demonstrates the relative importance of species locally known. This method can be used to choose species for garden cultivation or to create medical works with scientific backing. Results showed that *Azadirachta indica* and *Eucalyptus globulus* were the species with the highest use-value, probably because they are dominant vegetation and have high curative activity. The plant species cited by more than two informants are considered the most used medicinal plants (Randrianarivony *et al*., 2017).

Direct matrix ranking analysis indicated that *Arundinaria alpine* and *Eucalyptus globulus* were ranked 1st and 2nd, and hence are the most preferred medicinal plants by local people for multipurpose uses and are the most threatened species as the informants reported while *Maesa lanceolate* and *Prumnas Africana* were the least ranked species in multipurpose importance in the study area.

There is an abundant undocumented traditional knowledge of non-plant materials used to treat a wide range of livestock diseases in the study area that had

a significant effect on animal health practices. The survey also revealed that eleven non-plant remedies were used in the Dawuro zone. Some of the non-plant materials reported include hyena faece, wood ash, honeydew, oils, kerosene, local soap, salt, porcupine meat, dear faece, sharp hot iron or knife, milk, fermented *kocho* (*Ensete ventricosum*), porridge and the end product of *Ensete ventricosum* fluid traditionally called *Zaalima* which they believed were effective in ethnoveterinary management. Nearly similar ethnoveterinary studies conducted in Nigeria (Alhaji and Babalobi, 2015) share a similar concern on the knowledge of non-plant remedies found that sharp hot iron or branding for treatment of blackleg and inflammation due to trauma, salt used for appetite promotion, and vegetable oil for managing bloat. However, hyena faece, wood ash, dear feaces, porcupine meat, fermented *kocho* or *C'aalaa unc'c'a*, the end product of local kocho fluid (*Zaalima*), and honeydew have not been reported before another ethnoveterinary survey of non-plant remedies used in Ethiopia.

According to Souto *et al*. (2011), the use of zootherapy for medicinal purposes is part of a body of traditional knowledge that is increasingly becoming more relevant to discussions on conservation biology, public health policies, sustainable management of natural resources, biological prospection, and patents. From this perspective, the present study was also undertaken to document information about local ethnoveterinary practices by traditional herbalists in the Dawuro zone. Accordingly, the products and parts of porcupines, hyenas, cattle, bears, and aphids (insects) were used by the community in ethnoveterinary practices. Other studies have likewise highlighted these medicinal animal products used in ethnoveterinary practices from other parts of the world (Padmanabhan and Sujana, 2008; Souto *et al*., 2011; Vats and Thomas, 2015).

The driving factors for medicinal plants and non-plants use as ethnoveterinary treatments were easy accessibility, an inadequate number of formal veterinary clinics and veterinarians, primary healthcare system, apparent effectiveness, lack of adequate modern drugs supplies, long distance to veterinary clinic station, affordability, and low cost, and outbreaks; available in the area that would never be enough to provide healthcare services for more than 3,543,417 livestock population in the area. Moreover, almost all of the rural community lives in marginal areas which are not easily accessible to the rare modern veterinary services which are also known for their scorching prices unaffordable to the less economically endowed people living there. This finding is similar to the work of (Wabe *et al*., 2011; Lulekal *et al.,* 2012) in Ethiopia, South Africa (Soyehu and Masika, 2009), and Brazil (Souto *et al*., 2011).

Conclusions

The present study showed that the Dawuro community used a wide range of plant species for ethnoveterinary medicine purposes. A total of 92 plant species belonging to 44 families were documented; of which the family *Asteraceae* represented 10 species. Herbs and leaves were the most commonly used plants and plant parts, respectively. *Cyphostema* sp. and *Pentas schimperiana* showed the highest fidelity value as plant materials while dear feaces, and milk as non-plant remedies. Hotwire/knife were materials used for branding or any other manipulation. In a nutshell, this study attempts to contribute a piece of evidence for ethnoveterinary practice documentation, which could be instrumental for future scientific studies. However, the loss of indigenous knowledge due to oral transfer by and large; and the unwillingness of elder herbalists to share their knowledge, requires immediate intervention. Therefore, strong government policy support to safeguard plant species is indispensable. Besides, there is a need for research and development in the applications of ethnoveterinary knowledge; and evaluation of bioactive material in the plant material with safety, efficacy, and toxicity level.

Ethical approval

This study was approved by the Ethical and research review Board of the Office of Jimma University, College of Agriculture and Veterinary Medicine. The consent of each respondent was asked verbally to participate in the study. Consequently, the confidentiality of their traditional property owners was completely maintained during data collection.

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Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in immuno-compromised women and sheep of Bahir Dar City, Ethiopia

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Abstract

Toxoplasmosis is one of the most significant worldwide zoonotic infections caused by the protozoan parasite *Toxoplasma gondii*. It is a major public health and veterinary concern in terms of mortality and morbidity. The objective of this study was to estimate the seroprevalence and assess the potential risk factors of *T. gondii* infection among pregnant women, HIV/AIDS patients, and sheep in Bahir Dar city, North West Ethiopia. A cross-sectional study design and systematic random sampling techniques were employed. Serum samples were analyzed for anti-*T. gondii* antibodies using a commercially available Toxo-latex agglutination test kit. Logistic regression analysis was employed to test associations between variables. The seroprevalences of *T. gondii* infection in the examined pregnant women, HIV/AIDS patients, and sheep were 70.8% (95% confidence interval (CI): 65.1-73.4%), 70% (95% CI: 63.3-76.8%), and 43% (95% CI: 38.5 - 47.4%) respectively. Multivariable logistic analysis showed that *T. gondii* infection was significantly associated with knowing the zoonotic nature of toxoplasmosis (adjusted Odds ratio [AOR] = 0.197, 95% confidence interval [CI]: 0.107 - 0.35), eating raw meat (AOR=3.27, 95% CI: 1.89-5.65), and owning a cat (AOR=9.35, 95% CI: 4.00-21.84) in pregnant women and HIV/AIDS patients. Hence, comprehensive health education about toxoplasmosis, particularly for pregnant women and HIV/AIDS patients, avoiding consumption of uncooked meat, and cat contact for immune-compromised women are of paramount importance.

Keywords*:* Bahir Dar; HIV patient; pregnant woman; Seroprevalence; Sheep; T. gondii.

Introduction

Toxoplasmosis is an infection caused by a protozoan parasite *T. gondii* which is one of the most important zoonotic diseases worldwide (CDC, 2018). *T. gondii* is an obligate intracellular parasite that can infect humans as well as warm-blooded domestic and wild animals (Tenter *et al*., 2000).

T. gondii is a tissue cyst-forming parasite and has two alternative hosts, namely the definitive host and intermediate host. Sexual reproduction occurs in the definitive host and asexual replication occurs in the intermediate host (Dubey, 1996). Sexual reproduction occurs in cats with the ingestion of cysts from tissues of an intermediate host and sporulation occurs in the environment. Asexual development occurs in intermediate hosts in humans, warm-blooded animals, and birds after ingestion of sporulated oocyst (Tenter *et al*., 2000; Cenci *et al.,* 2013).

T. gondii can be transmitted between and within the hosts via ingestion of raw or undercooked meat containing viable cysts and accidental inoculation of tachyzoites (Khan and Khan, 2018). Ingestion of unwashed fruits or vegetables that have been contaminated with feces containing sporulated oocysts is also a means of transmission of the parasite (CDC, 2018). Congenital transmission through the placenta from the mother to her unborn child may occur during pregnancy in case of an acute phase of infection (Peyron *et al*., 2019). Contaminated injection, blood transfusion, and organ transplantation can transmit the infection if the donor has recently acquired toxoplasmosis and parasitemia at the time of blood collecting (Florence and Marie-Laure, 2012).

T. gondii in immune-compromised groups (pregnant women and HIV patients) is a major health concern as the disease is serious in terms of mortality and psychological squeal. In pregnant women, *T. gondii* infections may cause spontaneous abortion, stillbirth, prematurity, and serious fetal damage (Maldonado and Read, 2017). This parasite acquired in the first and second trimesters of pregnancy generally shows a more severe degree of illness in the newborn (Khan and Khan, 2018).

Toxoplasmosis is also a major cause of reproductive failure in sheep, goats, and pigs. Sheep are more exposed to toxoplasmosis due to feeding habits and high susceptible nature of it. They have a big potential to spread the tissue cysts of *T. gondii* to humans through the consumption of raw or undercooked meat

and offal (Gebremedhin *et al*., 2013). Thus, information on the prevalence of toxoplasmosis in sheep is useful for the assessment of the risk of meat of these animals to the public.

Despite the severe consequence of toxoplasmosis among pregnant women, HIV patients, and sheep there is no intervention measure in our study area and the knowledge about the epidemiological status of this parasite is limited in Ethiopia. *T. gondii* screening in pregnant women and HIV patients was not practiced and hence no follow-up has been put in place. Therefore, this study was conducted to estimate the seroprevalence and potential risk factors of *T. gondii* infection among pregnant women, HIV/AIDS patients at public hospitals, and sheep slaughtered at the abattoir in Bahir Dar city, northwest Ethiopia.

Materials and methods

Study area and period

This study was conducted in Bahir Dar City. Bahir Dar is found in the northwestern part of Ethiopia, which is 565 km away from Addis Ababa, the capital city of Ethiopia. The city is located near Lake Tana, the headwaters of the Blue Nile. Its latitude and longitude are 11°36'N and 37°23'E, respectively, humidity is 69% and temperature ranges from 10 to 38°C and the town has an elevation of 1840 meters above sea level (Bahir Dar Land Administration and Use Bureau, 2018). The area receives a mean annual rainfall of 750mm (Ethiopian Metrology Agency, 2018). The city administration has a total population of 455,901 including 222,474 males and 233,428 females (CSA, 2021). The number of animals in the city includes 46096 cattle, 12070 sheep, 4468 goats, 7736 equines, 76526 poultry, and 4021 bee colonies (Amhara Livestock Resource Development and Promotion Agency, 2019).

Study design

Hospital and abattoir-based cross-sectional study designs were conducted from September 2019 to May 2021 to estimate seroprevalence and associated risk factors of *T. gondii* infection among immunocompromised individuals who visited Felege Hiwot and Adiss Alem Hospitals and sheep slaughtered at Bahir Dar Municipal abattoir, Northwest Ethiopia.

Source and study population

The source population was all pregnant women who came to antenatal care centers at Felege Hiwot and Adiss Alem hospitals and all sheep that came to the abattoir for slaughter at Bahir Dar, Ethiopia during the study period. The study population consists of those pregnant women who attended antenatal service for visitors first at Felege Hiwot and Adiss Alem hospitals that fulfilled the inclusion criteria during the data collection period and the number of sheep that came for slaughter to the abattoir.

Eligibility criteria

Inclusion criteria

Pregnant women who were able to communicate, not critically ill, and able to see, and those who voluntarily provided vital information were included in the study

Exclusion criteria

Pregnant women and HIV patients who were critically ill, unable to communicate, unable to see, and not volunteered to provide vital information were excluded from the study.

Sample size determination and sampling method

Sample size determination

The sample size was determined using a single population proportion formula to estimate the seroprevalence of *T. gondii* infection among pregnant women and sheep. The sample size was calculated based on the following assumptions; the prevalence of *T. gondii* infection among pregnant women, 68.4% in Debre Tabor (Agmas *et al*., 2015); 95% level of confidence, and 5% margin of error. The sample size was calculated by using single population proportion formulas as follows (Thursfield, 2007).

$$n = \frac{(Z\alpha/2)^2 \, \mathrm{P\,exp}\ (1 - Pexp)}{\mathrm{d}^2}$$

n= $\underline{(1.96)^2}$ $\underline{[0.684\ (1\text{-}0.684)}$

$$\frac{(0.05)^2}{}$$

n=332 pregnant women

Where, n = sample size, d^2d^2=of error, and $Z\alpha/2Z\alpha/2$=the value of standard normal distribution corresponding to a significant level of alpha.

Similarly, for sheep, considering the previous study, the seroprevalence of *T. gondii* infection among sheep slaughtered for human consumption in central Ethiopia was 20% (Gebremedhin *et al.,* 2014). Therefore, the calculated minimum sample size for sheep slaughtered in Bahir Dar abattoir was 245. However, due to logistic limitations, only 150 slaughtered sheep were sampled.

Sampling method

The method for selecting the study individuals was a systematic random sampling technique in which study subjects were picked during the time of data collection until the required sample size was reached. Our preliminary assessment before the start of the study was based on the previous year's data and one-month data from the antenatal care registration book of the health institutions were taken to approximate the total number of pregnant women attending antenatal care during the study period. Based on that assumption, about 1,500 pregnant women were estimated to visit Felege Hiwot referral hospital. Similarly, about 1300 women were expected to visit Addis Alem hospital for antenatal care. The expected total sample size was 332 as computed above. Therefore, the K^{th} interval = N/n= 2800/332 = 8.

Thus using systematic random sampling every 8^{th} pregnant woman was included in the study. The sample size of each hospital was determined using a proportional allocation formula:

$$\mathbf{ni} = \mathbf{n} \times \frac{\mathbf{Nj}}{\mathbf{N}}$$

Where ni is the sample size of each hospital, n is the total sample size, Nj is the total number of pregnant women in each hospital, and N is the total number of pregnant women who attended all hospitals. However, the real number surges the calculated assumptions thus 200 from Felege Hiwot hospital and the rest 132 from Addis Alem hospital were selected.

Since our preliminary study showed a small number of sheep slaughtered; every 2nd sheep were included in the study. However, during the study period, a total of 830 sheep were slaughtered at Bahir Dar abattoir.

Method of data collection

Questionnaire survey

A structured and pre-tested questionnaire (on 5% of the sample size) was used to collect data from study participants. Questionnaire surveys were used to assess the socio-demography, behavioral, clinical, and obstetrics characteristics of pregnant women and HIV patients of Bahir Dar city. Health professionals holding BSc (Bachelor of Science) degrees and above as laboratory workers in health centers were engaged in data collection after obtaining training on how to collect data through face-to-face interviews with the respondents.

Sample collection for laboratory processing

Blood samples were aseptically collected from venous tissue from pregnant women during their first visit (n=332) at Felege Hiwot referral hospital (n=200) and Addis Alem hospital (n=132), using vacutainer tubes. Out of the 332 blood samples, 20 samples were obtained from HIV patients. From sheep presented at Bahir Dar abattoir, blood samples were collected from the jugular vein (n=150) before slaughtering. These samples were transported in an icebox with ice packs to the Amhara Public Health Institute laboratory; Bahir Dar city Ethiopia; for testing. The samples were centrifuged at 5000 rpm for 10 minutes to separate the serum from the other blood components. Until the samples were serologically examined, they were maintained at -20°C. The Latex agglutination slide test was used for the serological examination.

Study variables

Dependent variable

The dependent variable was a dichotomous variable of *T. gondii* infection's positive or negative (presence or absence of specific antibodies).

Independent variables

Independent variables included in the study were socio-demographic characteristics (age, source of cat food, owning cats at home, educational status, knowing the zoonotic nature of toxoplasmosis and knowing toxoplasmosis as a foodborne disease, the presence of rats in and around the house, housing condition, and cat litter cleaning activities), behavioral characteristics (habit of eating sheep raw meat (undercooked or '*tibis*'), the culture of eating raw fruits), clinical and obstetrical characteristics (presence of another disease, blood transfusion history, history of organ transplantation and stages of pregnancy).

Serological tests

Detection of *T. gondii* antibodies was done using a commercial latex agglutination test kit. The test was conducted according to the instructions of the latex agglutination test manufacturers. All collected serum samples of pregnant women, HIV patients, and sheep were tested for antibodies against *T. gondii*. The test kit was provided with a buffer, a freeze-dried positive and negative control that is latex suspension was sensitized with a *T. gondii* antigen. The presence or absence of a visible agglutination indicates the presence and absence of anti-toxoplasma antibodies in the sample tested. The test was considered positive when a layer of agglutination was formed, while it was considered negative when agglutination was not formed. The proper working of the latex agglutination test kit was checked by the use of positive and negative controls (Mazumder *et al*., 1988).

Data management and analysis

Raw data generated from field and laboratory findings were encoded into Microsoft Excel and exported into SPSS version 20. Descriptive statistics were performed to look into the data pattern frequency distribution of various risk factors and were applied to quantify the magnitudes of seroprevalence among study subjects and the percentages of the questionnaire survey. A binary logistic regression model was employed and variables having a p-value of < 0.25 were exported to a multivariable logistic regression model to assess the effect of confounders. Variance inflation factor (VIF) was used to check the multicollinearity problem. However, in the data set no collinearity problem was detected. The degrees of associations of risk factors with the occurrence of toxoplasmosis in pregnant women, HIV/ADIS patients, and sheep serum samples were quantified using an adjusted prevalence odds ratio (AOR). In all

the analyses, the confidence level and level of significance were held at 95% and ≤0.05 respectively.

Ethical consideration

Before the study, ethical clearance letters were obtained from Bahir Dar University College of Agriculture and Environmental Sciences (Ref. No:1/983/1.2.6; Date: 08/08/2019 G.C.). Furthermore, written informed consent was obtained from all study participants before involvement in the study. Confidentiality of the collected information and laboratory test results was maintained and appropriate counseling was done as per the routine procedures of Ethiopian hospitals. For those unable to read and write (illiterates) pregnant women's researchers read loudly and then asked about their voluntariness and their signatures with their thumbs obtained.

Results

Prevalence of *T. gondii* infection

In the present findings out of 332 pregnant women, 235 (70.8%, 95% Confidence Interval (CI): 65.1-73.4%) were seropositive for *T. gondii* infection. Of the 20 HIV/AIDS patients tested, 14 (70%, 95% CI: 63.3-76.8%) had *T. gondii* infection. Among 150 sheep tested, 65 (43%, 95% CI: 38.5 - 47.4%) of sheep were seropositive for *T. gondii* infection.

Socio-demographic characteristics of respondents

The majority of 213 (64.2%) of the pregnant women who participated in the present study were in the age range of 20-30 years old. Of the pregnant women participants in our study, 35(10.5%) were illiterate. Out of the pregnant women who participated in our study, 97(29.2%) had cats in their homes. The description of study subjects; socio-demographic characteristics are summarized in Table 1 below.

