Ethiopian Veterinary Journal

Year: 2021 Volume: 25, No. 1

2021

ISSN:1683-6324 (print) ISSN:2221-5034 (online)

Ethiop. Vet. J. is the Official Scientific Organ of the Ethiopian Veterinary Association

Ethiopian Veterinary Journal (*Ethiop. Vet. J.*)

Objectives and Scope

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.

Ethiop. Vet. J., is published by the Ethiopian Veterinary Association (EVA)

Ethiop. Vet. J., 2021, 25 (1)
ISSN: 1683-6324

Cover drawing by Wossene Abay and Yilma Jobre

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Prevalence, risk factors and antibiogram of *Escherichia coli* isolated from dogs in Ambo, Gojo and Bako towns of Oromia region, Ethiopia

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Abstract

Dogs are a potential reservoir for *Escherichia coli* and other zoonotic bacterial pathogens posing the risk of infection to humans and other animals. A cross-sectional study was used to collect 438 rectal swab samples from apparently healthy dogs of Ambo, Gojo, and Bako towns of West Shewa Zone with the objectives of investigating the prevalence, risk factors and antibiogram of *E. coli*. A questionnaire survey was administered at the household level to collect data on potential risk factors to acquire *E. coli* infection. Prevalence was determined by using standard bacteriological culture techniques. Further confirmation and antimicrobial susceptibility testing of *E. coli* using the minimum inhibitory concentration (MIC) method were conducted using Automated Phoenix Machine. The overall prevalence of *E. coli* was found to be 24.2% (95% confidence interval [CI]:20.26-28.49%). The isolation rate of *E. coli* was the highest in Bako 31.01%, followed by Gojo 30.99% and Ambo 18.49% towns. Univariable and multivariable logistic regression analyses revealed that there was a significant association between *E. coli* prevalence and towns and *Kebeles* ($P<0.05$). However, age, sex, the presence of other domestic animals in the household, types of the housing system, educational level of the dogs' owners, and type of feed provided to the dogs did not significantly correlate with the isolation of *E. coli* ($P > 0.05$). *E. coli* isolates were pan-susceptible to amikacin, ertapenem, imipenem, piperacillin-tazobactam, netilmicin, ampicillin, piperacillin, cefoxitin, ciprofloxacin, levofloxacin, and meropenem. A low level of resistance was found to aztreonam, ceftriaxone, (each 13.95%), cefazolin (22.73%), gentamicin (11.90%), and trimethoprim-sulphamethoxazole (6.97%). *E. coli* isolates

showed multidrug resistance to aztreonam, cefazolin, ceftriaxone, gentamicin, and trimethoprim-sulphamethoxazole (14.0%). This demonstrated *E. coli* prevalence and moderately low antimicrobial resistance suggests the possible risk of infection of humans. Therefore, raising public awareness about zoonotic canine disease prevention measures and good hygienic practices are essential..

Keywords: Antibiogram, Dog, *Escherichia coli*, Ethiopia, Prevalence, Risk factor.

Introduction

Dogs benefit humankind in several ways such as through guiding blind people, guarding, and hunting (Dantas-Torres and Otranto, 2014). The ownership of a dog has been associated with a decreased likelihood of high blood pressure, obesity, and cardiovascular disease (McConnell *et al*., 2011). However, there are several health hazards associated with owning dogs (Damborg *et al*., 2009) such as viral, bacterial, protozoal, and helminth infections; and bite and allergy. Considering the large number of people sharing their homes with dogs and the close relationship that many owners share with their dogs, the public health impacts of dogs as companion animals is important to consider, particularly with the One Health initiatives which aim to integrate human, animal and environmental health (Blaha, 2012).

Escherichia coli normally reside in the lower intestines of most warm-blooded mammals, including dogs. Normally, the presence of *E. coli* is benign and even beneficial, but in some cases, it can cause disease, especially in newborn puppies and in those with impaired local or systemic immunity (Beutin, 1999). The enteric *E. coli* are divided based on virulence properties into Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enteroinvasive (EIEC), Verotoxigenic (VTEC), Enterohemorrhagic (EHEC) and Enteroaggregative (EaggEC) (Frenzen and Drake, 2005).

The pathogenic strains or clones of *E. coli* which exist in canines are capable of causing significant morbidity and mortality in humans as well as in pets (Sanyal *et al*., 1997; Beutin, 1999; Sancak *et al*., 2004). Besides its role in gastrointestinal infections, *E. coli* can cause infections of the urogenital tract and systemic disease in dogs and cats (Beutin, 1999). Moreover, companion animals are of importance in global antimicrobial resistance research, particularly due to the consistent intimate contact they have with humans, and thus their pu-

tative role in the transmission of zoonotic resistant bacteria like *E. coli*, either directly or indirectly through contact and the environment (Guardabassi *et al*., 2004; Ewers *et al*., 2012).

It has been reported that canine feces represent a potential reservoir for the human acquisition of commensal bacteria, such as *E. coli*, that can act as a major source of antimicrobial resistance genes (Caprioli *et al*., 2000). *E. coli* have been extensively studied in farm animals as well as pets around the world (PuñoSarmiento *et al*., 2013). In Ethiopia, the presence of *E. coli* in general and *E. coli* O157: H7, in particular, have been studied in meat/carcass of ruminants (Hiko *et al.,* 2008; Mersha *et al.,* 2010; Taye *et al.,* 2013; Bekele *et al*., 2014; Dulo *et al*., 2015; Abdissa *et al.,* 2017; Atnafie *et al.,* 2017; Beyi *et al.,* 2017), bovine milk (Bihon *et al.,* 2018), in yogurt and cheese/cottage cheese (Tsegaye and Ashenafi, 2005), fruit juice (Mutaku *et al.,* 2005), in feces and skin (Mersha *et al.,* 2010), urine of pregnant women (Gessesse *et al.,* 2017). But, to the best of the author's knowledge, no published information is available regarding the epidemiology, antibiogram, and potential public health risks of *E. coli* in dogs. Thus, this study was conducted to estimate the prevalence of *E. coli* from rectal swabs of dogs, to assess the potential risk factors of infection and to determine the antibiogram of *E. coli* isolates in Ambo, Gojo and Bako towns of West Shewa Zone, Oromia region, Ethiopia.

Materials and methods

Study area

The study was conducted in three selected towns, namely Ambo, Bako, and Gojo, located in Oromia Region, West Shewa Zone. Ambo town is the administrative center of the Zone of West Shewa located 114 km west of Addis Ababa, the capital of Ethiopia, between latitudes of 8°59′N - 8.983°N and longitudes of 37°51′E - 37.85°E. The elevation of the town ranges from 1900 to 2275 meters above sea level (masl). Its temperature ranges from 19 °C to 29 °C with an average annual temperature of 22 °C and an average annual rainfall of about 900 mm. The town has a total human population of 74, 843 out of which 39,192 are males, and 35,651 are females (CSA, 2005).

Bako town, the administrative center of Bakotibe district, is located 260 Km West of Addis Ababa and lies between 9.1274° N and 37.0561° E. The elevation of the town ranges from 960 to 2450 masl. The mean annual rainfall is 886.5

mm. The monthly mean temperature of the town varies from 14.5°C in December to 21.6°C in June with an average annual temperature of 28°C (BTAC and Agricultural Bureau, 1998). The town has a total human population of 35,769 out of which 16,692 are male and 19,077 are females (CSA, 2005).

Likewise, Gojo town is the administrative center of Jeldu district of West Shoa Zone located 120 Km West of Addis Ababa, between 9.2659° N and 38.0817° E. The elevation of the town ranges from 1800 to 2550 masl. The mean temperature of the town is 20 °C and gains an annual average rainfall of 2500 mm. The human population of Jeldu district is 202,655 of which 102,796 are females and the remaining 99,859 males (CSA, 2008). The three towns have bimodal rainfall characterized by a small rainy season from February to May and a big rainy season from July to September. The dry season extends from October to January (CSA, 2007; CSA, 2015). There was no accessible recorded statistical information on the dog population in the three study towns.

Study populations and study animals

Study populations were dogs including local, exotic and crossbreeds owned by the households in the three selected towns of West Shewa zones. The study animals are dogs in the selected *Gotes* (a sub *Kebele* holding up to 50 households) and *Kebeles* (smallest administrative unit of a district/town) in the three towns.

Study design

A cross-sectional survey was used to study the prevalence, risk factors, and antibiogram of *E. coli* from rectal swab samples of dogs.

Sample size determination

The sample size was determined according to Thrusfield's (2005) formula with an expected prevalence of 50%, 5% desired absolute precision and 95% confidence interval since there is no previous study conducted on *E. coli* of dogs in Ethiopia.

N=1.962 Pexp (1-Pexp)/d2

Where n= required sample size, p=expected prevalence d= desired absolute precision. Therefore, the calculated sample size was 384. For better precision,

438 animals were investigated. There was no data on the dog population in the three towns. The distribution of the dog population in each *Kebeles* of the study towns was assumed uniform. There are three *Kebeles* in Ambo town while Bako and Gojo towns have two and one *Kebeles* respectively. The total sample size was allocated to Ambo (n=238), Bako (n=129), and Gojo (n=71) towns roughly proportional to the human population.

Study methodology

Questionnaire survey
A semi-structured pretested questionnaire was administered to dog owners during sample collection. Questions asked to dog owners include dog management, housing system, feeding, presence of other animals in the household, veterinary care, and educational level of the owners.

Data collection format was also prepared and used to record the sex (female, male), age (juvenile (6 weeks to 6 months), adolescent (7 months to 18 months), adult (19 months to 7 years), geriatric (greater than 7 years) (Kiflu *et al*., 2017)), breed (local, exotic & cross), district (Ambo, Bako, Gojo), housing (indoor, outdoor, mixed), feeding (household leftover, raw animal products, cooked animal products, mixed), of the all sampled dogs.