Table 1. Summary of respondent's sociodemographic characteristics in Bahir Dar city (n=332)

Variables	Categories	Number Tested n(%)	Seroprevalence Positive n (%)
Educational status	Illiterate	35 (10.5)	21 (60)
	Write and read	79 (23.8)	56 (70.9)
	Secondary	114 (34.3)	82 (71.9)
	College and above	104 (31.3)	76 (26.9)
Age (in years)	20-30	213 (64.2)	153 (71.8)
	31-40	112 (33.7)	77 (68.8)
	41-50	7 (2.1)	5 (71.4)
Owning cat	Yes	97 (29.2)	90 (92.8)
	No	235 (70.8)	145 (61.7)
Source of food	Municipal abattoir	1 (1.0)	1 (100.0)
	Slaughter slab	4 (4.1)	3 (75.0)
	Butcher	6 (6.2)	4 (66.7)
	House food	85 (87.6)	81(95.3)
Cat litter cleaning activity	Yes	64 (66.0)	61 (95.3)
	No	33 (34.0)	29 (87.9)
Knowing the zoonotic nature of toxoplasmosis	Yes	53 (23.9)	
	No	110 (33.1)	66 (60.0)
Know toxoplasmosis as a foodborne disease	Yes	204 (61.4)	153 (75.0)
	No	128 (38.6)	82 (64.1)
Have mud house	Yes	178 (53.6)	130 (73.0)
	No	154 (46.4)	105 (68.2)
Presence of rats at home	Yes	135 (59.3)	142 (72.1)
	No	135 (40.7)	93 (68.9)

Behavioral characteristics of respondents

Of the respondents, 175 (52.7%) had raw sheep meat eating habits, and among those 140 (80%) were seropositive for *T. gondii* infection. In addition, as shown

in table 2 below, 219 (66%) had the habit of eating raw fruit of which 159 (72.6%) were seropositive for *T. gondii* infection.

Table 2. Summary of respondent's behavioral characteristics

Variables	Seroprevalence		Total n (%)
	Positive n (%)	Negative n (%)	
Eating raw sheep meat			
Yes	140 (80.0)	35 (20.0)	175 (52.7)
No	95 (60.5)	62 (39.5)	157 (47.3)
Eating raw vegetable			
Yes	159 (72.6)	60 (27.4)	219 (66.0)
No	79 (67.3)	37 (32.7)	113 (34.0)

Clinical and obstetrics characteristics of respondents

Of the 332 pregnant women, 110 (33.1%) had limited information knowing the zoonotic nature of toxoplasmosis. Based on the presence of another disease history; 180(54.2%) had other diseases. The seroprevalence of *T. gondii* infection was highest in the first trimester 110 (72.8%), followed by the second trimester 69(70.4), and the third trimester 56 (67.5%) (Table 3).

Table 3. Clinical and obstetrics status of the respondents in Bahir Dar Public hospitals, Northwest Ethiopia

Variables	Seroprevalence		Total n (%)
	Positive n (%)	Negative n (%)	
Presence of other diseases			
Yes	133 (73.9)	47 (26.1)	180 (54.2)
No	102 (67.1)	50 (32.9)	152 (45.8)
Blood transfusion history			
Yes	18 (58.1)	13 (41.9)	31 (9.3)
No	217 (72.1)	84 (27.9)	301 (90.7)
Organ transplantation history			
Yes	3 (60.0)	2 (40.0)	5 (1.5)
No	232 (70.9)	95 (29.1)	327 (98.5)
Pregnancy stage			

1st trimester	110 (72.8)	41 (27.2)	135 (45.5)
2nd trimester	69 (70.4)	29 (29.6)	98 (29.5)
3rd trimester	56 (67.5)	27 (32.5)	83 (25.0)

Risk factors associated with seropositivity of *T. gondii* infection

In the present findings by using binary logistic regression model analysis; pregnant women who were in their second (COR= 0.89, 95% CI (0.51-1.56) and third trimester (COR= 0.77, 95% CI (0.43-1.38) had an almost similar risk of toxoplasmosis when comparing those who were in the first trimester. Bivariate analysis indicated that age, cat litter cleaning activity, knowing toxoplasmosis as a foodborne disease, educational status, presence of other diseases, eating raw fruit, blood transfusion history, presence of rat, organ transplantation history, and pregnancy stage did not show any statistically significant association with the seroprevalence of *T. gondii* infection (Table 4).

Table 4. Binary logistic regression result of *T. gondii* infection occurrence with different risk factors in Bahir Dar city, northwest Ethiopia

Independent Variables	Sero-prevalence		COR (95%CI)	p-value
	Positive n (%)	Negative n (%)		
Educational status				
Illiterate	21 (60)	14 (40)	0.55 (0.25-1.23)	0.15
Able to write and read	56 (70.9)	23 (29.1)	0.89 (0.47-1.72)	0.74
Secondary	82 (71.9)	32 (28.1)	0.94 (0.52-1.71)	0.85
College and above educated	76 (29.9)	97 (73.1)	1	
Age in years				
20-30	153 (71.8)	60 (28.2)	1	
31-40	77 (68.8)	35 (31.3)	0.86 (0.52-1.42)	0.05
41-50	5 (71.4)	2 (28.6)	9.80 (0.19-5.19)	0.98
Owning cat				
Yes	90 (92.8)	7 (7.2)	7.98 (3.54-17.99)	0.00
No	145 (61.7)	90 (38.3)	1	
Food source				
Municipal abattoir	1 (100.0)	0 (0.0)	0.00 (0.00)	1.00

Independent Variables	Sero-prevalence		COR (95%CI)	p-value
	Positive n (%)	Negative n (%)		
Slaughter slab	3 (75.0)	1 (25.0)	6.75 (0.57-80.27)	0.13
Butcher	4 (66.7)	2 (33.3)	10.13 (1.41- 2.75)	0.21
House food	81 (95.3)	4 (4.7)	1	
Cat litter cleaning activity				
Yes	61 (95.3)	3 (4.7)	0.36 (0.08-1.70)	0.20
No	29 (87.9)	4 (12.1)	1	
Knowing the zoonotic nature of toxoplasmosis				
Yes	169 (76.1)	53 (23.9)	2.13 (1.30-3.47)	0.00
No	66 (60.0)	44 (40.0)	1	
Presence of other diseases				
Yes	133 (73.9)	47 (26.1)	1.39 (0.86-2.23)	0.18
No	102 (67.1)	50 (32.9)	1	
Know toxoplasmosis as a food-borne disease				
Yes	153 (75.0)	51 (25.0)	1.68 (1.04 -2.72)	0.03
No	82 (64.1)	46 (35.9)	1	
Eating raw sheep meat				
Yes	140 (80.0)	35 (20.0)	2.61 (1.60-4.26)	0.00
No	95 (60.5)	62 (39.5)	1	
Eating raw vegetable				
Yes	159 (72.6)	60 (27.4)	0.78 (4.74-1.27)	0.31
No	79 (67.3)	37 (32.7)	1	
Blood transfusion history				
Yes	18 (58.1)	13 (41.9)	0.54 (0.25 - 1.14)	0.11
No	217 (72.1)	84 (27.9)	1	
Organ transplantation history				
Yes	3 (60.0)	2 (40.0)	0.61 (0.10 -3.74)	0.60
No	232 (70.9)	95 (29.1)	1	
Presence of rat				
Yes	142 (72.1)	55 (27.9)	1.17 (0.72 -1.88)	0.53
No	93 (68.9)	42 (31.1)	1	
Pregnancy stage				

Independent Variables	Sero-prevalence		COR (95%CI)	p-value
	Positive n (%)	Negative n (%)		
1st trimester	110 (72.8)	41 (27.2)	1	
2nd trimester	69 (70.4)	29 (29.6)	0.89 (0.51-1.56)	0.68
3rd trimester	56 (67.5)	27 (32.5)	0.77 (0.43-1.38)	0.39

Key: Statistically significant at P < 0.05, CI = Confidence interval, COR = Crude odds ratio,

In multivariable analysis, showed that significantly high seroprevalence of *T. gondii* infection in study participants who own cats (AOR =9.35, 95% CI (4.0 - 21.84), eating raw meat (AOR=3.27, 95% CI (1.89 - 5.65), and know about zoonotic nature of toxoplasmosis (AOR=0.20, 95% CI (0.11-0.35) (Table 5). In the present study, there was no significant statistical association between independent variables and seropositivity of *T. gondii* infection among immunocompromised women.

Table 5. Multivariable logistic regression result of *T. gondii* infection seroprevalence with different risk factors in Bahir Dar city, northwest Ethiopia

Variables	Sero-prevalence			
	Positive n (%)	Negative n (%)	AOR (95% CI)	p-value
Owning cat				
Yes	90 (92.8)	7 (7.2)	9.351 (4.0 -21.84)	0.00
No	145 (61.7)	90 (38.3)	1	
Knowing the zoonotic nature of toxoplasmosis				
Yes	169 (76.1)	53 (23.9)	0.20 (0.11-0.35)	0.01
No	66 (60.0)	44 (40.0)	1	
Presence of other diseases				
Yes	133 (73.9)	47 (26.1)	1.18 (0.68 -2.07)	0.20
No	102 (67.1)	50 (32.9)	1	
Know toxoplasmosis as a foodborne disease				
Yes	153 (75.0)	51 (25.0)	1.68 (1.04 -2.72)	0.56
No	82 (64.1)	46 (35.9)	1	
Eating raw meat				
Yes	140 (80.0)	35 (20.0)	3.271 (1.9 - 5.65)	0.000

No	95 (60.5)	62 (39.5)	1	
Blood transfusion history				
Yes	18 (58.1)	13 (41.9)	5.49 (0.23 -1.28)	0.18
No	217 (72.1)	84 (27.9)	1	

Key: Statistically significant at $p < 0.05$, AOR = Adjusted odds ratio, CI = Confidence interval.

Discussion

The present study reveals that, out of 332 pregnant women, 235 (70.8 %) of them were seropositive for *T. gondii* infection. This finding is in agreement with the report of 68.4% (Agmas *et al*., 2015) in Debre Tabor. The seroprevalence finding of *T. gondii* infection in this study is slightly lower than the reported results of 88.6% (Endris *et al*., 2014), 86.4% (Gebremedhin *et al*., 2013) and 85.3% (Abamecha *et al*. 2016). However, the *T. gondii* infection seroprevalence found in the present study was higher than the reports of 9.3% (Finda *et al*., 2015), 25% (Andiappan *et al*., 2014), 30.9% (Mwambe *et al*., 2013) and 23.6% (Mosti *et al*., 2012). The variations in the *T. gondii* infection result found in the present study in Ethiopia as well as in other countries could be due to variations in climatic conditions, cat management system, housing conditions, socioeconomic status, and feeding habits of study participants and diagnostic methods (Ibrahim *et al*., 2017).

This study identified statistically significant associations between owning cats and seropositivity. For pregnant women owning a cat, the probability of being infected by *T. gondii* infection is 9.35 times more likely as compared with pregnant women not owning a cat (AOR =9.35, 95% CI (4.0 - 21.84). This finding was in agreement with that of Zemene *et al*.(2012). This might be due to frequent contact with cat feces and accidental ingestion of oocysts. The cat carrier state remains a basic factor for the possible survival and spread of toxoplasma oocyst through their feces. The oocysts, in particular, can survive for long periods in sandy soil thus causing a source of accidental contamination (Tenter *et al*., 2000; CDC, 2018). Semi-urban peoples of Bahir Dar city have low living standards and mostly keep cats for hunting rats due to their homes being wood and mud infested by rodents.

Pregnant women who have raw meat eating habits had a significantly higher risk of being infected with *T. gondii*. They were 3.27 times more likely to be infected by *T. gondii* infection as compared with those who don't have raw meat eating habits (AOR=3.27, 95% CI (1.89-5.65). This finding was in agreement

with those of Fenta (2019), Abamecha and Awel (2016), Awoke (2015), and Mandour *et al*. (2017).

In the present study, knowing the zoonotic nature of toxoplasmosis (AOR=0.20, 95% CI (0.11-0.35) has a protective effect on the occurrence of *T. gondii* infection. This might be due to their awareness they protect themselves from contacting reservoir host cats. However, no significant difference was observed between the different educational levels of respondents. The reason might be in different educational levels; there was relatively similar awareness about *T. gondii* infection.

The current study also revealed that out of the 150 sheep investigated, 65 (43%) were seropositive for *T. gondii* infection. The seroprevalence of *T. gondii* infection in this study was lower than that of the 77% result recorded elsewhere (Elmore *et al*., 2010). However, the *T. gondii* seropositivity found in the present study was higher than the 19.5% reports of Opsteegh *et al.* (2014). The management system, feed type, and geographic location might bring such different findings. The highest prevalence recorded in our study area might be because of free grazing farming practices that access freely contaminated pastures by *T. gondii* oocysts. Sheep are important reservoirs for *T. gondii* pathogens because of their grazing character in oocyst-contaminated pastures. In-home-based consumption, sheep are the most common farm animal slaughtered in individual households in our study area. Raw meat of sheep consumed in Bahir Dar like other goat and beef, especially undercooked thick muscle (*kitfo/libleb or tibs)*, tongue, and liver. There is also cross-contamination of cooking materials that contact with the raw meat of sheep thus transmitting to immune-compromised women (CDC, 2018).

Toxoplasma gondii infection is a neglected zoonotic disease, but its high prevalence among immunocompromised women in our study showed that it could lead to transmitting the disease vertically to their unborn babies (Cenci *et al*., 2013). Different scholars indicate that in women, infection with *T. gondii* affects the pregnancy thus leading to abortion and stillbirth. Vertical transmission affecting the newborn leads to psychomotor retardation, schizophrenia spectrum disorders, hydrocephalus, etc. Similarly, anxiety, depression, suicide attempts, and hearing problems are seen in immunocompromized women. It also causes significant economic loss due to treatment costs, labor costs, poor

growth performance, high mortality, and morbidity (Mizani *et al*., 2017; Fallahi *et al*., 2018).

The limitation of this study was that the kit did not measure the acute or chronic nature of toxoplasmosis. In addition, not all the calculated sample size for sheep was tested due to a shortage of kits.

Conclusions

This study gives an insight that the occurrence of *T. gondii* infection among pregnant women, and HIV/AIDS patients at public hospitals of Bahir Dar city, northwest Ethiopia is high. Our study confirms that *T. gondii* infection remains a major public health importance in our study area. Thus, it might cause significant economic and health loss due to high mortality and morbidity. *T. gondii* infection occurrence among pregnant women was found to be significantly associated with owning cats at home and eating raw meat. In the present finding, the seroprevalence of *T. gondii* infection in sheep was high in the study area. Thus, the higher seroprevalence in these encountered animal species used as a food source revealed the potential risk of *T. gondii* infection presented to people through the consumption of their raw meat.

Taking the result of this study into account, we strongly advise better cat management activities be practiced to minimize *T. gondii* oocyst contamination. It is also advised to avoid eating raw or uncooked meat through integration with other disease control programs. A compulsory continuous health care awareness creation about *T. gondii* infection like other diseases should also be given. Building belief among scientists (i.e. veterinarians and public health workers) to generate a robust one-health approach; applied and coordinated interventions, long term strategy to manage *T. gondii* infection dynamics will also be essential.

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Dog demography, rabies awareness and dog bite cases in Bishoftu town, Ethiopia

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Abstract

Rabies is a zoonotic viral disease that affects humans and animals and dogs are the primary source of infection. Information on domestic dog populations is vitally important for rabies control. The present study is to assess the demography of dogs, the incidence of dog bites, and the knowledge, attitude, and practice (KAP) of the communities concerning rabies in Bishoftu town. Cross-sectional household surveys were conducted from April to June 2021 involving a questionnaire survey and collection of retrospective patient records from healthcare facilities. A total of 244 respondents were interviewed using a structured questionnaire format to determine their KAP about rabies and dog ownership. Data on the incidence of dog bites and suspected cases of rabies from November 2019 to June 2021 were retrieved from the records of four health centers in Bishoftu town. In interviewed households (HH) 51.2 % owned at least one dog with a dog: human ratio of 1:9 and 1.54 dogs/household. In the participating households, 71% of dogs had been vaccinated against rabies. Almost all, 99% of the respondents had heard of rabies and identified the mode of transmission of rabies. However, 61.8%, had a moderate level of knowledge and 59.8% had an intermediate level of attitude, and 64.3% satisfactory level of appropriate rabies prevention practices score. There was a statistically significant association between knowledge score and age, occupation, and source of information as well as attitude score with age and source of information ($p<0.05$). Respondents who attend higher education were more likely to have higher practice scores in the prevention of rabies ($p <0.05$). There were significant correlations among the participant's knowledge, attitude, and practice ($p<0.01$). A total of 612 victims were registered and received the post-exposure vaccine in Bishoftu over two years (2019 to 2021). We identified critical knowledge gaps related to factors influencing rabies prevention and control. The veterinary and public health sectors should play an important role in educating

and raising community awareness. Moreover, the production and use of the banned nerve tissue vaccine in the public health sector in Ethiopia need attention.

Keywords: Knowledge; Attitude; Practice; Bishoftu; dog demography; Rabies.

Introduction

Dogs have been known to have a close relationship with humans since their domestication which made them a source of several diseases to humans such as rabies (Rinzin *et al*., 2016). Rabies is a deadly viral zoonosis, which causes encephalitis in warm-blooded animals and humans caused by the rabies virus, which belongs to the genus *Lyssavirus*. The virus is transmitted to humans from rabid animals through the bite and sometimes scratches. Despite the invention and application of the rabies vaccine since long time ago, rabies still causes deadly diseases worldwide (Zulu *et al*., 2009) It has been anticipated that about 60,000 people die every year due to canine rabies globally, of which the highest death is in Asia and Africa; dogs (almost 99%) remain the major animal reservoirs in these continents (Undurraga *et al*., 2017).

In Ethiopia, 10 years (1990-2000) retrospective data-based study revealed that annually, on average, about 2200 people received post-exposure anti-rabies treatments and 96.2% of whom were bitten by dogs. During the same period, 322 fatal human rabies cases were recorded and 95% of these were acquired from dogs (Yimer *et al*., 2002). During the next decade (2001-2009) the number of fatal cases increased to 386 ranging from 35 to 58 per year. The magnitude of post-exposure vaccination escalated to 17,204 mostly from Addis Ababa. During this period a total of 3,460 brains were examined from animals involved in human bites and 75% of them were positive. A total of 130,673 doses of rabies vaccine were administered to humans and 85,055 doses were administered to animals during this period (Deressa *et al*., 2010). Moreover, a hospital-based study from the southern Ethiopian pastoral area revealed that 98% of rabies suspected animals bite were due to dog bites (Gum *et al*., 2019).

Dogs are classified as owned, that are restricted within a fenced yard or in the homestead under the owners' supervision, or ownerless/stray dogs that are not under direct human control or not prohibited from freely roaming (Smith *et al*., 2019). In Ethiopia, both owned and ownerless dogs bite humans and con-

tribute to the transmission of rabies. Between the years 2009 and 2011, a total of 1,088 dogs and cats were examined for rabies, of which 73.62% of dogs and 5.1% of cats were found positive. The majority of the rabid dogs were ownerless or those whose ownership was not known (Reta *et al.*, 2014). The annual mortality due to rabies was estimated at 10,000 people making Ethiopia to be one of the worst-affected countries in the world. The incidence, however, varies both temporally and spatially. Dog-borne rabies remains a public health concern in the country, especially in urban settings where there is a large pet dog population (Shite *et al.*, 2015). Studies showed that the dog population in urban areas including Africa (Reece, 2000) creates favorable conditions for the transmission of dog-borne rabies to humans (Baneth *et al.,* 2016; Damborg *et al.,* 2016). It is highly associated with the presence of a large population of uncontrolled and stray dogs (Hampson *et al.,* 2015). Poor public awareness of rabies is considered one of the bottlenecks to the prevention and control of the disease in Ethiopia (Jemberu *et al.,* 2013). In addition, knowledge about dog demography and quantification of rabies-suspected animal bites are relevant for the prevention and control of dog-borne zoonosis as used in several countries, is limited (Gsell *et al.*, 2012; Rinzin *et al.,* 2016; Tiwari *et al.,* 2019).

Rabies is endemic in several parts of Ethiopia. However, data on dog demography such as population densities, population structure, and owner characteristics is extremely scarce. Reliable information on dog demography and ecology is important for developing and implementing vaccination programs and estimating the vaccine coverage needed to control dog-borne rabies (Rinzin *et al*., 2016). In Bishoftu town, the current study area, there are several slaughterhouses and slabs where leftovers are thrown to scavengers, which attract several stray dogs. As a result, there is a large number of roaming dogs in the town. Hence the total dog population of the town is on the rise. This study, therefore, was carried out to identify the status of dog demography, pinpoint the impacts of dog demography on the occurrence of rabies in animals and humans, and assess knowledge, attitude, and practices of the community in Bishoftu town, central Ethiopia.

Materials and methods

Study areas

This study was conducted in Bishoftu town which is located at a distance of 45km southeast of Addis Ababa, Ethiopia (Figure 1). The town is located in Ada'a district, East Shewa zone of Oromia Regional State at 9° N and 40° E having an average altitude of 1850 meters above sea level. The area receives an annual rainfall of 866 mm with bimodal occurrence. The mean annual maximum and minimum temperatures of the area are 26°C and 14°C, respectively, with a mean relative humidity of 61.3%. The town is home to 127,678 people (59,589 men and 68,090 women) (CSA, 2015) and is divided into 14 administrative units. Besides, since the town is surrounded by several beautiful lakes, there are several resorts visited by several non-inhabitants. Although an estimate of the dog population of the town is lacking, there is a large number of dogs in the town.

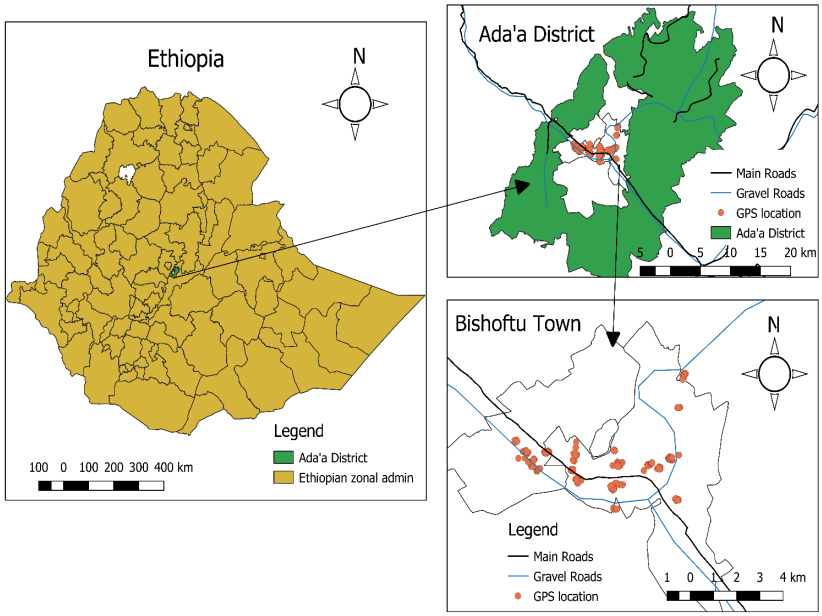


Figure 1. Map of Ethiopia depicting the location of study areas. The specific area where data was collected is depicted using GPS data.

Study design

A community-based cross-sectional study was conducted from April 2021- June 2021 to gather data on dog demography and the community's KAP using a questionnaire. A publicly available map of Bishoftu town was downloaded from Google Earth (https://earth.google.com/web.) and the entire residential areas were divided into non-overlapping approximately equal-sized blocks demarcated by main roads and adjacent blocks were marked using four different colors: red, yellow, blue, and green (Figure 2A). Hence, Bishoftu town was divided into 80 blocks. One of the four colors was randomly chosen using a lottery system. Hence, the blue color was selected. Twenty blocks were identified but due to a shortage of logistics and time, only 16 of the selected blocks were included in this study (Figure 2B). The sixteen selected blocks were physically visited to mark their boundaries on the ground using turning roads and fixed landmarks such as hotels, buildings, and long fences. All living compounds within the marked boundaries in the selected blocks were included in the survey.

Two hundred and forty-four households were interviewed in this study. Only voluntary households were interviewed. The structured questionnaire was prepared and finally populated to Epcollect5 mobile and web application data gathering platform. Both open and close-ended questions were included. The questionnaire was first prepared in English and translated to Afan Oromo and Amharic for appropriateness and clarity in approaching the study participants. Before the interview began, they were briefed about the purpose of the study and were asked for their consent. The questionnaire focused on socio-demographic characteristics of the interviewed household, dog ownership, confinement status of every dog owned by the household, rabies vaccine coverage in dogs, dog-to-human population ratio, factors related to rabies awareness, and dog-related zoonosis, community knowledge on rabies, rabies prevention measures, treatment practices, and animal bite management and healthcare seeking behavior. All the information obtained from the study participants was kept confidential. Then, the responses from respondents were counted and scored. This score was then pooled together, and the mean score was computed to determine the overall KAP of respondents from zero to three with the right answers receiving the highest points.

Retrospective data collection

Retrospective data were retrieved from case books and registries from November 2019 to June 2021 in Bishoftu town. Registered data on dog bites and suspected cases of rabies were collected from the records books of four health stations (Zqual, Cheleleka, Babugaya, and Keta Health Centers) that provide rabies vaccines for the community inhabiting the town. Information on the date of the bite, species of animal which bite, sex of the victim, and body part bitten was retrieved from the patient recording books.

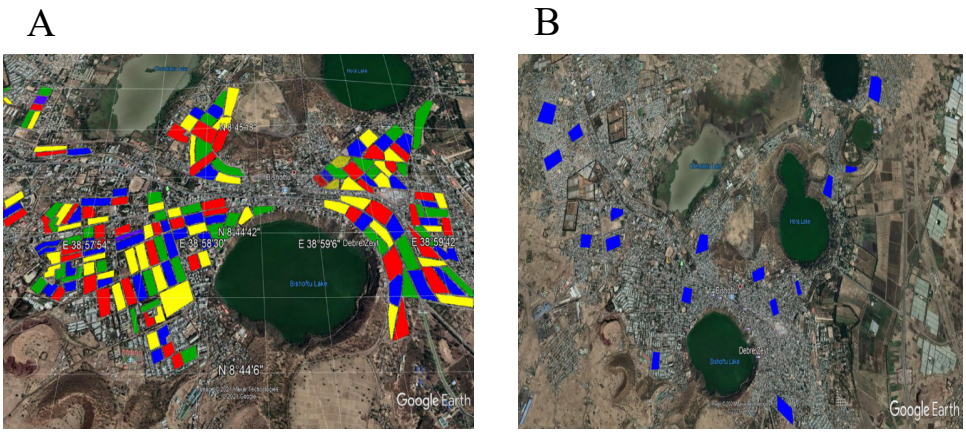


Figure 2. Map of Bishoftu town depicting all blocks and those included in the study: A) 80 approximately equal-sized non-overlapping blocks marked with four different colors; B) the randomly selected 16 Blue colored blocks included in this study

Data analysis

After collecting the data using the Epcollect5 platform, the data was downloaded and converted into Microsoft Excel 2010 spreadsheet and coded. All the descriptive and analytical statistics analyses were performed by using a statistical package for social sciences (SPSS) version 25. Data obtained were presented using tables and charts. Pearson's chi-square test and fisher exact test were used for calculating the association of all independent variables (gender, age, education level, occupation, dog ownership, and source of information on rabies) and each dependent variable (i.e. respondent knowledge, attitude, and practice level regarding the question). Descriptive statistics were used for

calculating the frequency and percentage of both dependent and independent variables. The p-values were used to describe statistical significance (judged as significant when the p-value is less than 0.05). The models were tested for correlations between each dependent variable.

Ethical clearance

This study was approved by the ethical review board of Addis Ababa University, Aklilu Lemma Institute of Pathobiology (Ref ALIPB IRB/16/2012/20, 09 March 2020). Each participant was informed about the aim of the study and verbal informed consent was obtained from each respondent. Participation in the study was voluntary and respondents were free to withdraw from the study at any time. Their consent was recorded (marked) on the questionnaire paper, the interview was anonymous, and data remained confidential throughout the study.

Results

Socio-demographic characters of the participants

A total of 244 households were interviewed during the study period. In study households, the average number of persons per household was 7, ranging from 1-30 per household. The age of the respondents ranged from 14 to 80 with a median age of 35 years. Fifty-nine percent of the respondents were female. Forty-point-two percent of the respondents attended secondary school and 41.8 % of them were college or university graduates (Table 1).

Table 1. The Socio-demographic characteristics of the respondents interviewed in this study

Characteristics	Category	N	%
Gender			
	Male	100	40.98
	Female	144	59.73
Age group			
	Range (Min,-Max)	14-80	
	Median age	35	
	<18	18	7.38
	19-35	73	29.92
	>36	153	62.70
Educational statues			
	No formal educations	15	6.15
	Primary education (1-8 grades)	29	11.88
	Secondary education (9-12 grades)	98	40.16
	Technical / Higher education	102	41.80
Occupations			
	Employed/ Professional	76	31.15
	Private business	58	23.77
	Housewife/ Unemployed	74	30.33
	Student	36	14.75
Head of household (HH)			
	Yes	163	66.80
	HH representative	81	33.2

Dog demography and rabies vaccine coverage

Households owned at least one dog was 51.2 % with a total number of 190 dogs, ranging from 1 to 4 dogs per household. There were on average 1.54 dogs per household. The estimated dog-to-human ratio for the households studied was 1:9. The female: male ratio of dogs was 1 to 3.75. About 71.2% of the HH possess indigenous breeds, whereas 16.8% and 12% were crossbreeds and exotic, respectively. In the present study area, 88% of owners kept dogs for house guarding purposes. Forty-six percent of the households confine their

dogs while 53% of the households let their dogs freely roam and scavenge in the environment.

Of a total of 190 dogs owned by the interviewed households, 71.58% (95% confidence interval [CI]: 64.05 – 77.39), (136) of them were vaccinated against rabies (Table 2). However, only 15 households had vaccination certificates. The respondents disclosed various reasons for not having their dogs vaccinated against rabies which include: distance from veterinary clinics (37%), lack of knowledge about the existence of rabies vaccine for animals (35%), and lack of awareness about the source of vaccines (16.9%). Other reasons include the inability to handle their dogs and fear of behavioral change after vaccination.

Table 2. The demographic structure of the dog population owned by the 125 households included in this study

Variable	Category	N(HH)	%
Sex of dog			
	Male	150	78.95
	Female	40	21.05
Age of dog			
	Adult >1year	180	94.74
	Puppy <1year	10	5.26
Breed			
	Local	136	71.58
	Cross	36	18.95
	Exotic	18	9.47
Source of dog			
	Received as gift	144	75.79
	Bought	30	15.79
	From own bitch	16	8.42
Reason for dog ownership			
	Guard dogs/protection	158	83.16
	Love and affection/pet	32	16.84
Confinement of dog			
	Allow to roaming	102	53.68
	Never allowed to leave the premises	88	46.32

Variable	Category	N(HH)	%
Feeding of dog			
	Household member	169	88.95
	Neighbors /household	15	7.89
	Roaming around	16	8.42
Rabies vaccination status			
	Vaccinated	136	71.58
	Not vaccinated	54	28.42
Housing system			
	Specially constructed house/cage	76	40.00
	Anywhere on the premises	66	34.74
	On house passageway/corridor	48	25.26

Knowledge, Attitude, and Practice of the community concerning rabies

Out of the total respondents, 10.7% of them correctly identified the features of rabies as an infectious disease caused by a virus, and 96.3% of respondents were aware of the transmission of the infection to humans. Only 8.1% of the respondents correctly described the symptoms of rabies in humans and 60% of them described the symptoms of rabies in animals. In this study, 96% of the respondents correctly identified the different modes of transmission of rabies whereas only 5.3% of the respondents were aware of pre-exposure vaccines in humans and 76.6% of them revealed that stray dogs transmit the disease to humans. The data showed that 67.6% of the respondents correctly identified rabies prevention measures, and 34% of them correctly mentioned actions to be taken against rabies suspected animals (Table 3).

Table 3. Knowledge, attitude, and practice of respondents about rabies in Bishoftu, Ethiopia

Code	Questions	Correct answer		Partially correct answer		Incorrectly answered	
		n	%	n	%	n	%
K1	What causes rabies in a dog?	26	10.7	-	-	218	89.3
K2	Is rabies a transmissible disease humans?	235	96.3	-	-	9	3.7
K3	Is rabies a fatal disease in humans and animals?	184	73.4			60	24.6
K4	Which animals can be affected by rabies?	63	26	131	53.7	50	20.5
K5	Which species of animals are sources of rabies to humans?	112	46	91	37	41	16.8
K6	What are the symptoms of rabies in animals?	148	60.7	79	32.4	17	7
K7	What are the symptoms of rabies in humans?	20	8.1	132	54.1	92	37.7
K8	How rabies can be transmitted to humans?	234	96	1	0.4	9	3.7
K9	Do you know the rabies vaccine for humans that work before a dog bite?	13	5.3	-	-	231	94.7
	Attitude question						
A1	Do you think rabies can be cured once the animal show the symptoms	135	54.1	-	-	109	44.7
A2	Do you think rabies can be cured once a person shows the symptoms	74	30.3	-	-	170	69.7

Code	Questions	Correct answer		Partially correct answer		Incorrectly answered	
A3	Do you agree with the statement that stray dogs can cause a problem for human	192	76.6	-	-	52	21.3
	Practice question						
P1	How can rabies be prevented in dogs?	165	67.6	36	14.8	43	17.6
P2	As a first-aid measure, what would you do after a bite?	29	12.1	148	60.7	67	27.4
P3	What action do you take on rabies suspected animals?	83	34.1	152	62.3	9	3.7

K=knowledge, A= attitude, P= practice

The data showed that 61.8%, 59.8 %, and 64.3% of the respondents had a moderate level of knowledge, attitude scores, and appropriate practices, respectively toward rabies (Table 4).

Table 4. Number of questions, range, scores, and levels of knowledge, attitude, and practice regarding rabies

				Level (%) N=244		
Variable	Number of question	Range score	Total score (mean ±SD)	Low	Moderate	High
Knowledge	10	0-15	10.4±2.5	19.3	61.8	18.9
Attitude	3	0-3	2.5±1.2	17.2	59.8	23
Practice	3	0-8	5.5 ±2	17.2	64.3	18.4

Determinants of Knowledge, Attitude, and Practice concerning rabies

The results showed that there was a significant association between knowledge scores and the age of the respondents ($x^2 = 15.027$; p=0.003); those respondents who were older than 36 years had good knowledge than the other respondents (Table 5). Similarly, it was associated with the sources of information ($x^2 = 15.007$; p=0.009). The source of information was significantly associated with attitude score (17.697. p = 0.003) (Table 6).