Sample collection and transportation

From each "*Kebeles*," four "*Gotes*" were randomly selected using the list of *Gotes* in each *Kebeles* (sampling frame) provided by local administrators. The index household in a *Gote* was randomly selected and subsequent households were surveyed door to door. Before the fecal swab samples collection, 70 % ethyl alcohol impregnated cotton was used to clean the perianal area of each dog. Prior to sample collection, restraining of dogs was performed manually using movable metallic crush. The sterile cotton-tipped applicator stick was inserted 2-4 cm into the rectum, slightly rotated to collect fecal material, then gently removed, put into peptone water, and immediately transported to Ambo University Zoonoses and Food Safety Laboratory using an icebox with ice packs. In the laboratory, the samples were processed immediately, otherwise stored at 4^{o}C until examined in 2 days.

Isolation of *Escherichia coli*

The swab samples were incubated overnight in buffered peptone water at 37°C for 24 hours. Then, a loopful of bacteria from peptone water was cultured on MacConkey agar (BM020, Sisco Research Laboratories Pvt. Ltd., India) for 18 to 24 hours at 37°C. Typical lactose fermenting pink color colony was picked and streaked again on Eosin Methylene Blue (EMB) agar (BM020, Sisco Research Laboratories Pvt. Ltd, India). Green metallic sheen isolates were considered to be *E. coli* and the presumptive colonies were transferred on nutrient agar. Then, isolates were biochemically tested using the IMVIC (indole, methyl red, Voges Proskauer, and citrate) test and on triple sugar iron agar (TSI) and motility. The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas. The isolates were also revealed a positive reaction to the MR test and the Indole test but a negative reaction to the VP test. TSI agar medium gave yellow color in both the slant and butt with gas production.

Identification and antimicrobial susceptibility test

Further Identification (ID) and antimicrobial susceptibility test (AST) were performed at the International clinical laboratory (ICL) located in Addis Ababa, Ethiopia. Fifty-seven isolates of *E. coli* identified by biochemical tests were transported to ICL using a cold chain. Then, isolates were sub-cultured on MacConkey agar media and incubated for 24 hours. After all bacterial colonies and materials were collected to the inoculation site, Phoenix ID broth was labeled. Using an aseptic technique, fresh colonies of the same morphology were picked up with the tip of a sterile cotton swab and suspended in the phoenix ID broth (4.5ml) then the tube was capped tightly, vortexed for five seconds and allowed to settle for approximately ten seconds for air bubbles to surface. After this, the tube was inserted into the BD Phoenix Spec™ nephelometer to measure inoculum density which was set to 0.5 McFarland for the panel type being run (range of 0.5-0.6 is acceptable). The density of the organism was adjusted by either adding colonies from the isolate or diluting the broth with fresh Phoenix ID broth and re-measuring the turbidity. Then, the Phoenix AST broth tube (8 ml) was labeled with the sample code and one free-falling drop of AST Indicator solution was added to it and was inverted to mix. Then, 25µL of the bacterial suspension from the ID broth tube was transferred into the AST broth tube using a sterile pipette. Then, the AST tube was capped and inverted several times to be mixed. After waiting for few seconds for air bubbles,

ID tube inoculum was poured into the fill port on the ID side of the panel (Gram-negative tray with *E. coli* ATCC 25922 used as quality control organism) and the fluid was allowed to traverse down the tracks. Then, AST broth inoculums were poured into the fill port on the AST side of the panel (85 well sides) and the fluid was allowed to traverse down the tracks before moving the panel. Then the panel closure was snapped on and the panels were inserted in the transportation caddy. Before insertion of the panel into the Phoenix 100 machine, all panels were logged into the panel log in soft key. After the panel soft key was opened, all information was fed into the software and saved. The panels were inserted into the BD Phoenix 100 machine. The load panel key on the instrument was pressed to hear the audible signal sounds, then, the door unlocked icon appeared and the instrument door was opened and the panel holder where there was no panel in place was selected. Then, the bottom part of the panel was placed in the panel holder and pressed downward; the top of the panel was pivoted back into the panel holder and then allowed to move upward. Lastly, the instrument door was closed.

The antimicrobials for testing drug resistance were selected based on information obtained from local health authorities (personal communication) about the antibiotics that are used to treat human and animal *E. coli* infections in Ethiopia. *E. coli* isolates resistant to three or more classes of antimicrobials were considered to be multidrug-resistant (MDR) (Magiorakos *et al.*, 2012). The Minimal Inhibitory Concentration (MIC) Interpretive Standards were shown in Table 1.