Table 5. Relationships among knowledge scores on rabies and some key independent Variables between study respondents

	High	Moderate	Low			
Variables	**No (%)**	**No (%)**	**No (%)**		**Df**	**P-value**
Head of households				4.225	2	0.121
Yes	36 (22.09)	101 (61.96)	26 (15.95)			
No	11 (13.58)	50 (61.73)	20 (24.69)			
Sex				0.071	2	0.965
Male	20 (20.00)	61 (61.00)	19 (19.00)			
Female	27 (18.75)	90 (62.50)	27 (18.75)			
Age (year)				15.027	4	0.003
<18	4 (22.22)	9 (50.00)	5 (27.78)			
19-35	7 (9.59)	44 (60.27)	22 (30.14)			
>36	36 (23.53)	98 (64.05)	19 (12.42)			
Education status				11.598	12	0.479
No formal educations	2 (13.33)	11 (73.33)	2 (13.33)			
Primary education (1-8 grades)	4 (13.79)	19 (65.52)	6 (20.69)			
Secondary education (9-12 grades)	18 (18.37)	65 (66.33)	15 (15.31)			
Technical / University	23 (22.55)	56 (54.90)	23 (22.55)			
Occupation				9.721	6	0.137
Employed/ Professional	17 (22.37)	50 (65.79)	9 (11.84)			
Privet business	11 (18.97)	37 (63.79)	10 (17.24)			
Housewife /Do not have work	14 (18.92)	46 (62.16)	14 (18.92)			
Student	5 (13.51)	18 (48.65)	14 (37.84)			
Dog ownership				2.250	2	0.325

	High	Moderate	Low			
Variables	**No (%)**	**No (%)**	**No (%)**		**Df**	**P-value**
Yes	28 (22.40)	72 (57.60)	25 (20.00)			
No	19 (15.97)	79 (66.39)	21 (17.65)			
Source of information				15.007	6	0.009
Formal	11 (29.73)	24 (64.86)	2 (5.41)			
Informal	31 (16.23)	118 (61.78)	42 (21.99)			
Mixed source	4 (33.33)	8 (66.76)	0.00			

Table 6. Relationship between rabies attitudes score and some significant independent variables among study participants

	High	Moderate	Low			
Variables	**No (%)**	**No (%)**	**No (%)**		**Df**	**P-value**
Head of households				4.162	2	0.125
Yes	27 (16.6)	112 (68.7)	24 (14.7)			
No	18 (27.2)	45 (55.6)	18 (22.2)			
Sex				0.827	2	0.830
Male	18 (18)	63 (63)	19 (19)			
Female	27 (18.8)	94 (65.5)	23 (16)			
Age (years)				9.133	4	0.072
<18	5 (27.8)	9 (50)	4 (22.2)			
19-35	17 (23.3)	39 (53.4)	17 (23.3)			
>36	23 (15)	109 (71.2)	21 (13.7)			
Education status				12.333	6	0.060
No formal educations	1 (6.7)	6 (40)	8 (53.3)			
Primary education (1-8 grades)	6 (20.7)	17 (58.6)	6 (20.7)			

	High	Moderate	Low			
Variables	**No (%)**	**No (%)**	**No (%)**		**Df**	**P-value**
Secondary education (9-12 grades)	18 (18.4)	65 (66.3)	15 (15.3)			
Technical / University	20 (29.6)	67 (65.7)	15 (14.7)			
Occupation				4.175	6	0.678
Employed non-health Professional	17 (22.4)	48 (63.2)	11 (14.5)			
Privet business	12 (20.7)	37 (63.8)	9 (15.5)			
Housewife /Do not have work	8 (12.2)	51 (68.9)	14 (18.9)			
Student	7 (19.4)	21 (53.3)	8 (22.2)			
Dog ownership				4.099	2	0.129
Yes	22 (17.6)	87 (69.6)	16 (12.8)			
No	23 (19.3)	70 (58.8)	26 (21.8)			
Source of information				17.697	6	0.003
Formal	5 (13.5)	30 (81.1)	()			
Informal	35 (18.3)	120 (62.8)	36 (18.8)			
Mixed source	5 (41.7)	6 (50)	1 (8)			

Both levels of education and sources of information were associated with practice scores (12,333. p = 0.046 and 17,697. p = 0.003), respectively (table7)

Table 7. Relationship between rabies preventive practice scores and some significant independent variables among study participants

	Good	Satisfactory	Low			
Variables	**No (%)**	**No (%)**	**No (%)**		**Df**	**P-value**
Head of households				4.162	2	0.125
Yes	27 (16.6)	112 (68.7)	24 (14.7)			
No	18 (22.2)	45 (55.6)	18 (22.2)			

	Good	Satisfactory	Low			
Variables	**No (%)**	**No (%)**	**No (%)**		**Df**	**P-value**
Sex				0.380	2	0.827
Male	18(18)	63(63)	19 (19)			
Female	27(18.8)	94 (65.3)	23 (16)			
Age (year)				9.133	8	0.051
<18	5 (27.8)	9 (50)	4 (22.2)			
19-35	17 (23.3)	39 (53.4)	17 (23.3)			
>36	23 (15)	109 (71.2)	21 (13.7)			
Education status				12.333	6	0.046
No formal educations	1 (20.7)	6 (40)	8 (53.3)			
Primary education (1-8 grades)	6 (20.7)	17 (58.6)	6 (20.7)			
Secondary education (9-12)	18 (18.4)	67 (68.4)	13 (13.3)			
Technical / University	20 (19.6)	67 (62.7)	15 (14.7)			
Occupation				3.936	6	0.691
Employed/ Professional	17 (22.4)	48 (63.2)	11 (11.5)			
Privet business	12 (20.7)	37 (63.8)	9 (15.5)			
Housewife /Do not have work	9 (12.2)	51 (68.9)	14 (19.9)			
Student	5 (13.5)	18 (48.6)	14 (37.8)			
Dog ownership				4.099	2	0.129
Yes	22(17.6)	87(69.6)	16(12.8)			
No	23(19.3)	70(58.3)	26(21.8)			
Source of information				17.697	6	0.003
Formal	5 (13.5)	30 (81.1)	2 (5.4)			
Informal	35 (18.3)	120 (62.8)	36 (18.8)			
Mixed source	5 (41.7)	6 (50)	1 (8.3)			

KAP correlation analysis

Linear correlation analysis was done based on individual scores to determine the relationship between Knowledge, Attitude, and Practice. There was a significant positive correlation between Knowledge and Attitude ($p< 0.01$), between knowledge and practice ($p<0.05$), and between attitude and practice ($p=0.007$). People with good knowledge scores had a good score for attitude and practice than others.

Results of retrospective survey on animal bite cases in humans during 2019-2021

During the period from November 2019 to Jun 2021, a total of 612 bites by rabies suspected animals were recorded in Bishoftu town all of whom received post-exposure vaccines. The average incidence of bite cases was 204 cases per year. A total of 355 (58.01 %; CI: 53.98, 61.95) of the victims were male and 257 (41.99 %; CI: 38.05, 46.02) were female (Table 8.). Individuals aged between 14 and 29 years were most commonly bitten by dogs followed by those aged between 30 and 64 years whereas the lowest bite cases were recorded in older individuals. The results of this study also showed that the leg (68%) was the most commonly bitten body part, followed by the hand (22.7%), face (4.8%), thigh (2.8%), abdomen (0.9%), and breast (0.8%). Out of the 612 rabies/bite cases, 608 (99.35 %; CI: 98.34, 99.82) of them were by dogs, 2 (0.33; CI: 0.04, 1.18) were by cats whereas donkey and horse inflicted 1 case each.

Table 8. Gender and age-wise distribution of rabies cases in humans (2019-2021)

Age in year	Gender		Total (%)
	Number of Males (%)	Number of Females (%)	
≤5	31 (5.1)	18 (3)	49 (8.1)
6-13	88 (14.3)	64 (10.4)	152 (24.8)
14-29	128 (21)	87 (14.2)	215 (35.2)
30-64	97 (15.8)	79 (13)	176 (28.8)
≥65	11 (1.8)	9 (1.4)	20 (3.2)
Total	355 (58.01)	257 (41.99)	612 (100%)

Discussion

Dog demography and vaccination coverage

Rabies remains an important disease both in humans and animals in the study area where the Fermi-type vaccine has been widely used. The knowledge of the dog population and demography is essential for planning an effective control of dog-transmitted rabies. During this study, about 190 dogs were found in the homestead of the selected households. That is, more than half of the households surveyed own dogs. This suggests the potential for the spread of rabies in such very crowded residential areas in which dogs roam freely. Similar dog ownership structures were previously reported in Ethiopia although the proportion of households owning dogs varies. For instance, a slightly higher proportion was reported from the West Shoa zone of the Oromia Region 65.1% (Gebremedhin *et al*., 2020). In contrast, a lower proportion of dog ownership was reported from Karayu pastoralism in Eastern Ethiopia 33 % (Tschopp *et al*., 2016). Elsewhere in Africa, 79.6 % of the population own dogs in Côte d'Ivoire (Kallo *et al*., 2020), 63 % in Kenya (Kitala *et al*., 2001). Initially, all households were visited to collect information on dog ecology and demography based on WHO guidelines and to collect serum for rabies antibody detection. A second visit was made 1 year later, to obtain follow-up data on births, deaths, dog movements and other events since the first visit. Dog ownership was common, with a range of 53--81% (mean=63%, 14 % in Tanzania (Gsell *et al*., 2012) such information is still rare for African domestic dog populations, particularly so in urban areas. This study describes the demographic structure and population dynamics of a domestic dog population in an urban sub-Saharan African setting. In July to November 2005, we conducted a full household-level census and a cross-sectional dog demography survey in four urban wards of Iringa Municipality, Tanzania. The achievable vaccination coverage was assessed by a two-stage vaccination campaign, and the proportion of feral dogs was estimated by a mark-recapture transect study. The estimated size of the domestic dog population in Iringa was six times larger than official town records assumed, however, the proportion of feral dogs was estimated to account for less than 1% of the whole population. An average of 13% of all households owned dogs which equalled a dog:human ratio of 1:14, or 0.31 dogs per household or 334 dogs km-2. Dog female:male ratio was 1:1.4. The average age of the population was 2.2 years, 52% of all individuals were less than one year old. But mortality within the first year was high (72%, 17% in South Africa (Conan *et al*., 2015) and 35 % in Ghana (Tasiame *et al*., 2019) this information is unavailable in Ghana. This study describes dog population structure in Kumasi, Ghana. A

household cross sectional survey was conducted from January through April 2016 in Ayeduase and Kwame Nkrumah University of Science and Technology (KNUST). The reported variation between studies and countries may be due to variations in sociocultural attitudes to keeping the dog, population density, agroecology, and livelihood.

The survey showed that 71.0% of the dog population was vaccinated with the anti-rabies vaccine, which is according to the recommendations of the World Health Organization for the control of canine rabies. The vaccine coverage reported in this study is higher than the vaccine coverage reported in other African countries such as Congo (24.0%), Malawi (59.0%), Nigeria (47.9 %), Chad (0.5 %), South Africa (56.0%), Madagascar (22.0%), Kenya (29.0%) and Zambia (20.0%) (De Balogh *et al*., 1993; Jonasson, 2014; Kitala *et al*., 2001; Mbilo *et al*., 2019, 2017; Ratsitorahina *et al*., 2009)190 households were interviewed. In the urban study area (Mutendere, a low income suburb of Lusaka. The higher vaccine coverage in the current study area may be due to the rabies vaccination campaign by the College of Veterinary Medicine and Agriculture, the availability of rabies vaccine at the National Veterinary Institute in Bishoftu, and the provision of door-to-door vaccination by private veterinary practitioners. Moreover, the price of anti-rabies vaccines is also within the reach of average people (ETB 100 – 150 which is equivalent to USD 2 – 3) per dose of the vaccine, which has contri*but*ed to adequate vaccine coverage. In most African countries where dog vaccination is not free, dog owners are generally unwilling to pay for dog rabies vaccination because they see no direct economic and/or health benefits. This is supported by the fact that the vast majority of humans who have been bitten were not bitten by their dogs (Jibat *et al*., 2015) where the majority of the human cases results from dog bites. Mass dog vaccination is considered to be the most effective method to prevent rabies in humans. Our objective was to systematically review research articles on dog rabies parenteral vaccination coverage in Africa in relation to dog accessibility and vaccination cost recovery arrangement (i.e.free of charge or owner charged. Even though in the present study the dogs' vaccine coverage in Bishoftu town is 71% and within the range of disrupting urban rabies transmission in African cities (Zinsstag *et al*., 2017)dog-transmitted human rabies persists and has reemerged in Africa. Two consecutive dog vaccination campaigns took place in Chad in 2012 and 2013 (coverage of 71% in both years the present retrospective survey of rabies-suspected bites in four health centers in Bishoftu, 99.4% of them were bitten by dogs. This may be due to the current vaccine coverage did not include

ownerless dogs and free-roaming dogs, which may sustain rabies transmission in the area.

In the present study, the average number of dogs per household was 1.54. A comparable result was reported from the Philippines, 1.8 dogs/household, while variable results were reported from Ethiopia 1.1 dogs/household (Gebremedhin *et al.,* 2020), Philippines 1.21 dogs/household (Dizon *et al*., 2022) and Nigeria 1.1 dogs/household (Kwaghe *et al.*, 2019). The observed differences between studies and countries may be due to socio-cultural ties between dogs and humans in different societies. It is these close ties, if not handled carefully and appropriately, that result in the incidence of rabies. For example in developed countries where there are strict dog ownership regulations and awareness about the importance of veterinary services and certification, the incidence of dog-borne rabies is low. In contrast, although the mean number of dogs per household is similar, the incidence of rabies is higher in developing countries due to a lack of regulations, sufficient veterinary services, and certifications. In addition to the average number of dogs per household, the dog: human ratio (1:9) observed in this study was comparable with reports from Ethiopia 1:7 (Gebremedhin *et al.,* 2020), Tunisia 1:11 (Kalthoum *et al.,* 2021) and Nigeria 1.1: 6 (Kwaghe *et al.,* 2019). The observed differences may be due to differences in human and dog population density in the given communities and countries. Ninety-four percent of the dog population in the study area were adults and 78 % were male, which is in agreement with the observations reported in sub-Saharan Africa(Conan *et al.*, 2015; Mauti *et al.,* 2017; Mbilo *et al.,* 2019; Ratsitorahina *et al.*, 2009; Sambo *et al.*, 2018). In our study, the majority of the community prefers to own a male dog over a female dog, which may be due to the perception of considering a male dog as an effective guard dog or maybe to control breeding, since female dogs give birth to more puppies in a short period. We also observed the predominance of male dogs in our study. Some studies explain the predominance of male dogs in Tanzania and South Africa (Conan *et al*., 2015; Knobel *et al*., 2008).

Community Knowledge Attitude and Practice (KAPs) on rabies

In this study, the overall KAP revealed that the majority of the respondents had a moderate level of knowledge, attitude, and appropriate practices toward rabies. A moderate level of knowledge and attitude is an attribute that can be considered for the control of rabies. Since the community had a moderate level of knowledge it would be easier to participate in the control activities. This

study also identified factors responsible for positive practices and knowledge related to rabies. For example, 52.0% of the respondents in this study had attained primary and secondary schools, which helped them to understand the disease. Similar results have been reported elsewhere in the world such as in Rwanda, Kenya, Tanzanian, and India (Ntampaka *et al*., 2019; Sambo *et al*., 2014; Sivagurunathan *et al*., 2021). The survey revealed that the communities are well aware of rabies. This finding was consistent with results from northern Ethiopia (Gebeyehu and Teshome, 2016) and Tanzania (Sambo *et al*., 2014). In contrast, this finding is lower than the study conducted in Kombolcha, Southern Wollo (Gebremeskel *et al*., 2019), Philippines (Davlin *et al*., 2014), Thailand (Kongkaew *et al*., 2004), Cambodia (Lunney *et al*., 2012). However, it is higher than the study conducted in the Dedo district of the Jimma zone (Abdela *et al*., 2017). These differences may be due to differences in the source of information, the composition of the selected population, and the way by which the information was collected.

The majority of respondents identified bite as a means of disease transmission and also many participants (44%) believed that any direct or indirect contact with saliva could transmit rabies. Fifty-point-four percent (50.4 %) of the respondents, mentioned water and feed shortages and being exposed to a long drought be associated with the disease. This idea is most likely explained by the belief that stress such as hunger and thirst may induce the development of clinical rabies in asymptomatic carrier dogs (Wilde *et al*., 2005) nevertheless, remains a public health threat in many parts of the world. Lack of motivation by governments, cultural issues and inadequate funding remain barriers. This is amazing since the number of human rabies deaths worldwide is greater than that from polio, meningococcal meningitis, Japanese encephalitis, yellow fever, SARS, bird flue and other scourges that attract more attention. Safe and effective vaccines are now widely available. Reduced dose effective and less expensive post-exposure vaccination regimens have helped eliminate nerve tissue vaccines in Thailand, Philippines and Sri Lanka. India and Pakistan, the major users of dangerous nerve tissue derived Semple type vaccine, are now considering following suite. Immediate wound care and prompt use of a potent vaccine will save a majority of infected persons. Rabies immunoglobulin, injected into and around bite wounds, provides added safety for the severely exposed. The high cost of rabies immunoglobulin and tissue culture vaccines are remaining barriers, but new manufacturers and the use of intradermal vaccination schedules can reduce costs. Ultimately, it is the need to control

rabies in dogs that must occupy most of our attention. The tools are available, but attitudes must change before they can be applied. There have been many new developments since publication of the last WHO rabies expert committee report in 1992 (new version in print. However, the idea of clinical rabies development is a controversial issue (Jemberu *et al*., 2013) we estimated the incidence of rabies in humans and domestic animals, and assessed the people's awareness about the disease in North Gondar zone, Ethiopia. The incidence of rabies in humans and domestic animals was prospectively followed up for one year period based on clinical observation. A questionnaire was also administered to 120 randomly selected dog owners and 5 traditional healers to assess the knowledge and practices about the disease. We found an annual estimated rabies incidence of 2.33 cases per 100,000 in humans, 412.83 cases per 100,000 in dogs, 19.89 cases per 100,000 in cattle, 67.68 cases per 100,000 in equines, and 14.45 cases per 100,000 in goats. Dog bite was the source of infection for all fatal rabies cases. Ninety eight percent of the questionnaire respondents were familiar with rabies and mentioned dog bite as a means of transmission. But discordant with current scientific knowledge, 84% and 32% of the respondents respectively mentioned any type of contact (irrespective of skin condition.

The public needs to be aware of the signs of rabies to identify suspected animals and take appropriate measures, such as informing the veterinary personnel. Most of the study participants correctly described the symptoms of rabies in animals. An accurate description of the clinical signs of rabies reported by the study population shows that the disease is among the predominant problems in the study area. The majority of respondents mentioned paralysis as a possible sign of rabies, which is typical of the "dumb" or paralytic form of rabies. This may be cases of paralytic rabies being detected. However, only 8.1% of respondents correctly mentioned symptoms of rabies in humans. Human rabies has a non-specific clinical manifestation, which can lead to a misdiagnosis of the disease (Mbilo *et al*., 2017). In contrast, although rabies is invariably fatal once clinical signs develop in both animals and humans, 70% of the respondents to this survey were unaware that rabies is fatal.

Many of the respondents exhibited a lack of detailed knowledge about the prevention of rabies and the risks associated with its occurrence. According to WHO guidelines, wounds must be washed with water and soap for 15 minutes and disinfected immediately before being admitted to the hospital. It helps

to remove animal saliva from bite sites and reduce viral load. We found that the majority of respondents had no awareness of the importance of immediately washing wounds with water and soap. Similarly, only 12.0% of the respondents were aware of first aid measures that should be taken after an animal bite. Studies conducted in Tanzania, Uganda, Chad, South Africa, India, and Pakistan where rabies is endemic, also showed that a lower proportion of people practice wound washing with soap and water (Hergert and Nel, 2013; Ichhpujani *et al*., 2008; Mbilo *et al*., 2017; Sambo *et al*., 2014) a disease of antiquity continues to be a major public health problem in India. Multiple factors contribute to high mortality and morbidity due to animal bites. An effective strategy for control of rabies takes into account the epidemiology of animal bites, rabies and factors influencing post exposure treatment. The study was carried out as a part of Agreement for Performance of Work (APW). In this study, two-thirds (67.6 %) of the participants knew that vaccinating dog is an effective measure to prevent rabies in humans and animals. Restriction of movement of dogs, killing stray dogs and preventing dogs from starving, hunger, and drought are also mentioned as rabies prevention measures. The level of practice toward rabies prevention measures among the study participants was satisfactory.

Schoolmates, friends, family members, and neighbors played a more significant role in disseminating information about rabies than mass media, health centers, and veterinary services. In this study, the majority of the respondents showed that they gained knowledge about rabies from the informal sources mentioned here. In consent to our observation, a study carried out in Côte d'Ivoire (Tiembré *et al*., 2014) revealed that 82.9 % of the study participants knew about rabies and 88.6 % of them revealed that the sources of information were schools. This demonstrates the important roles that schools play in creating awareness about rabies. The medical and veterinary services did not play an important role in disseminating information. However, the community in the study area practices inadvertent killing of rabies suspected dogs without the knowledge of veterinary authorities, obscuring the diagnosis and surveillance of the disease. Similar findings were also reported in Ethiopia (Gebeyehu and Teshome, 2016), Kenya (Muriuki, 2016), and Congo (Mbilo *et al*., 2019).

Animal bites cases in humans

The incidence of bites by rabies-suspected animals reported in this study is considerable. In the absence of appropriate diagnosis and surveillance programs the bites, most of the time result in rabies. Since society mostly kills suspected dogs, it is inevitable to provide post-exposure vaccines to the victims. This contributes to the huge health and economic burden associated with rabies. This finding is similar to the report of Beyene *et al.* (2018).In contrast, it is lower than what was reported in Addis Ababa (Aklilu *et al*., 2021). Males were most frequently bitten than females. Similar observations were reported in other parts of Ethiopia (Aklilu *et al*., 2021), Nigeria (Ikye-Tor *et al*., 2020), Tanzania (De Nardo *et al*., 2018)many endemic countries are not able to assess their rabies burden and implement appropriate solutions. This study reports the incidence of animal bites considered at risk of rabies transmission, along with rates and determinants of the adherence to post-exposure prophylaxis (PEP, and the USA (Tuckel and Milczarski, 2020). This could be attributed to the outdoor activities in which male individuals are engaged, whereas females are more likely to remain indoors for socio-cultural and religious reasons. Most bites occurred in individuals who were older than 14 years as this is the productive age when most individuals especially males are engaged in various activities and are exposed to stray and roaming dogs and other carnivores. We also observed that most of the dog bites were on the legs and hands. It is a fact that these body regions are most likely used by people to protect themselves from dog attacks (Guy *et al*., 2001)their homes, and their victims were gathered in a detailed telephone survey of general veterinary clientele in the Canadian provinces of New Brunswick, Nova Scotia, and Prince Edward Island. All of the dogs had bitten either someone living in the same household, or someone who was a frequent visitor and was well known to the dog. There were 117 male and 110 female dogs included in this case series. Significantly more female dogs were neutered (p=0.03.)

In addition, post-exposure prophylaxis was given to victims bitten, without confirmation of the rabies status of the animal. Three types of rabies vaccines are available; outdated nerve tissue vaccines, cell culture vaccines, and embryotic egg vaccines. The cell culture and embryonated egg vaccines are the ones recommended for use by WHO. They are considerably safer and well-tolerated. In developed countries, nerve tissue vaccines have been replaced by cell culture and embryonated egg vaccines. In contrast, nerve tissue vaccines can

cause severe adverse reactions that can last for 4–7 months in 0.03–0.08% of cases, including the risk of rabies due to incomplete virus inactivation, are less immunogenic and also contribute to vaccine failure. The World Health Organization has banned this vaccine since 1980. However, in developing countries, including Ethiopia, the mammalian neural tissue vaccine is still widely used and needs attention. While cell culture is effective and immunogenic, however, it is also expensive (Beyene *et al*., 2018) there is a need for quantitative data on the public health burden and costs of diseases to support intervention prioritization. This study aimed at estimating the health burden and post-exposure treatment (PET).

Limitations of the study

The demography of stray dogs was not included in the present study and the relatively small sample size used in our study may affect the quality of the work.

Conclusions

Although the overall level of knowledge, attitude, and practice towards rabies of the majority of the respondents were moderate and, was gained from informal sources, there are still KAP gaps regarding preventive measures, the deadly nature of the disease, and lack of awareness of the first aid measures to be taken after a case of rabies suspected animal bite. Furthermore, this study showed the presence of high dog populations with the potential to cause health and economic burdens in Bishoftu town. A high incidence of rabies-suspected animal (dog) bites was recorded in the study town. Production and use of banned nerve tissue vaccine in Ethiopia need government attention to replace the recommended vaccine.

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Foot and Mouth Disease in Adama and Boset districts, East Shewa zone, Ethiopia: Seroprevalence and Virus serotyping

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Abstract

Foot and mouth disease (FMD) is consistently ranked as the most economically important viral disease, and ranks among the top five important livestock diseases in Ethiopia. The status of FMD is barrier for the international trade of livestock and livestock products; thus, continuous epidemiological studies are necessary to develop efficient control strategies. This study determined the seroprevalence of FMD, associated risk factors for its occurrence, and FMD serotypes in the Adama and Boset districts of the East Shewa zone of Oromia Regional State, Ethiopia. A total of 305 blood samples were randomly collected from cattle and subjected to a 3ABC enzyme-linked immune-sorbent assay (ELISA) to detect antibodies against non-structural proteins of FMD virus (FMDV) and Solid-Phase Competitive ELISA for FMDV serotype identification. Evaluation of the associated risk factors was conducted using a multivariable logistic regression model. In this study, an overall seroprevalence of 28.2% (95% CI: 25.7% - 36.2%) was recorded. The risk of being FMD positive was significantly higher in exotic crossbreds [38.60%; OR= 2.23 (95% CI: 1.34 – 3.71); p<0.001] as compared to local breeds. The seroprevalence of FMD was significantly higher in large herd sizes [54.41%; OR=4.2 (95%CI: 3.05 - 15.87), P < 0.001] compared to medium and small herd sizes. Three FMD serotypes namely serotype O, A, and SAT-2 were identified. Besides, mixed serotypes O and A, serotypes O and SAT-2, serotypes A and SAT-2, and serotypes A, O, and SAT-2 were recorded. By considering the detection of multiple serotypes of the FMDV and the lack of cross-protection among the serotypes, the use of polyvalent FMD vaccine against the serotypes of the virus circulating under field conditions is highly recommended.

Keywords: 3ABC-ELISA; Ethiopia; Foot and Mouth Disease; Risk factors; Seroprevalence; Serotypes.

Introduction

Ethiopia owns the largest livestock population in Africa with an estimated cattle population of 70 million (CSA, 2021). Livestock production plays an important role in the economies of farmers and pastoralists, and the country at large. The Ethiopian livestock contribution to the national economy is estimated at 19% of the total gross domestic product (GDP), 40% of the agricultural GDP, and about 20% of the country's export earnings (Shapiro *et al.,* 2017). Moreover, the livestock sector plays a vital role in the livelihood of millions of households in Ethiopia as a source of meat, milk, drought power, and income. The contribution of livestock to the country's economy primarily in terms of foreign currency gains via the export of live animals, meat, skin, and hides. Although there is substantial international demand for live animals and animal products, transboundary livestock diseases are a significant factor that hampers the trade of animals and animal products to the international market (Shapiro *et al.,* 2015; Jemberu, 2016). Among the transboundary animal diseases, foot and mouth disease (FMD) is the most important that has a significant socio-economic impact on Ethiopia.

Foot and mouth disease is a highly contagious viral disease of cloven-hoofed animals. The disease is characterized by fever and vesicular eruptions on the tongue, feet, snout, and teats, and sudden death in young animals. A non-enveloped RNA virus within the family *Picornaviridae* and genus *Aphthovirus* causes foot and mouth disease. Foot-and-mouth disease virus (FMDV) exists in seven distinct serotypes; A, O, C, Southern African Territories (SAT)-1, SAT-2, SAT-3, and Asia-1, and numerous and constantly evolving variants showing a spectrum of antigenic diversity. There is no cross-protection and immunity between the different serotypes (Davies, 2002). Therefore, disease control via vaccination must consider the evolving variants and the serotypes that are circulating in the field.

In Ethiopia, FMD is consistently ranked as the most economically important viral disease, and among the top five important livestock diseases (Shiferaw et *al.,* 2010; Jibat et *al.*, 2013). Among the seven serotypes of FMD viruses, currently, four of them (serotype O, A, SAT-1, and SAT-2) have been reported

in different geographical settings of Ethiopia (Ayelet *et al.,* 2009; Negusssie *et al.,* 2011; Mohammed *et al.,* 2022). The national FMD outbreak report revealed that the disease is endemic in all regional states affecting more than a quarter of the country every year (Ayelet *et al.,* 2009; Jemberu, 2016). The status of FMD is an important determinant for international trade of livestock and livestock products (ILRI and MoA, 2015), and the existence of FMD is an effective barrier from the international markets (Jibat *et al.*, 2013; Shapiro *et al.*, 2015).

Outbreaks of FMD frequently occur in different parts of Ethiopia. This is mainly due to the lack of effective vaccines and vaccination strategies, the absence of livestock movement control, and the absence of systematic disease surveillance and reliable epidemiological data. Considering the various serotypes of FMDV and the absence of cross-protection among the different serotypes, there are strong recommendations to use polyvalent vaccines incorporating the dominant circulating serotypes in the country. To achieve this, continuous epidemiological studies including the identification of FMDV serotypes circulating in major livestock populations of the country are important. Therefore, this study was intended with the objectives of estimating the seroprevalence, assessing risk factors, and identifying circulating serotypes of FMDV in Adama and Boset districts of East Shewa, Oromia Regional State, Ethiopia.

Materials and methods

Study area

The study was conducted in two districts; namely, Adama and Boset districts of East Shewa Zone, Oromia Regional state, Ethiopia, which is located from 7^0 33′0″ to 9^0 08′56″N and 38^0 24′10″E to 40^0 05′ 34″E (Figure 1). About 93% of the total area of the zone is located along the Rift Valley. East Shewa Zone has a total of 1,147,173 cattle (CSA, 2021).

Adama is one of the districts in the East Shewa Zone and it is located at 8.54^0N 39.27^OE at an altitude of 1,712 masl. It receives an average rainfall of approximately 600 – 1,150 mm with annual average minimum and maximum temperatures of 18^0C and 32^0C (ESZLFD, 2017). Boset district is characterized by a semi-arid climate. Its' mean annual rainfall is ranged from 600 to 900 mm, while the mean annual temperature is 26 – 34°C. Almost 90% of the district is found in less than 1,500 masl (BDANRO, 2017). These districts were selected based on their large livestock population, accessibility for transportation, presence of huge fattening farms, and main routes for live animal exports.

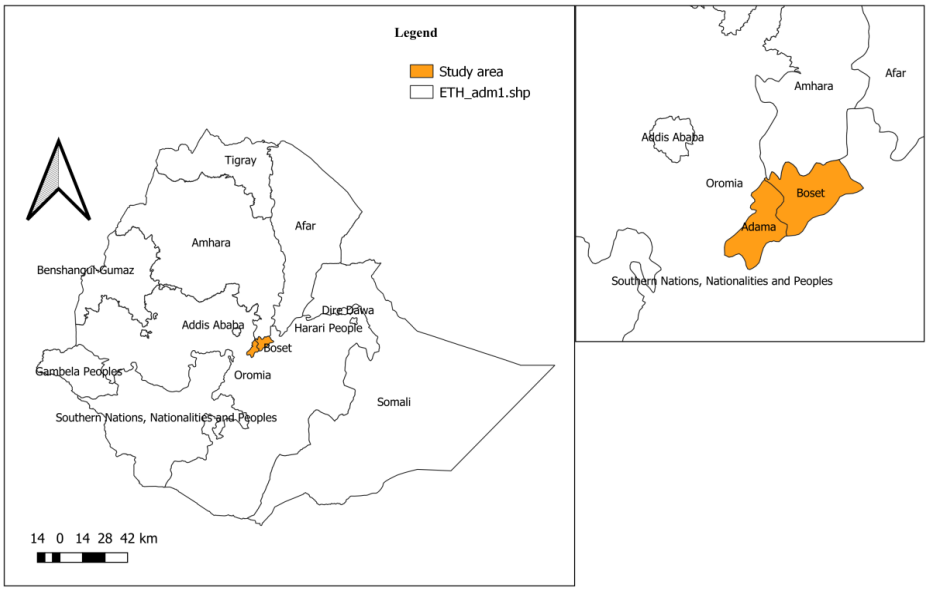


Figure 1: Map of Ethiopia showing the study areas. This map was developed from the Ethiopian

Administrative boundaries shapefile 2021 using QGIS version 3.1.1.2.

Study population, study design, and sample size

Study animals were local zebu and exotic crossbred cattle managed under extensive and semi-intensive systems. Cattle were kept together with either small ruminants (mixed herd composition) or only cattle without mixing with other livestock species. To avoid maternal immunity interference, cattle that were in age of < 6 months were not sampled. Animals were grouped into two age categories; 6 months to 2 years (young) and above 2 years (adult). During sampling, information such as age, sex, breed, herd size, composition, and previous vaccination status for the last six months was collected to determine whether FMD seropositivity was associated with potential risk factors. Herd sizes were categorized arbitrarily into small herds (< 15 cattle), medium herds (15 – 30 cattle), and large herds (> 30 cattle), in which the largest herd size contained 43 heads of cattle.

From January 2021 through June 2021, a cross-sectional study design was used. A multistage sampling method was applied to select 5 *Kebeles* from Adama district and 3 *Kebeles* from Boset district. A simple random sampling technique was used to select 305 cattle from the population.

The sample size calculation was determined according to Thrusfield (2007). The minimum sample size required for the study was calculated based on 26.8% individual animal-level prevalence of FMD (Sulayeman *et al.,* 2018) foot-and-mouth disease (FMD, with a 95% confidence interval, and 5% absolute precision. Accordingly, 305 blood samples were collected.

Blood sample collection

Samples were carefully collected following all ethical procedures for sampling, enrollment and sample collection. Blood samples were collected from the jugular vein of cattle using plain vacutainer tubes. About 10 ml of blood was drawn from each randomly selected animal and kept overnight in an upright position at room temperature. The serum samples were harvested, labeled, and transported using an icebox containing an icepack to Animal Health Institute (AHI), serology laboratory in Sebeta, Ethiopia. The samples were kept at -20°C until the serological test conducted.

Laboratory Diagnosis

The sera samples were tested for the presence of antibody against FMDV using competitive 3ABC ELISA (ID Screen®, ID Vet, Grabels, France) at the Animal Health Institute, Sebeta, Ethiopia. This test detects antibodies produced against non-structural proteins of the virus and can thus distinguish between FMDV infected animals and vaccinated with purified FMD vaccine. The tests were performed according to the manufacturer's instructions.

FMDV serotyping

Serum samples exhibiting strong antibodies against FMDV non-structural protein using 3ABC ELISA was selected for FMD serotyping. A total of 59 strong positive serum samples were selected and subjected to antigen-capturing sandwich ELISA (IZSLER, Brescia Italy). The assay was a monoclonal antibody based on an indirect sandwich ELISA that can detect and typing of

FMDV of serotypes O, A, C, Asia-1, SAT-1, and SAT-2. The test was performed according to the manufacturer's manual.

Statistical analysis

Data generated from the serological test was recorded in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 16 (STATA, 2019). A logistic regression analysis was used to measure the association of the risk factors with the seropositivity of FMD. The association of risk factors was screened out by using univariate logistic regression analysis and variables with p-value < 0.25 (maximum likelihood ratio test) were offered to the final multivariable model. The risk factors having a p-value < 0.05 were considered statistically significant. The multicollinearity and goodness fit of the models was checked using the variance inflation factor (VIF) and Hosmer and Lemeshow tests.

Results

Among the 305 sera tested using 3ABC-ELISA, a seroprevalence of 28.2% (95%CI: 25.7% - 36.2%) was detected.

Potential risk factors screened with univariable logistic regression analysis

With the univariate model, risk factors of age, sex, herd composition, and study districts, although observed with higher seroprevalence records in adult cattle, males, unmixed cattle herd, and Boset districts, respectively; and were found to be statistically not significant ($p > 0.05$). Significantly higher seroprevalence was recorded in exotic crossbreds [38.60%; OR = 2.23 (95% CI: 1.34 - 3.71), $p = 0.002$] and large herd size [54.41%; OR= 4.35 (95%CI: 2.34 - 8.10), $p<0.001$] with the model as compared to local breed and small-medium sized herds, respectively (Table 1).