Table 1. Minimal Inhibitory Concentration (MIC) Interpretive Standards for *Enterobacteriaceae*

Antimicrobial agents	Minimum Inhibition Concentration (MIC)		
	Susceptible at < µg/ml	Intermediate	Resistance at ≥ µg/ml
Amikacin	16	32	64
Amoxicillin-Clavulanate	8/4	16/8	32/16
Ampicillin	8	16	32
Ampicillin-Sulbactam	8/4	16/8	32/16
Aztreonam	4	8	16
Cefazolin	2	4	8
Cefepime	2	-	16
Cefoxitin	8	16	32
Ceftazidime	4	8	16
Ceftriaxone	1	2	4
Cefuroxime	8	16	32
Ciprofloxacin	0.06	0.12-0.5	1
Ertapenem	0.5	1	2
Gentamicin	4	8	16
Imipenem	1	2	4
Levofloxacin	2	4	8
Meropenem	1	2	4
Netilmicin	8	16	32
Piperacillin	16	32-64	128
Piperacillin-Tazobactam	16/2	32/4-64/4	128/4
Ticarcillin	16	32-64	128
Ticarcillin clavulanate	16/4	32/2-64/2	128/2
Tigecycline	-	2	8
Trimethoprim-Sulphamethoxazole	2/38	-	4/72

Source: CLSI, 2015

Data management and analysis

All data collected from the questionnaire survey and laboratory analysis were entered into the Microsoft-Excel spreadsheet. Descriptive statistics were used to summarize the data. STATA version 14.0 software (Stata Corp. College Station, USA) was used to analyze the data. The association between the prevalence of *E. coli* infection in dogs and potential risk factors was first analyzed using a Chi-square test followed by univariable and multivariable logistic regression analyses.The association of the explanatory variables was first tested using univariable logistic regression. Non-collinear variables with *P*-

value<0.25 in univariable analyses were entered into a multivariable logistic regression model. The interaction of variables that remained in the full model was carried out. A likelihood ratio test with a *P*-value of less than 0.05 suggested an interaction between the two variables being tested and was retained in the final model. In all the analyses *P*-value of less than 0.05 was considered significant.

Results

Prevalence

A total of 106 of 438 (24.2%, 95% confidence interval [CI]: 20.26-28.49%) dogs tested positive for *E. coli*. The Chi-square test showed that there was a significant difference in prevalence among the study towns ($P<0.05$) (Table 2, 3).

Table 2. Prevalence of *E. coli* in dogs of Ambo, Bako, and Gojo towns of West Shewa Zone, Oromia, Ethiopia

Study area/ towns	No. of samples tested	No. positive	Prevalence (%)	95% CI
Ambo	238	44	18.49	13.77-24.01
Bako	129	40	31.01	23.16-39.75
Gojo	71	22	30.99	20.54-43.08
Total	438	106	24.20	20.26-28.49

Significant association of towns and *E. coli* prevalence in dogs (χ^2=1.38, p-value = 0.01); No. = number, CI = confidence interval.

Risk Factors

Univariable logistic regression analysis revealed a significant association between the *E. coli* prevalence and district towns. The odds of the *E. coli* prevalence from Bako town was 1.98 (95% CI: 1.21-3.26, P =0.007) times higher than for dogs at the Ambo town. Similarly, the odds of the *E. coli* prevalence from the Gojo town was 1.98 (95% CI: 1.09 - 3.61, P=0.026) times higher than that of Ambo town. There was a significant difference in the prevalence of *E. coli* among sampled *Kebeles*. Univariable logistic regression revealed that the odds of *E. coli* infection in *Kebele* 03 of Ambo town was 38.77 (95% CI: 5.11-294.10: $P\leq$ 0.001) times higher than that of Ambo 01 *Kebele*. The univariable analysis also showed that there was no significant association ($P>0.05$) between *E. coli* prevalence and age, sex, breed, presence of other domestic animals in the home, education of the owner, the gender of the owner and visiting veterinary clinics (Table 3).

Table 3. Results of univariable logistic regression analyses of *E. coli* carriage and its association with the putative risk factors in dogs of Ambo, Bako, and Gojo towns