Table 1. Univariable logistic regression analysis results of potential risk factors for FMD seropositivity

Variable	No. tested	Positive (%)	OR(95%Conf. Interval)	p-value
Study Districts				
Boset	155	67 (43.27)	Ref	
Adama	150	19 (12.67)	0.19 (0.11 - 0.34)	0.071
Breed				
Local	191	42 (21.99)	Ref	
Cross	114	44 (38.60)	2.23 (1.34 - 3.71)	0.002
Sex				
Male	60	23 (38.33)	Ref	
Female	245	63 (25.71)	0.56 (0.31 - 1.01)	0.053
Age				
Young	100	25 (25.00)	Ref	
Adult	205	61 (29.76)	1.27 (0.73 - 2.19)	0.387
Herd Size				
Small	144	31 (21.53)	Ref	
Medium	93	18 (19.35)	0.87 (0.46 - 1.68)	0.687
Large	68	37 (54.41)	4.35 (2.34 - 8.10)	<0.001
Herd composition				
Cattle, sheep, and goats	175	47 (26.86)	Ref	
Cattle only	130	39 (30.00)	1.17 (0.71 - 1.93)	0.547

Multivariable logistic regression rnalysis of risk factors with FMD seropositivity

From six hypothesized risk factors, only two variables (i.e. breed and herd size) showed a significant association and were included in the multivariate logistic regression model. The multivariate logistic regression analysis revealed the risk of being FMD seropositive was significantly higher in exotic crossbreds with a prevalence of [38.60%; OR= 2.23 (95% CI: 1.34 – 3.71; p<0.001] as compared to local breeds. The seroprevalence of FMD was significantly higher in large herd sizes [54.41%; OR=4.2 (95%CI: 3.05 - 15.9, p < 0.001] compared to medium and small herd sizes (Table 2).

Table 2. Multivariate logistic regression analysis of risk factors with FMD seropositivity

Variable	No. tested	Positive (%)	OR (95% Conf. Interval)	p-value
Breed				
Local	191	42 (21.99)	Ref	
Cross	114	44 (38.60)	2.23 (1.34 - 3.71)	<0.001
Herd Size				
Small	144	31 (21.53)	Ref	
Medium	93	18 (19.35)	0.91 (0.42 - 1.94)	0.802
Large	68	37 (54.41)	4.2 (3.05 - 15.9)	<0.001

Identification of FMD Serotypes

Sera samples exhibiting strong antibodies against FMDV non-structural protein using 3ABC ELISA was selected for FMD serotyping. From the total of 59 strong positive sera samples selected and tested using antigen-capturing sandwich ELISA, three FMD serotypes (Serotype O, A, and SAT-2 were identified. Serotype O was recorded with the highest proportion (69.36%) followed by serotype A (45.63%), and SAT -2 (28.73%). In this study mixed serotypes such as serotypes O and A (3.38%), serotypes O and SAT-2 (1.69%), serotypes A and SAT-2 (27.12%), and A, O, and SAT -2 (18.64%) were also recorded (Table 3).

Table 3. FMD serotypes identified at Adama and Boset districts

Types of FMD serotypes identified (%)				Mixed serotype identified (%)			
No. sera tested	O	A	SAT-2	O & A	O & SAT-2	A & SAT-2	A, O & SAT -2
59	41 (69.36)	27 (45.63)	17 (28.73)	2 (3.38)	1 (1.69%)	16 (27.12%)	11 (18.64)

Discussion

In Ethiopia, FMD is consistently ranked as the most economically important viral disease, and among the top five important livestock diseases (Shiferaw *et al.,* 2010; Jibat *et al.,* 2013). The status of FMD is an important determinant of international trade of livestock and livestock products (ILRI and MoA, 2015), and the existence of FMD is an effective barrier from the international

markets (Jibat *et al.,* 2013; Shapiro *et al.,* 2015). In this study, an overall FMD seroprevalence of 28.2% was recorded in cattle in the study districts. This is in agreement with those of other previous reports in a different part of Ethiopia by Sulayeman *et al*. (2018) foot-and-mouth disease (FMD, Shurbe *et al*. (2022), and Dubie and Negash (2021) who reported a seroprevalence of 26.8% in Addis Ababa, Arsi and East Shewa, 26.8% in Gamo zone, and 19.8% in Afar region, respectively. However, the seroprevalence of this study was higher than Negusssie *et al.* (2011) (5.6%) and slightly higher than the study of Bayissa *et al.* (2011) who reported seroprevalence of 23%. The observed difference in prevalence in the current study could arise from the difference in sampling method, study design, agroecology, unrestricted herd mobility, and animal management systems.

In the current study, significantly higher seroprevalence was recorded in exotic crossbreds (38.6%) compared to local breeds. The risk of being FMD seropositive was significantly higher in exotic crossbreds [OR=5.51 (95% CI: 2.37 - 12.82), p<0.001] compared to the local breed. This might suggested that the local breed cattle are relatively resistant to the FMDV compared to exotic breeds. This finding is in agreement with Sulayeman *et al.*(2018), Negussie *et al.* (2011), and Ahmed *et al.* (2020) who reported a significant variation in seroprevalence among the different breeds of cattle. Similarly, Hunter (1998) reported that the direct impact of FMD on livestock in sub-Saharan Africa depends on the breeds of animals used. FMD is more severe in European breeds of cattle than the other breeds (Quinn *et al.*, 2005).

In this study, herd size was categorized into three groups namely < 15 cattle (small), 15 - 30 cattle (medium), and > 30 cattle (large). The seroprevalence of FMD was significantly higher in large herd sizes [54.41%; OR=4.2 (95%CI: 3.05 - 15.87), p<0.001] compared to medium and small herd sizes. Similar trends were reported by Sulayeman et al.(2018) and Dubie and Negash, (2021) where higher seroprevalence of FMD was observed in larger herd sizes. This suggests that higher herd size has a greater chance of animal contact and hence virus transmission between cattle herds.

Previous reports on FMDV serotypes in Ethiopia showed that FMD outbreaks were associated with serotypes O, A, C, SAT-2, and SAT-1 as diagnosed clinically, serologically, virologically, and molecular techniques during the period 1981-2018 (Jemberu *et al.*, 2016). In this study, three FMD serotypes namely serotype O, A, and SAT-2 were identified. The current result was in agree-

ment with the previous studies conducted by Ayelet *et al.* (2009), Jemberu *et al.* (2016), Gizaw *et al.* (2020), and Mohammed *et al*. (2022) stated that FMD serotype O, A, and SAT-2 were recorded from outbreaks in the central parts of the country. Serotype O was recorded with the highest proportion followed by serotype A, and SAT-2. This was in agreement with the studies conducted by Ayelet *et al.* (2009), Negussie *et al.* (2011), and Gizaw *et al.* (2020). However, Jemberu *et al.* (2016) recorded serotype SAT-2 in a higher proportion compared to serotype A. Mixed serotypes with serotypes O and A (3.38%) serotype O and SAT-2 (1.69%); serotypes A, O, and SAT-2 were identified. This suggests the possibility of simultaneous infection with different serotypes of the FMD virus which is in agreement with Arzt *et al*. (2021) reported the occurrence of simultaneous infection of individual hosts by multiple FMD virus strains. In FMD-endemic countries, vaccination is a highly effective method for preventing FMD. In Ethiopia, the National Veterinary Institute (NVI) is producing inactivated FMD vaccine containing a cocktail of serotypes O, A, and SAT-2. Due to the growing trend of private investments in the livestock sector, vaccination of cattle against FMD has been practiced (Ayelet *et al.,* 2013; Jemberu *et al.,* 2020) in some market-oriented dairy farms and feedlot operations in urban and peri-urban areas. However, the FMD vaccines used in many FMD-endemic developing countries are of questionable quality (Sutmoller *et al.,* 2003). Quality of vaccine is a critical requirement for the successful control of animal diseases through immunization. Various factors may limit their effectiveness such as low potency, poor antigenic match between the field and vaccine strains, as well as short duration of protection (Ferrari *et al.,* 2016).

Conclusions

Foot and Mouth Disease (FMD) is a highly prevalent disease in the study areas. FMD serotypes O, A, and SAT-2 were identified in the study areas. Mixed serotypes O and A, serotypes O and SAT-2, serotypes A and SAT-2, and Serotype A, O, and SAT -2 were recorded. It was concluded that by considering the detection of multiple serotypes of the FMDV and the lack of cross-protection among the FMD virus serotypes, the use of polyvalent FMD vaccine against the serotypes of the virus circulating under field conditions is highly recommended.

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Ethical considerations

Ethical approval for this study was granted from the animal research ethical review committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University (Reference number: VM/ERC/07/04/13/2021). All methods were performed in accordance with relevant guidelines and regulations. Before conducting the research, cattle owners were informed of the objectives and the benefits of the study and they gave consent for their animal's inclusion in the study. Cattle owners gave verbal consent for their animal's inclusion in the study because they were unable to write and read. These consents were taken in the presence of a third independent party.

Authors' contributions

AA and AM were involved in data collection and laboratory work. HN and YJ were involved in the design, statistical analysis, and interpretation of data; drafted the paper and involved in the critical revision of the paper. All the authors have read and approved the paper for submission.

Competing of interest

The authors declare that they have no competing/conflict of interests.

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Feeding frequency and its associated effects on the production and survival rate of *Glossina fuscipes fuscipes*

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Abstract

Tsetse flies are large blood-sucking flies of the genus *Glossina*, which are exclusively found in Africa. They are important vectors of *Trypanosomes*, which cause sleeping sickness in humans and African Animal Trypanosomosis (AAT) in livestock. Due to the lack of effective vaccines to control this disease, vector control has been an ideal strategy. There are many vector control methods developed so far. Sterile Insect Technique (SIT) is one of such method which depends on the mass production of male flies in the laboratory, sterilization, and release of these flies in the target area. The study was conducted to evaluate the effect of different feeding frequencies on the production and survival rate of *Glossina fuscipes fuscipes* to identify the best feeding frequency that could optimize the production and minimize the cost of production in a mass-rearing insectary facility at Kaliti Tsetse Fly Mass Rearing and Irradiation Centre, Addis Ababa, Ethiopia. Three experimental groups were established based on feeding frequency regimes. The first group was fed three times per week, the second four times per week, and the third group five times per week. Parameters that are routinely used for assessing colony performance such as; survival rate, fecundity, pupae per initial females (PPIF), and pupal quality were evaluated for each treatment group and statistical comparisons were made between the groups. The results revealed that feeding frequency had a crucial effect on fly production and survival. The lowest results for all parameters were obtained in flies fed three times per week and relatively the best results were obtained in the four-times-per-week feeding regime. Therefore, we recommend a further study on aspects that are not addressed in this study, such as emergency rate and progeny quality, to apply the four times per week feeding regime as it will have a positive economic impact that could enable

more resources to be re-injected into the insectary compared to five or more feeding regimes which are currently in use in the study site.

Keywords: Feeding regime; *G. f. fuscipes;* Mass-Rearing Productivity; Survival Rate.

Introduction

Tsetse flies are blood-sucking flies of the genus *Glossina* that belong to the family Glossinidae (Radostitis *et al*., 2007). They are found exclusively on the African continent, between 5°N to 20°S latitudes (Warnes *et al*, 1999). In Ethiopia, the vector distribution is mainly confined to the southern and western regions between longitudes of 33° and 38 °E and latitudes of 5° and 12 °N (Kotye, 2006). Among 31 species of tsetse flies found in Africa, five species, namely, *Glossina pallidipes*, *G. morsitans*, *G.f. fuscipes*, *G. tachinoides,* and *G. longipennis* are known in different regions of Ethiopia (Amhara, Benishangul Gumuz, Gambella, Oromia, Sidama, and Southern and Southwestern regions) (Langridge, 1976).

Tsetse flies can be ranked among the most destructive pests and are the vectors of the causative agents for sleeping sickness in humans and African Animal Trypanosomosis (AAT) or Nagana in livestock (Vreysen, 2001). Due to the lack of effective vaccines and high costs of disease treatment, disease control via control of the tsetse vector is the most effective strategy. So far, many chemical and biological methods of tsetse fly control have been developed, each of which has its advantages and limitations. Currently, the vector control interventions involve the use of insecticides either through sequential aerosol spraying technique (SAT); ground spraying; insecticide-treated targets or insecticide-treated animals – live baits; the use of traps, and the Sterile Insect Technique (SIT) (WHO, 2011).

Sterile Insect Technique is a biological insect control method based on genetics. It involves the mass production of male flies in a laboratory, sterilization, and release of these flies onto the target area. As compared to other techniques, the SIT is non-intrusive to the environment, has no adverse effects on non-target organisms, is species-specific, and can easily be integrated with biological control methods such as parasitoids, predators, and pathogens (Leak, 1999). There is no threat of resistance development to the effects of sterile

males if adequate quality assurance is assured during the production process and the sterile insects cannot be established in released areas, as is the case with other biological control programs (Vreysen, 2001). On the other hand, the SIT necessitates efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen, 2005). Sterile Insect Technique is an ideal mechanism to be combined with other methods of control and it fits well with the concept of integrated pest management (IPM). The control of diseases within an area-wide integrated pest management approach (AW-IPM) using the SIT has shown its effectiveness in some Sub-Saharan African countries for example, in Zanzibar (Vreysen *et al*., 2000). Motivated by these encouraging results, the SIT project in Ethiopia was initiated and designed in 1997 with the support of the International Atomic Energy Agency (IAEA) in the southern rift valley area. Its ultimate objective was to create a tsetse-free zone in a 25, 000 square kilometers area suitable for agricultural development. To achieve this objective, Kaliti Tsetse Fly Mass Rearing and Irradiation center was inaugurated on 3 February 2007 with the mission of creating a zone free of the tsetse fly in the Southern Rift Valley of Ethiopia. When completely equipped and operational, the new facility will have a colony capacity of approximately 7 million female flies and produces over 700,000 sterile male flies per week, enough to treat approximately 7000 km^2 at a time (IAEA, 2007).

Currently, two species of flies namely, *G. pallidipes* and *G. f. fuscipes* are mass-reared and efforts have been made to satisfy the needs of sterile males for the program of the country, Ethiopia and other countries infected by the same tsetse species both in quantity and in quality. Despite the coordinated effort to meet the objectives, the demand is not satisfied yet indicating the need to assess and solve the problems that are hindering the effort. The quality of the insects can be impaired by many factors in the rearing facility including crowding, rearing procedures, diets, insect pathogen load, deterioration of the strain, laboratory adaptation, genetic drift, and others or through handling, irradiation, packaging, release methods, etc. (Simmons *et al*., 2010).

Feed and feeding strategy are among the vital factors that determine the success of mass production in tsetse fly insectary facilities. The amount of blood imbibed during the inter-larval period is the major factor determining the productivity and survival rate of tsetse, *Glossina* species. Therefore, a qualitative and quantitative amount of blood is needed for the maintenance of fly colonies (IAEA, 2007) in vitro. This in turn requires more resources to be invested to

maintain production at a possible optimum level with affordable cost. As a result, optimized feeding strategies which are both efficient and cost-effective are mandatory in any given insectary facility. With the above background, the objectives of the present study were to test various feeding regimes and recommend the best, both in cost-effectiveness as well as to optimize the production of sterile male flies of G. *f. fuscipes* in tsetse mass-rearing facilities.

Materials and methods

Study area

This experimental study was conducted at Kaliti Tsetse Fly Mass Rearing and Irradiation Centre in Addis Ababa. Addis Ababa is the capital city of Ethiopia with geographical locations of 9.03° North; 38.74° East, latitude and longitude, respectively. The center was inaugurated on 3 February 2007 with a mission of creating a zone free of tsetse flies in the Southern Rift Valley of Ethiopia and, when completely equipped and operational, the facility was supposed to have a colony capacity of approximately 7 million female flies and will be able to produce over 700,000 sterile male flies per week enough to treat approximately 7,000 Km^2 at a time (IAEA, 2007).

Study population and study period

The study was conducted from March to May 2022 to evaluate the effects of feeding frequency on G. *f. fuscipes.* The experimental flies used for this study, 450 (360 females and 90 males flies, were obtained from the same production batch of pupae that originated from the main colonies of the insectary (Kaliti Tsetse Fly Mass Rearing and Irradiation Centre). Immediately after they had emerged from incubated pupae flies were rendered to immobility by using a chiller (+4 - sex-sorted and placed separately in colony cages with 50 (40 females per 10 males) flies per cage called colonies, following the predefined sex ratio (4:1) and maintained in a replicate of cages diameter 20 cm and width of 5 cm netting on top and bottom for feeding and collection of the hatched larvae (FAO/IAEA, 2006) within the rearing section (room) as the main colony throughout the experimental period.

Study design

An experimental study was conducted on feeding frequency and its associated effects on G. *f. fuscipes,* which is one of the *Glossina* species reared at Kaliti

Tsetse Fly Mass Rearing and Irradiation Centre. Three experimental groups with a total of 450 tsetse flies were established based on feeding frequency regimes in a week. These groups were coded as F3, F4, and F5 based on a feeding frequency regime representing a feeding frequency group of three, four, and five times per week, respectively. Each group had 150 flies and a replicate of 3 mating cages with 50 (40 female and 10 male) flies per cage. All groups were maintained in the same controlled and optimum environmental conditions of an insectarium similar to the general colony of the center, at a temperature of 23 – 25 °C and a relative humidity (RH) of 75 – 80% (FAO/ IAEA, 2006). Then, they were subjected to this experimental study, and the experimental parameters such as the average productivity, parameters measured as the number of Pupae Per Initial Female (PPIF), fecundity, survival rate (mortality), and the number of pupae production, were measured and analyzed for all the tested groups accordingly.

Feeding frequency test: The first group (F3) was fed three times (Monday, Wednesday, and Friday), the second group (F4) four times (Monday, Tuesday, Thursday, and Saturday), and the third group (F5) five times (Monday. Tuesday, Thursday, Friday and Saturday). All experimental groups of flies were fed with a similar quality of blood factor from the same batch of defibrinated gamma irradiated bovine blood meal using an in vitro silicon membrane system which was previously collected aseptically and frozen at -20 °C (IAEA, 2007). The feeding system was installed in a climatic room that maintained a similar environmental condition of 25 ± 1 °C and 50 ± 5% humidity for all treatments. The flies remained in the feeding room for less than 30 minutes and were placed under similar and optimum environmental conditions (FAO/ IAEA, 2006). The holding room temperature and humidity were adjusted and monitored daily from the data logger set in the rearing room. Pupae were collected daily and a mortality check was performed weekly. The weekly datasets of the colonies, in different feeding regimes, were measured by testing tsetse production and survival parameters including fecundity, Pupae per Initial Female (PPIF), mortality rate and pupae production, weight, and pupal size (classes are graded based on their size).

Fecundity test: Fecundity was expressed as the number of pupae produced per female per 10 days, by considering day 18 immediately after they emerged from the pupae stage as the first larviposition day (FAO/IAEA, 2006).

Pupae sorting and grading test: The collected Pupae from larviposition cups on daily bases throughout the experimental period and sorted into normal and aborted L3 by visual observation. Then the normal pupae were categorized into five size classes by a sorting machine. The standard system has five collecting shuts labeled, A (smallest) to E (largest); the length of the collection area has been adjusted to correspond to the five weight classes that previously have been defined to tsetse pupae of *G. fuscipes* (Zelger and Russ, 1976).

Mortality test: Mortality was recorded daily for each test group throughout the experimental period. Dead flies were sorted into blood-fed and starved fly mortalities. The mortality rate was calculated according to Standard Operating Procedures (SOP) for Mass-Rearing Tsetse flies (FAO/ IAEA, 2006).

Data analysis

The data from this experimental study results were organized, coded, and analyzed by Stata version 12.0 software. One-way analysis of variance (ANOVA) was used to investigate the link between parameters and feeding frequency. After a significant ANOVA, paired mean comparison tests were used to examine differences between pairs of feeding regimes. Statistical significance was set at the conventional level of "less than 5%" for all analyses.

Results

Pupae production and pupal size class distribution

The first larviposition date for all blood-feeding treatment groups (3 times per week (F3), 4 times per week (F4), and 5 times per week (F5)) was the same as on day 18. The highest number of pupae was produced by the flies fed 5 times per week (n = 390) followed by flies fed 4 times per week (n = 386) and 3 times per week (n = 271) respectively (Table 1).

Table 1. The effect of feeding frequency on pupal production, pupal size, and PPIF among the test groups – F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

Treatment groups	Total pupae produced	Pupal size classes distribution percentage within the group					PPIF
		A	B	C	D	E	
F3	271	49 (18.08%)	78 (27.80%)	105 (38.70%)	36 (13.30%)	3 (1.12%)	2.26
F4	386	40 (10.36%)	87 (27.54%)	137 (35.50%)	115 (29.80%)	7 (1.80%)	3.22
F5	390	38 (9.70%)	86 (22.00%)	112 (28.70%)	127 (32.60%)	27 (6.90%)	3.25

The results showed that the size-wise distribution of the pupae was not the same both within and among the test groups. The highest percentage of the total pupae produced was concentrated in class A – C sized pupae in the F3 feeding treatment group covering about 84.58% of the total pupae produced by the group, while class C – E sized pupae constituted the highest percentage in F4 and F5 feeding treatment groups contributing about 67.10% and 68.20% of their overall production respectively. Additionally, the highest number of the smallest size class pupae (class A) were produced by F3 (18.0%) followed by F4 (10.36%), and the least was produced by F5 (9.70%) and the reverse was true for the largest pupal class (class E) covering 6.90%, 1.80% and 1.20% of the total pupal production of their respective group, F5, F4, and F3 respectively. The pupal size classes' distribution for each test group is shown in figure 1.

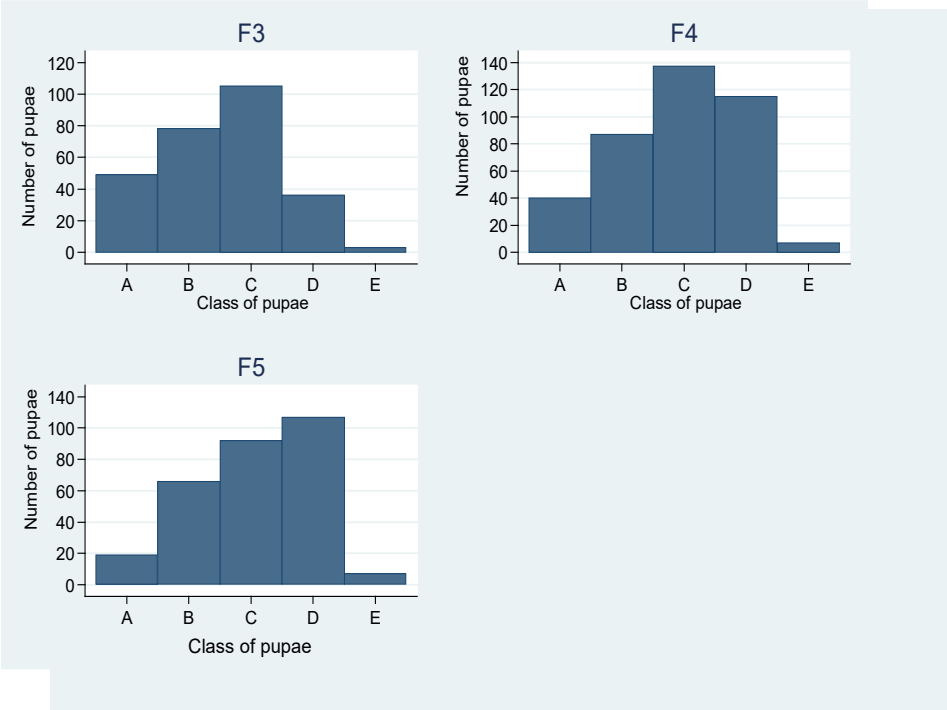


Figure 1. Pupal size classes' distribution of test group, F3 (three blood meals per week), F4 (four blood meals per week), F5 (five blood meals per week).

Pupae per initial females (PPIF) and fecundity

Pupae per initial females (PPIF), which is expressed by the total pupae produced by initial females at a given time was evaluated for each test group. The result revealed that the highest overall PPIF was produced by group F5 (3.25 PPIF) followed by group F4 (3.22 PPIF) and the least was by group F3 (2.26). Similarly, fecundity, which is defined as pupae per female per 10 days (P/F/10 days), was assessed for each group. The lowest fecundity was observed for the feeding treatment group F3 with a mean fecundity of 0.559 ± 0.15 pupae/females/10 days compared to both F4 and F5 with a mean fecundity of 0.721± 0.15 and 0.697± 0.01 pupae/females/10 days in that order. A statistically significant link was found between feeding frequency and fecundity (ANOVA, $p>0.02$ df = 2). The mean difference was significant for F3 *vs* F4 and F3 *vs* F5, but not for F4 *vs* F5 (Table 2).

Table 2. Pairwise comparisons of means of fecundity between test groups – F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

Paired groups	Contrast	Std. Err.	t	P>t	[95% Confidence Interval]	
F4 vs F3	0.162	0.059	2.753	0.014	0.035	0.289
F5 vs F3	0.138	0.059	2.345	0.027	0.017	0.259
F5 vs F4	-0.024	0.059	-0.408	0.687	-0.145	0.097

Figure 2 shows the trend of PPIF for the three treatment groups over the study period.

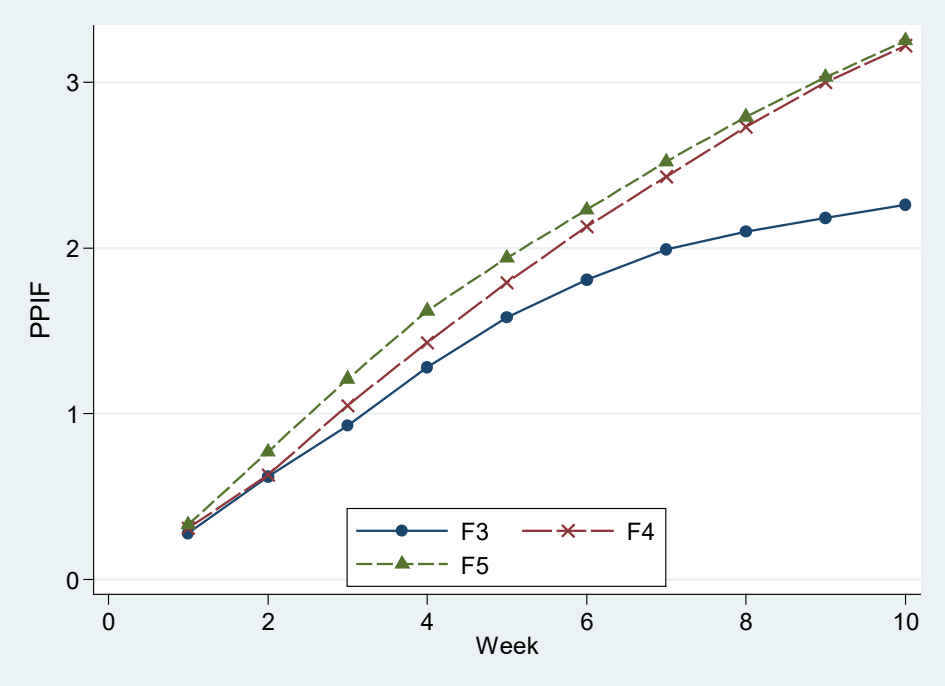


Figure 2. The trend of PPIF for the three treatment groups, F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

Fly mortality and survival rate

The overall female mortality rate was highest for females fed 3 times per week (41.60%) followed by females fed 5 times per week (29.80%) and the lowest was for flies fed 4 times (28.60%). The dissection result of the dead female flies indicated that only 1.80% of the total dead flies had blood in their abdomen (blood mortality), 0.42% died of unknown causes and all the rest died due to starvation. The statistical analysis showed that there was no link between mortality and the causes of mortality ($p > 0.05$). Nevertheless, the frequency of blood feeding had a significant effect on female survival and/or mortality (ANOVA, $p > 0.044$ $df = 2$). Females' mortality was highest for females fed 3 times per week (41.60%) followed by females fed 5 times per week (29.80%) and the lowest was for flies fed 4 times (28.60%) with an average daily mortality rate of 1.15%, 0.79% and 0.82% for F3, F4, and F5 respectively. When a pairwise mean comparison was made between the groups, mortality in F3 was significantly higher than in both F4 and F5. However, there was no statistically significant difference in females' mortality rate of treatment groups F4 and F5 (Table 3).

Table 3. Pairwise comparisons of means for mortalities between test groups – F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week).

Paired groups	Contrast	Std. Err.	t	P>t	[95% Confidence Interval]	
F4 vs F3	-3.000	1.260	-2.381	0.030	-5.704	-0.296
F5 vs F3	-2.727	1.260	-2.164	0.039	-5.301	-0.154
F5 vs F4	0.273	1.260	0.216	0.830	-2.301	2.846

Figure 3 shows the trend of survival rate for the three feeding frequency groups over the study period.

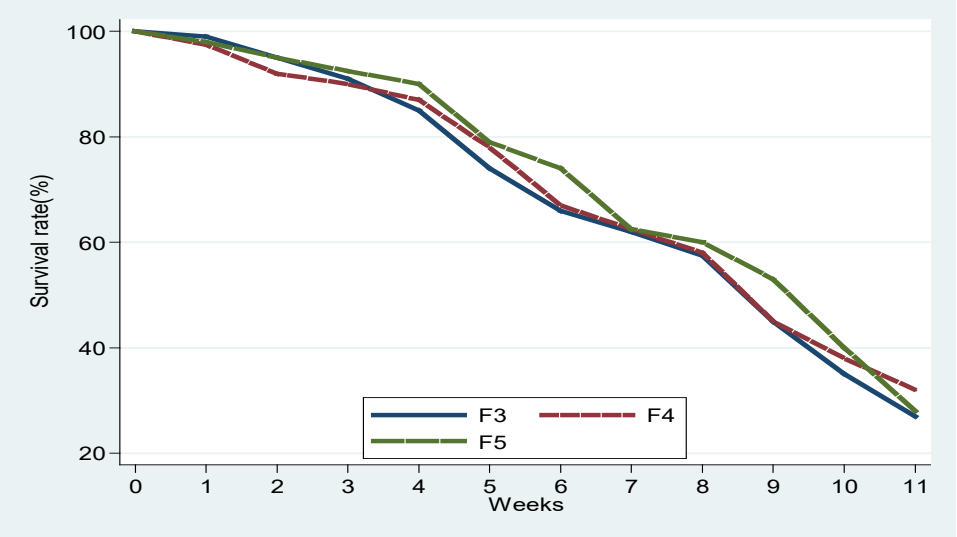


Figure 3. The trend of survival rate for flies fed at different frequencies per week, F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week).

Discussion

In this study, the effect of different feeding frequencies on the survival and production of *G. f. fuscipes* was assessed to identify the best feeding frequency that could be applied in tsetse fly mass-rearing facilities. Survival, fecundity, pupae per initial females (PPIF), and pupal quality are known to be the crucial parameters routinely used for assessing colony performance in all standardized insectary facilities. Hence, these parameters were evaluated for each treatment group and statistical comparisons were made between the groups.

The current results revealed that feeding frequency had a crucial effect on the productivity and survival rate of *G. f. fuscipes* subjected in the study. Flies fed four times per week and five times per week produced significantly more pupae with a total pupal production of 386 and 390 pupae, respectively, compared to those fed three times per week that produced only 271 pupae throughout the study period. This finding is in agreement with the findings of other *Glossina* species, *G. pallidipes,* by Tsegaye *et al.* (2020) who reported low pupal production for flies fed three times per week compared to five times per week.

It is a fact that pupal class is a good overall quality indicator of the effectiveness of colony maintenance where each weight class can be defined using a size-sorting machine. The mean pupal weights should approximate the values developed by Zelger and Russ (1976), and no more than 10% of the puparium should be in weight class A (FAO/IAEA, 2006). Based on this fact, the results of this study indicated that flies fed four and five times per week produced higher quality pupae than flies fed three times per week. Likewise, the highest percentage of pupae, which are considered to be of high-quality classes (class C and D), was highest in F5 followed by F4, although the difference between the two was not significant. These results were in line with the findings of Kettle (1995) who found that the weight of a pupa depends on the amount of blood taken by a female during pregnancy, with a highly significant correlation between puparia weight and quality of blood ingested. The reason for this finding could be due to the nutrient shortfall needed for the required performance in flies fed three times per week as stated by previous findings, which stated pupal quality is an indication of the nutritional status of the fly and is reflected by pupal weight and size (IAEA, 2007).

Regarding PPIF, the overall commutative Pupae per Initial Female (PPIF) for the group of flies subjected to five times per week feeding regime produced 3.25 PPIF which was the highest of all, although the difference was not significant compared to four times per week feeding frequency regime with overall PPIF of 3.22. On the other hand, flies subjected to the three-times-per-week feeding frequency regime produced an overall PPIF of 2.26, which was significantly lower than the four and five-times-per-week regimes. Similarly, the lowest fecundity was observed for the feeding treatment group F3 with a mean fecundity of 0.559 ± 0.15 pupae/females/10 days compared to both F4 and F5 with a mean fecundity of 0.721± 0.15 and 0.697± 0.01 pupae/females/10 days in that order. The data on PPIF for *G. f. fuscipes* and *G. pallidipes* showed a wide range of PPIF depending on different factors. Results range from 4 – 7 using different food sources obtained by Langley and Pimley (1979) indicating that the PPIF could be influenced not only by the frequency of feeding but also by the quality of the feeding source. In the same way, seven pupae per initial female were reported when flies were fed on cow blood, and 6.8–7.8 when fed on rabbits (Mews *et al*., 1976). The values of PPIF can vary greatly, from 1.6 to 14 and the PPIF required for the establishment of a colony should be ≥3. Thus, the results for both F4 (3.22) and F5 (3.25) were above the previously established limit and satisfactory, but the values obtained for F3 colonies in our study were lower than the limit required to establish a fly colony in a given insectary facil-

ity. On the other hand, studies on tsetse fly-rearing facilities stated that for a steadily growing tsetse colony, it was important to make sure that the colony's daily mortality was kept below 1% and the fecundity (pupae/female/10 days) was above 0.60 (Jordan, 1980). Thus, the fecundity of the F4 and F5 groups in this study was above the average acceptable level whereas F3 was below the average lower limit.

The results of this study on mortality and survival revealed that the overall female mortality was highest for females fed 3 times per week (41.60%), followed by females fed 5 times per week (29.80%), and the lowest was for flies fed 4 times (28.60%) with an average daily mortality rate of 1.15%, 0.79% and 0.82% for F3, F4, and F5, respectively. The daily mortality rate recorded in the two feeding regimes (F4 and F5) was less than the acceptable daily mortality rate (1.20%) suggested by the IAEA (2007). In contrast to this, the daily mortality for F3 was above the acceptable level of daily mortality, highlighting that applying this feeding regime affects the steady growth of the tsetse colony. These results are in agreement with the previously obtained data on other *Glossina* species, *G. pallidipes* and *G. morsitans morsitans,* which proved the lowest survival and productivity of three times per week feeding as compared to four and six times per week feeding regimes indicating a positive correlation between feeding frequencies and adult fly survival as well as fecundity (Langley and Stafford, 1990). According to these authors, the minimum number of blood meals per cycle (an inter-larval period of 9 days) which did not cause a decline in reproductive performance was 4 times for *G. m. morsitans* and five for *G. pallidipes.* Gaston *et al.* (1993) also stated that the flies fed every third day, and always engorged fully at every opportunity, whereas flies offered food every second day refused to feed at all or did not engorge fully at every opportunity. Furthermore, previous studies indicated that in the laboratory, female flies which take a full blood meal every third day perform less (survival and productivity) than those which take on average rather smaller blood meals every second day (Langley and Pimley, 1979). The best outcomes for F4 in nearly all parameters evaluated here could be because the meal, which is critical for normal larval development, was more likely to be attained with the 4 meals per week regime and hence the flies have more opportunity to have a meal at the critical time. On the other hand, even though a feeding frequency of five times per week optimized the number of blood-fed flies, it is important to note that the tsetse fly needs on average 2 days to completely digest a blood meal (McCue *et al*., 2016).

Conclusions

In conclusion, the best results for almost all parameters evaluated in this study were obtained in treatment F4 (4 blood–feedings per week), and the lowest results were obtained in treatment F3 (3 blood feedings per week) indicating that feeding flies four times per week instead of five or more times per week will have no adverse effect on colony production performance. In contrast to this, treatment F3 (3 blood–feedings per week) led to significant negative effects on female survival and production parameters although maintenance remained acceptable in some regards. It is, therefore, recommended to feed tsetse flies, particularly *G. f. fuscipes* species, four times per week in the tsetse flies' factory. Furthermore, the effects of the feeding regime on tsetse fly emergence rate and progeny quality need to be assessed.