Variables	Categories	No. tested	No. positive (%)	OR (95% CI)	p-value
Districts/ towns	Ambo	238	44 (18.49)	1.00	
	Bako	129	40 (31.01)	1.98 (1.09-3.61)	0.026
	Gojo	71	22 (30.99)	1.98 (1.21-3.26)	0.007
Kebeles	Ambo 01	73	1 (1.37)	1.00	
	Ambo 02	85	15 (17.65)	15.43 (1.98-119.94)	0.009
	Ambo 03	80	28 (35.00)	38.77 (5.11-294.10)	≤0.001
	Bako 01	63	15 (23.81)	22.50 (2.88-175.99)	0.003
	Bako 02	66	25 (37.88)	43,90 (5.74-336.03)	≤0.001
	Gojo	71	22 (30.99)	32.33 (4.22-247.77)	0.001
Sex	Female	103	23 (22.33)	1.00	
	Male	335	83 (24.78)	1.15 (0.68-1.94)	0.612
Age	Adult	269	57 (21.19)	1.00	
	Juvenile	49	15 (30.61)	1.64 (0.84-3.22)	0.15
	Adolescent	76	21 (27.63)	1.42 (0.79-2.54)	0.237
	Geriatric	44	13 (29.55)	1.56 (0.77-3.17)	0.220
Breed	Exotic & cross	77	16 (20.78)	1.00	
	Local	361	90 (24.93)	1.27 (0.69- 2.31)	0.44
Feeding	HH leftover	82	18 (21.95)	1.00	
	Raw AP	96	25 (26.04)	1.25 (0.63 -2.51)	0.525
	Mixed	203	50 (24.63)	1.16 (0.63- 2.14)	0.631
	Cooked AP	57	13 (22.81)	1.05 (0.47- 2.36)	0.905
Housing	Indoor	93	21 (22.58)	1.00	
	Outdoor	159	38 (23.90)	1.08 (0.59- 1.98)	0.811
	Mixed	186	47 (25.27)	1.16 (0.64- 2.09)	0.622
Visiting Vet. clinic	No	385	95 (24.68)	1.00	
	Yes	53	11 (20.75)	0.80 (0.40-1.62)	0.533
Presence of other DA	Yes	184	41 (22.28)	1.00	
	No	254	65 (25.59)	1.20 (0.77- 1.88)	0.425
Educ. of the owner	Tertiary	145	31 (21.38)	1.00	
	Illiterate	78	23 (29.49)	1.54 (0.82- 2.88)	0.179
	Primary	105	27 (25.71)	1.27 (0.71- 2.30)	0.423
	Secondary	110	25 (22.73)	1.08 (.60-1.96)	0.797
Gender of the owner	Female	129	25 (19.38)	1.00	
	Male	309	81 (26.21)	1.48 (0.89-2.45)	0.129

CI= Confidence Interval OR= Odds Ratio, Educ. = Education, Vet. = Veterinary, AP=animal products, HH=household, DA=domestic animals

All risk factors are non-collinear (r<0.6) except *Gote* and *Kebele* which are collinear with each other (r=0.756). The variable "*Kebele*" was selected for further multivariable logistic regression analysis (Table 4).

Table 4. Multivariable logistic regression of *E. coli* carriage and potential to risk factors

Variables	Categories	OR (95% CI)	*P*-value
Districts/Towns	Ambo	1.00	
	Bako	31.09 (4.04-239.18)	0.001
	Gojo	43.45 (5.64-334.70)	≤0.001
Kebeles	Ambo 01	1.00	
	Ambo 02	15.16 (1.95-118.08)	0.009
	Bako 01	0.51 (0.24-1.10)	0.087
	Gojo	-	-
	Ambo 03	38.08 (5.01-289.68)	≤0.001
Age	Adult	1.00	
	Adolescent	1.54(0.83-2.85)	0.173
	Geriatric	1.11(0.53-2.31)	0.781
	Juvenile	1.51(0.74-3.07)	0.256
Gender of the owner	Female	1.00	
	Male	1.41(0.83-2.40)	0.205

Antimicrobial susceptibility

Due to a financial limitation, out of 106 *E. coli* isolates obtained from this study, 43 isolates were selected using a simple random sampling technique and subjected to antimicrobial susceptibility test using the phoenix machine. Overall, 76.7% (33/43), 6.98% (3/43), 2.33% (1/43), and 14.0% (6/43) of the isolates were pan-susceptible, resistant to one class of antimicrobial, two class of antimicrobial and ≥ 3 classes of antimicrobials (multidrug-resistant (MDR)), respectively. The tested isolates were 100% susceptible to 12 antimicrobials; viz. amikacin, netilmicin, ampicillin, piperacillin-tazobactam, piperacillin, cefoxitin, ciprofloxacin, levofloxacin, colistin, ertapenem, meropenem, and imipenem. The most common resistance phenotypes were recorded for cefazolin (22.73%), aztreonam (13.95%), ceftriaxone (13.95%), gentamicin (11.90%), and trimethoprim-sulfamethoxazole (6.97%) (Table 5).

Table 5. Results of antimicrobial susceptibility testing of 43 *E. coli* isolates from dogs of Ambo, Bako, and Gojo towns of West Shewa Zone, Oromia, Ethiopia.

Antimicrobial Class	Name of Antimicrobials	MIC or Conc.	No. Res. (%)	No. Int. (%)	No. Sus. (%)
Aminoglycosides	Amikacin	<=4	0 (0.0)	0(0.0)	43 (100)
	Gentamicin	<=1	5 (11.90)	0(0.0)	37 (88.10)
	Netilmicin (n=19)	<=1	0 (0.0)	0 (0.0)	19 (100)
Beta-lactam	Amoxicillin-Clavulanate(f) (n=20)	4/2	1(5.0)	2(10.0)	17(80.0)
	Ampicillin (n=19)	<=2	0 (0.0)	0 (0.0)	19 (100)
	Ampicil-Sulbactam	<=4/2	1(2.44)	6(14.63)	34(82.93)
	Aztreonam	<=1	6(13.95)	0(0.0)	37(86.04)
	Piperacillin-Tazobactam	<=4/4	0 (0.0)	0 (0.0)	43 (100)
	Ticarcillin-(n=17)	32/2	0(0.0)	5(29.41)	12(70.59)
	Piperacillin (n=18)	<=4	0(0.0)	0(0.0)	18 (100)
Cephalosporins	Cefazolin (n=22)	<=2	5(22.73)	0(0.0)	17(77.27)
	Cefepime	<=1	0(0.0)	6(13.95)	37(86.04)
	Cefoperazole-sulbactam(n=9)	<=0.5/8	0(0.0)	6(66.67)	3(33.3)
	Cefoxitin(n=22)	<=4	0(0.0)	0(0.0)	22 (100)
	Ceftrazidime	<=0.5	0(0.0)	2(4.65)	41(95.34)
	Ceftriaxone	<=0.5	6(13.95)	0(0.0)	37(86.04)
	Cefuroxime(n=21)	<=2	1(4.76)	0(0.0)	20 (95.24)
Quinolone	Ciprofloxacin(n=42)	<=0.125	0(0.0)	0(0.0)	42(100.0)
	Levofloxacin(n=34)	<=1	0(0.0)	0(0.0)	34(100.0)
Polymyxin	Colistin (n=9)	<=1	0(0.0)	0(0.0)	9 (100)
Carbapenem	Ertapenem	<=0.25	0(0.0)	0(0.0)	43 (100)
	Meropenem	<=0.125	0(0.0)	0(0.0)	43 (100)
Carbapenams	Imipenem	<=0.25	0(0.0)	0(0.0)	43 (100)
Glycylcyclines	Tigecycline	<=0.5	0(0.0)	2(4.65)	41(95.34)
FPI	Trimethoprim-Sulamethoxazole	<=1/19	3(6.97)	0(0.0)	40(93.02)

FPI=folate pathway inhibitor

Among the antimicrobials used, multidrug-resistant (resistant to three or more antibiotics) was observed against amoxicillin-clavulanate (f), ampicillin-sulbactam, aztreonam, cefazolin, cefepime, ceftriaxone, gentamicin, ticarcillin-clavulanate, ticarcillin-clavulanate, tigecycline, and trimethoprim-sulfamethoxazole. Multidrug resistance (MDR) was observed in 6 *E. coli* isolates (14.0%, 6/43). The maximum number of antimicrobials class to which an isolate demonstrated resistance was 5 (Table 6).

Table 6: Drug-resistant pattern of *E. coli* isolates from dogs in Ambo, Bako and Gojo towns of West Shewa Zone

Antimicrobials	Number of isolates (%)
AMC, AM	1 (2.33)
CLN, TIG	1 (2.33)
ATM, CRO, GM	3 (6.98)
ATM, CZ, CRO, GM	1 (2.33)
ATM, CXM, CRO, SXT	1 (2.33)
ATM, CZ, CRO, GM, SXT	1 (2.33)
Total	**8 (18.6%)**

AMC: Amoxicillin-Clavulanate AM: Ampicillin CLN: Colistin TIG: Tigecycline CZ: Cefazolin ATM: Aztreonam CRO: Ceftriaxone CMX: Cefuroxime GM: Gentamicin SXT: Trimetho-Sulphamethoxazole.

Antimicrobial resistance did not show a statistically significant association with towns (P>0.05). But, antimicrobial resistance in Ambo was higher 23.81% (5/21) when compared with Bako (21.43%) and Gojo (12.5%) towns (Fig.1).