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SHORT COMMUNICATION

A pilot survey on factors limiting veterinary service delivery systems at animal health facilities in Hawassa, Shashemene, and Negele Arsi, Ethiopia

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Abstract

A pilot questionnaire survey was carried out from December 2021 to June 2022 at animal health facilities in Hawassa city and Shashemene and Negele Arsi towns in Ethiopia. The objective of the survey was to determine what factors in the study area limited the provision of veterinary services at animal health facilities like veterinary clinics, veterinary pharmacies, and veterinary administrative offices. Government veterinary staff, clinical veterinarians, and private veterinary pharmacists were purposefully chosen based on their availability at each study area, whereas livestock owners (clients) were chosen at random at the point of service. A semi-structured questionnaire with multiple-choice and open-ended questions was administered to 100 study participants. It was discovered that the public sector was generally preferred by 74% of livestock owners and the private sector was preferred by 26%. In terms of infrastructure coverage, the current animal health service delivery system was found to be deficient (35.7%). Drug shortages were a major problem for most public veterinary service providers (66.7%), while expensive drug prices were a problem for private veterinary service providers (34.0%). Additionally, it was determined that poor management (57.1%), a lack of funding (14.3%), a lack of commitment (28.6%), and a lack of attention to the veterinary sector (78.6%) were among the factors that hindered the provision of proper veterinary services in these areas. To carry out efficient veterinary clinical activities, the existing public veterinary institutions should be given sufficient financial resources, qualified personnel, and physical resources.

Keywords: Animal health; Hawassa; Negele Arsi; Shashemene; veterinary service delivery.

Introduction

Agriculture forms the foundation of the economies of the majority of developing nations. The majority of the countries in eastern and southern Africa derive more than 30% of their GDP from agriculture (Institute of Biodiversity Conservation, 2004). By supplying cheap labor, capital investment, foreign currency, and markets for manufactured consumer goods, increasing agricultural productivity encourages industrial growth (Upton, 2004). One of the most crucial ways to raise living standards in many developing nations is through livestock production. Livestock is essential to national economies and rural communities in Sub-Saharan African nations (Sibanda *et al*., 2014). About 12–16% of the country's total GDP, 35% of the agricultural GDP, and 60%–70% of Ethiopians' means of subsistence are derived from it (Halderman and Nelson, 2004).

Ethiopia has 61.5 million cattle, 33 million sheep, 39 goats, 59.4 million chickens, 11.96 million equines, 1.76 million camels, and 7.1 million beehives. Cow milk is produced at a rate of 3.3 billion liters annually, camel milk at a rate of 282.2 million liters annually, eggs at a rate of 151.47 million, and honey at a rate of 58.6 million kg annually (CSA, 2019). More than half of all industrial raw materials are supplied by it, making it the most significant economy in terms of foreign exchange (Hoekstra *et al*., 2011). The productivity of the livestock sector is lower than the potential of the average African production rate despite the significance of livestock to the majority of the population and the economy as a whole (Duguma *et al*., 2012a). Diseases, an out-of-date management approach, poor genetics, food shortages in both quantity and quality, and a lack of a well-developed market infrastructure are all to blame (Duguma *et al*., 2012b).

Numerous signs point to serious shortcomings in veterinary services in Sub-Saharan Africa and the Horn of Africa, especially when it comes to investigating, reporting, managing, and quickly responding to outbreaks of livestock disease (OIE, 2019). The majority of African nations, including Ethiopia, have experienced a decline in animal health services as a result of several factors, including underdeveloped economies with increased state obligations, inadequate and erratic access to foreign currency, global economic fluctuations, and

the presence of various production systems within a single economy in a country (MOARD, 2010). Farmers treat diseases in their livestock with a variety of veterinary drugs, and uneducated people sell these substances as common market goods (Moritz *et al*., 2013; Vougat Ngom, 2017). Drug abuse in food-producing animals is exacerbated when metabolite residues in tissue, which indirectly affect the respective consumers' health, are present (Sanders, 2007).

Four categories of veterinary services are distinguished: (1) curative services, which include disease diagnosis and treatment; (2) preventive services, which include immunization and vector control; (3) production of veterinary pharmaceuticals; and (4) human health protection, which includes sanitary inspection of animal products (De Haan and Umali, 1992).

A crucial step toward helping rural poor farmers benefit from the rising demand for livestock products while also lowering risks to both animal and human health is improving veterinary service delivery to combat and control emerging and re-emerging animal diseases (Wymanns *et al*., 2007; Hall *et al*., 2004). Despite a few pilot studies in particular regions of the central highlands of Ethiopia, mostly for academic purposes, there is a lack of comprehensive, well-documented, and reliable information about the state of the veterinary services delivery system, as well as significant obstacles impeding the effectiveness of these services in both public and private veterinary services. The factors limiting the provision of veterinary services at animal health facilities in Hawassa, Shashemene, and Negele Arsi, Ethiopia, are similarly undocumented. Therefore, the objective of this pilot survey was to determine what factors were restricting the provision of veterinary services at animal health facilities in Hawassa, Shashemene, and Negele Arsi, Ethiopia.

Materials and methods

Description of study areas

From December 2021 to June 2022, the study was carried out in Hawassa city, Shashemene, and Negele Arsi towns, Ethiopia.

The capital of Sidama Regional State, Hawassa, is situated 275 km South of Addis Ababa.

1750 meters above sea level, it is located between 6°83' and 7°17' N and 38°24' and 38°72' E. It has an average annual temperature of 20 °C and 955 mm of precipitation (Fesseha *et al*., 2020).

Shashemene, on the other hand, is a town and woreda in the West Arsi zone of the Oromia Regional State. It is situated between 7°12′ N and 38°36′ E, 250 km South of Addis Ababa. Its elevation ranges from 1,672 to 2,722 meters above sea level. The town's annual rainfall varies between 1,500 and 2,000 mm, and its temperature ranges from 12 to 28 °C (CSA, 2003).

In addition, Negele Arsi is a town situated in the West Arsi Zone of Oromia Regional State, in southeast Ethiopia, on the paved road North of Shashemene. It is roughly 225 kilometers from Addis Ababa and is situated between 7°21' N and 38°42' E. The altitude of the town is 2043 meters above sea level (ORS, 2004).

Study design and sample size determination

Data were gathered using a cross-sectional study design that included an observational assessment and a semi-structured questionnaire. Using the formula given by Arsham (2007), the sample size for the questionnaire survey was calculated, with a standard error of 5% and a 95% confidence interval. As a result, the sample size was determined using the formula below:

Where, N is the required sample size, which is the total number of respondents to the questionnaire survey; SE is the standard error (0.05) at a 95% confidence interval. As a result, it was calculated that there were 100 participants in total.

Study participants

Veterinarians from public clinics, private veterinary pharmacists, and employees of veterinary service facilities were all purposefully selected as study participants based on their availability at the veterinary service facilities. However, a simple random sampling technique was used to choose livestock owners (clients). As a result, the questionnaire survey was completed by 50 livestock owners (clients), 13 veterinarians from public clinics, 23 veterinary pharmacists, and 14 members of the veterinary administrative staff.

Questionnaire survey

A semi-structured questionnaire with multiple choice and open-ended questions was used with 100 participants in the study area. To ensure consistency, the questionnaire was also translated from English into regional tongues. The respondents were interviewed in person for the questionnaire. People who work in veterinary service facilities, especially public clinic vets and private veterinary pharmacists, were questioned about the biggest challenges they faced and whether drug smugglers were present or not. The questionnaire also asked about the staff members' knowledge of other limitations on the area's veterinary service delivery systems, such as management-related restrictions and inaccessible veterinary facility infrastructure.

Observational assessments

Both private and public veterinary service facilities received regular visits. There were many facilities, including veterinary clinics, but not all of them used laboratory diagnostics. Drugs were unavailable or improperly stored, animal holding areas were dirty, lab equipment was inadequate, there were no quarantine or isolation areas for disease outbreaks, and there were no suitable operating rooms or emergency rooms.

Data management and statistical analysis

The data gathered during the survey period was entered into Microsoft© Office Excel before being encoded and analyzed using STATA statistical software version 14. The chi-square ($\chi 2$) test was used to examine the relationship between various risk factors. To analyze qualitative data, descriptive statistics were used. Frequencies and percentages were used to summarize the output. The threshold for statistical significance was set at $p<0.05$.

Results

Demography of study participants

A total of 100 participants took part in the study, including 50 livestock owners (clients), 13 public clinic veterinarians, 23 veterinary pharmacists, and 14 veterinary administrative staff (Table 1).

Table 1. Summary of study participants

Participants	Number of participants and their geographical locations			
	Hawassa	Shashemene	Negele Arsi	Total
Clients	20	15	15	50
Public clinic veterinarians	6	4	3	13
Veterinary pharmacists	10	6	7	23
Government staff	5	5	4	14
Total	41	30	29	100

Veterinary service delivery constraints identified

In the three study areas, various restrictions on the provision of veterinary services were found, the majority of which were connected to the prescription of drugs without laboratory confirmation of cases (94.4%), the inaccessibility of infrastructure (71.4%), a lack of drugs (66.7%), the presence of drug smugglers (58.3%), and delays in disease reporting (40.0%) (Table 2).

Table 2. Identified constraints of veterinary service delivery

Questions	Number of respondents (N)	
	Yes (%)	No (%)
Are there sufficient drugs with types and abundances available in your pharmacy?	12 (33.3)	24 (66.7)
Do you know that there are smugglers who sell drugs?	19 (52.8)	17 (47.2)
Have you faced expired drugs?	15 (41.7)	21 (58.3)
Do you treat an animal with specific drugs as requirements?	14 (38.9)	22 (61.1)
Do you depend on the lab result for a prescription?	2 (5.6)	34 (94.4)
Is there a shortage of skilled manpower in the Vet Clinic?	7 (50.0)	7 (50.0)
The animal health staff in these areas are adequate in number and can cover the area and address the vet. Needs of livestock keepers?	9 (64.3)	5 (35.7)
Is there infrastructure inaccessibility for delivering vet Service to the intended receiver?	4 (28.6)	10 (71.4)
Are there communication problems between the animal's owner and the veterinary service provider?	24 (48.0)	26 (52.0)
Do you take your animal to a veterinary clinic quickly once they are diseased?	30 (60.0)	20 (40.0)

Preference for veterinary service facilities among clients

About 74% of clients were discovered to frequently use public veterinary services for the care of their animals, while 26% of clients preferred private veterinary services (Table 3).

Table 3. Preference for veterinary service facilities among

Services	Frequency	Percent (%)
Public veterinary service	37	74.0
Private veterinary service	13	26.0
Total	50	100

Limitations in providing proper veterinary services to livestock owners (clients)

In Shashemene (16.7%), distance from the service center is a minor constraint, while it was the biggest issue for livestock owners in Hawassa (83.3%) in providing proper veterinary care. Distance from service centers was not cited in Negele Arsi, though, as a barrier to offering clients quality veterinary care. Other factors were also identified, including farmers needing drugs without bringing their animals, low perception of veterinary services, and bringing animals after attempting self-treatment on several occasions (Table 4).

Table 4. Limitations in providing proper veterinary services to livestock owners (clients)

Limitations related to clients in the provision of veterinary services	Number of responses in each area (%)			
	Hawassa	Shashemene	Negele Arsi	Total
Distance from the service center	5 (83.3%)	1 (16.7%)	0 (0.0%)	6 (16.7%)
Low perception	2 (33.3%)	1 (16.7%) 3 (50.0%)		6 (16.7%)
Coming after several trials by themselves	3 (37.5%)	4 (50.0%)	1 (12.5%)	8 (22.2%)
Farmers need drugs without bringing their animals	6 (37.5%)	4 (25.0%)	6 (37.5%)	16 (44.4%)

Constraints in public veterinary clinics and veterinary administration

The public veterinary clinics encountered a variety of restrictions, the majority of which had to do with management. For instance, the limitations included the sector receiving little attention, poor management, a lack of commitment, and a lack of funding (Table 5).

Table 5. Constraints related to public veterinary clinics and veterinary administration

Constraints related to public veterinary clinics and veterinary administration	Number of participants (%)			
	Hawassa	**Shashemene**	**Negele Arsi**	**Total (%)**
Lack of awareness about veterinary importance	1 (20.0)	0 (0.0)	0 (0.0)	1 (7.1)
Shortage of budget	1 (20.0)	1 (20.0)	0 (0.0)	2 (14.3)
Minimal attention is given to the sector	3 (60.0)	4 (80.0)	4 (100.0)	11 (78.6)
Lack of commitment	2 (40.0)	1 (20.0)	1 (25.0)	4 (28.6)
Shortage of manpower	0 (0.0)	1 (20.0)	0 (0.0)	1 (7.1)
Lack of incentive/bonus	1 (20.0)	0 (0.0)	0 (0.0)	1 (7.1)
Poor management (administration)	2 (40.0)	3 (60.0)	3 (75.0)	8 (57.1)

Limitations related to private veterinary service delivery system

Various constraints on the delivery of private veterinary services were identified. Non-professional involvement in the treatment of diseased animals, high service or drug costs, and treatment without a proper diagnosis was frequently reported (Table 6).

Table 6. Constraints related to the private veterinary service delivery system

Major constraints in private veterinary service delivery system	Number of participants (%) (N=50)			
	Hawassa	Shashemene	Negele Arsi	Total (%)
Use of material without sterilization	3 (75.0)	1 (25.0)	0 (0.0)	4 (8.0)
Provision of services without proper diagnosis	2 (20.0)	3 (30.0)	5 (50.0)	10 (20.0)
High service or drugs cost	6 (35.3)	4 (23.5)	7 (41.2)	17 (34.0)
Inadequate qualification (non-professional involvement)	9 (47.4)	7 (36.8)	3 (15.8)	19 (38.0)

Discussion

It is widely acknowledged that a variety of factors, including farmers' perceptions of the services, wealth position, and household head education level, among others, influence the provision of high-quality veterinary care within a particular agroecology/production system (Gizaw *et al*., 2021). Both the public and private sectors of Ethiopia's current animal health system offer poor-quality and limited-reach services. The government subsidizes the cost of clinical case treatment services and drug costs, making public veterinary services the preferred option over private ones in terms of effectiveness and cost (Girma, 2008). According to recent findings, the majority of livestock owners in the study area (74%) prefer public veterinary services (public veterinary clinics) to private veterinary service providers because these services are more sustainable than private veterinary services and have qualified professionals on staff.

The primary factors that cause livestock owners (clients) to dislike public veterinary services include drug shortages, weekend and holiday closures, a lack of punctuality, the absence of emergency services, and distance from the veterinary service facilities. On the other hand, some livestock owners (26%) preferred private veterinary clinics because of their punctuality, accessibility to a wide range of veterinary medications, and availability of home-to-home services for emergency cases, including on weekends and holidays. On the other hand, private veterinary care was more expensive than public veterinary care. These results were in line with the findings of Friederike (2003), which pointed out several problems that the government's veterinary service faced.

One problem was how to coordinate the numerous actors despite resources being limited, communication issues existing, and more importantly how to keep a steady and reliable data flow. Respondents to the current study attributed the inability to maintain full-service supply to a lack of resources, a lack of knowledge, budgetary restrictions, and insufficient government attention to the industry.

In the study area, challenges with the handling, management, and marketing of illegal drugs are frequent. According to veterinary administrative staff members who were interviewed, the main barriers to customer satisfaction in public veterinary clinics are a lack of basic infrastructure, including recovery rooms for sick animals, veterinary medications, suitable buildings with the necessary equipment, and laboratory equipment. Budget constraints and a lack of properly trained personnel were also cited as major bottlenecks in the sector.

Ethiopian livestock performance continues to be largely attributed to animal diseases, creating a growing supply-demand imbalance for livestock products (Agrawal, 1995). Over the past three decades, conventional veterinary services have been crucial in the country's efforts to control and prevent livestock disease. However, they are unable to offer complete coverage of preventive and curative healthcare due to a lack of labor, logistical problems, unpredictable pharmaceutical supply, and the high cost of medicines and equipment. Additionally, many livestock farmers, especially those in rural areas, are far from veterinary clinics and even those who do have access to them might not be able to afford them (Sori *et al.*, 2004).

The current study revealed that, even though the majority of farmers lack formal education, they still purchase medications from veterinary drug stores and care for their animals on their own. As a result, farmers were unable to properly apply the medication because they could not read the drug labels. In a similar vein, Kebede *et al.* (2014) reported that farmers in North Gondar, Ethiopia, were indiscriminately using drugs from the black market to treat their animals. Irrational drug use includes over-prescription (prescription of drugs when none are clinically necessary), omission (not prescribing needed medications for conditions), inappropriate dosage (too high or low), incorrect duration (too short or long), incorrect selection (drug and organism mismatch), unnecessary risk, and others (Brahma *et al.*, 2012).

Discussions were held with respondents at the veterinary administration during the observational assessments, and they noted that inadequate budgetary provisions for preventive veterinary services were the main obstacles to the implementation of disease control services like vaccination and quarantine. Additionally, the staff members mentioned a lack of facilities for veterinary clinical activities and case diagnosis in laboratories. It was subsequently established that the majority of sick animals were identified based solely on clinical symptoms without the support of relevant laboratory tests. These results corroborated a study by Kebede *et al.* (2014) that found the only clinical tool available to diagnose ill animals in all veterinary clinics examined in North Gondar between 2011 and 2012 was a thermometer.

Conclusions

The main factors limiting the veterinary delivery system in Hawassa, Shashemene, and Negele Arsi, Ethiopia, are a lack of laboratory facilities, a lack of drugs or a high cost of drugs, unclean restraint areas, a lack of quarantine and isolation areas for sick animals, a lack of suitable rooms for surgical and emergency cases, and inappropriate drug storage. It was evident that neither the public nor the private sectors' offerings of services were adequate to satisfy customers (clients). Additionally, it is difficult to maintain full-service provision due to customer distance from the service center, lack of knowledge about veterinary services, and client perception of veterinary services.

Accordingly, based on the foregoing concluding remarks, the following suggestions are made: a thorough analysis of the factors limiting the veterinary service delivery system should be carried out at the national level, awareness-raising events for both clients and professionals should be planned in consultation with the relevant government body, private-public veterinary service partnerships should be started, and short-term capacity building training should be provided to veterinarians.

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We thank all participants of the study for their willingness to participate in the study.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' contributions

STT – developed questionnaire, collected data, data analysis, and writing up of the first draft; TSM –developed questionnaire, data curation and analysis, and writing up the final draft; TEB – helped in data curation and analysis of data

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Acknowledgements

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