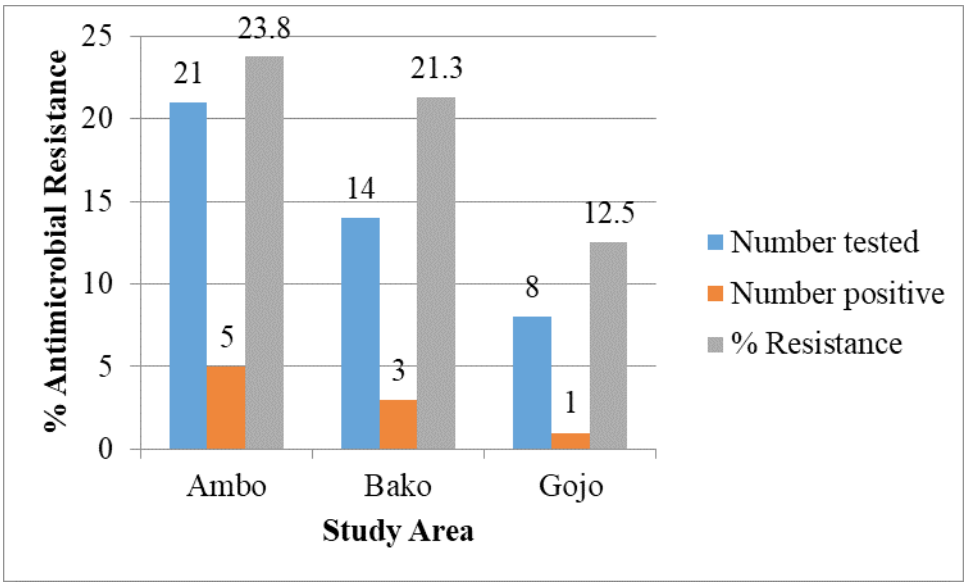

Fig 1. Distribution of antimicrobial resistant *E. coli* in the study towns

Discussion

Although most dogs carry commensal bacteria in their gut, *E. coli* is one of the most common opportunistic pathogens causing enteritis or diarrhea in addition to extra-intestinal infections in animals and human (Beutin, 1999). Besides this, dogs harboring *E. coli* could serve as a reservoir of antimicrobial resistance determinants (Johnson and Russo, 2002). Antimicrobial resistance is a well-known global challenge in the management of bacterial infections. In the current study, a 24.2% *E. coli* isolation rate was obtained from rectal swab samples of apparently healthy dogs. To the best of the authors' knowledge, this is the first study on prevalence, risk factors, and antibiogram of *E. coli* isolates from dogs in Ethiopia. This finding was comparable with the 21% isolation rate of hemolytic *E. coli* from the feces of healthy bitches in Australia (Chen *et al*., 2003). The present finding was higher than the finding of Beutin *et al.,* (1993) who reported a 4.8% isolation rate from healthy dogs but lower than the reports of previous studies (Hammermueller *et al.,* 1995; Beutin, 1999; Salvadori *et al*., 2003) who detected approximately 59-61% isolation of *E. coli* from dogs with diarrhea. The present result is also lower than 37.14% *E. coli* isolation from dogs in Alexandria (Younis *et al*., 2015). The difference in the prevalence of *E. coli* among studies might be due to the difference in the season of the study (Rowland, 1986), age, immune status, stage of infection, number of samples analyzed (Shaheen *et al*., 2011; Yunis *et al*., 2015), sampling techniques, and health status of dogs sampled (sick/hospitalized dogs vs healthy). The other reason for the difference in prevalence among studies might be due to the volume of fecal samples collected (rectal swab vs. large volume of feces). The use of rectal swabs in the present study might have underestimated the prevalence of *E. coli* infection in dogs due to the small volume of feces used for analysis. The health status of the sampled dogs could be also another source of variation among the reports in that a higher prevalence is more likely from diarrheic dogs than apparently healthy dogs. Although cases of diarrheic dogs were not registered and quantified during the current study, it is known that *E. coli* infected dogs with diarrhea defecate frequently and uncontrollably, contaminate the environment, and spread the bacteria more than non-diarrheic dogs (Hammermueler *et al*., 1995; Torkan *et al*., 2016).

The significantly high prevalence of *E. coli* in dogs of Bako and Gojo towns might be related to the relatively lower hygiene of dogs, and inadequate sanitation of dogs' feeds, and dog environment. Although indoor dogs are expected to be at lower risk of *E. coli* infection than outdoor dogs, in our study no signifi-

cant difference was observed. Perhaps, due to the raw animal product feeding as well as the poor hygienic status of homemade diets of indoor dogs similar to that of outdoor dogs. Outdoor dogs feed on contaminated feeds such as leftover feed, dead poultry, and other animals, which might serve as vehicles for *E. coli* transmission to dogs.

In the present study, several potential risk factors for the carriage of *E. coli* in the household of dogs in Ambo, Gojo, and Bako towns of West Shewa Zone were investigated. Among these risk factors, multivariable logistic regression analysis revealed that study sites (towns) and *Kebeles* were important predictors of *E. coli* infection in dogs. The reason behind the high prevalence in Gojo and Bako towns as compared to Ambo town is not well known. However, it might be due to the poor sanitary practices in the households and its surroundings, and inadequate sanitary facilities for people. Because animal feces are plentiful source of *E.coli* infection (Bach *et al*., 2002), high in dog (Gebremedhin *et al*., unpublished) and other livestock populations in the towns of Bako and Gojo compared to Ambo may have contributed to the high *E. coli* prevalence. Michel *et al*. (1999) also pointed out that living in an agricultural area where cattle are raised could be a significant risk factor for the acquisition of *E. coli*. Moreover, the cool and humid climatic condition of Gojo town is suitable for prolonged survival and infectivity of the bacteria. The significant difference in the prevalence of *E. coli* infection between *Kebeles* of the study area might be because of the variation in the number of butcher shops, restaurants and cafeterias providing *E. coli* contaminated leftover foods to dogs coupled with variation in sanitary practices including the location of the slaughterhouse that might directly or indirectly influence across *Kebeles*.

Antimicrobial resistance in bacteria is a phenomenon that has been in constant evolution since the introduction of antimicrobial drugs. Several factors are known to promote bacterial resistance including failure of a treatment regimen, prophylactic use of antimicrobials, and the use of antimicrobials as growth promoters as well as using antimicrobials commonly used in humans' practice (DACA, 2009). Antimicrobial resistance has been suggested as one important therapeutic problem in veterinary and human medicine (DACA, 2009). In this study, antimicrobial resistance of *E. coli* isolated from apparently healthy dogs was investigated against 25 antimicrobial drugs using the MIC technique. Overall, moderately low antimicrobial resistance was found (20.93%). The antimicrobial susceptibility of *E. coli* isolates to multiple antimicrobials was also detected. The *E. coli* isolates were pan-susceptible to er-

tapenem, imipenem, piperacillin-tazobactam, amikacin, netilmicin, ampicillin, cefoxitin, ciprofloxacin, levofloxacin, colistin, and meropenem. This might be due to the less availability and utilization of these drugs in the country and study area. Susceptibility of *E. coli* isolates to ciprofloxacin, ceftazidime, aztreonam, cefepime, ceftriaxone and gentamicin could also be attributed to their inadequate utilization in canine clinical practice in Ethiopia. The lower level of antimicrobial resistance observed against aztreonam, ceftriaxone, (13.95% each), cefazolin (22.73%), gentamicin (11.90% each) and trimethoprim-sulphamethoxazole (6.97%) in the present study is in line with the 79% antimicrobial susceptible *E. coli* isolates reported previously (Windahl *et al*., 2014). Unlike the present study, increased detection of pathogenic and non-pathogenic *E. coli* that are resistant to antimicrobial drugs have been previously reported (Hammermueler *et al*., 1995; Ewers *et al*., 2012).

The present findings are also in line with the study conducted by Wedley (2017) who reported the pan-susceptibility to piperacillin-tazobactam and high susceptibility to aztreonam. Dogs are generally the close companions of their human caretakers thereby providing opportunities for the exchange of antimicrobial-resistant bacteria. The MDR in the current study (14.0%) is not greatly different from previous reports such as 15.3% (Wedley, 2017) and 15% (Wedley *et al*., 2011) MDR in healthy dogs in the UK. Relatively high prevalence of MDR *E. coli* in sick dogs (29%) have been reported in the U.S.A (Shaheem *et al*., 2010). A lower level of MDR *E. coli* (11%) has also been reported in healthy dogs of the U.S.A (Davis *et al*., 2011). This variation in the level of antimicrobial resistance could probably be attributed to the expression of resistant gene coded by the pathogen, which is associated with the emerging and re-emerging aspects of the isolates in different agro-ecology (Reubaen and Owuna, 2013). Differences in the prevalence of resistance observed might also be due to differences in the interpretation of the zone sizes, MICs observed, or differences in how intermediate measurements were classified (Wedley *et al*., 2017).

Low level or absence of individual antimicrobial resistance in *E. coli* isolates in the present study might be an indication of the low level or absence of antimicrobial drug usage in dogs in the study areas for prophylaxis or treatment purpose. This observed a lower level of resistance to some antimicrobial drugs might also be due to acquiring either from contaminated feed and improperly managed leftover household human foods containing AMR *E coli*, which were normally given to dogs. Feeding of dogs with animal products containing high levels of antimicrobial residues may contribute to the emergence of antimicro-

bial resistance in bacteria recovered from dogs (Prescott *et al*., 2002). Thus, it is fundamental that veterinarians' guide dog owners towards the use of treatment regimens for sick dogs and the establishment of long-term preventive programs against infectious diseases such as diseases caused by *E. coli*.

The emergence of antimicrobial resistance or MDR (14.0%) *E. coli* in household dogs, though at a lower level, is of public health and economic importance in that it indicates the potential of the spread of AMR *E. coli* from dogs to humans and other animals. Therefore, attention should be given to the management of dogs, and the provision of safe feed and water, which might contain antimicrobial residues contributing to the source of the resistance. According to the reports of DACA (2009), antimicrobial resistance has been incriminated with higher mortality and morbidity, increased costs of treatment, and loss of productivity.

The limitations of this study is that data on dog hygiene and environmental hygiene was not captured during questionnaire survey. Besides, molecular studies aimed at determining virulence genes and pathotypes of *E. coli* was not done to determine the pathogenicity of *E coli* isolates in the current study due to the limitation of resources and facilities. Nevertheless, isolation of *E. coli* is of great public health concern because there is close contact between dogs and humans and poor awareness and hygiene are common, as some isolates might be of zoonotic risk for humans coupled with the potential transfer of MDR genes. Although we couldn't prove the pathogenicity of the *E. coli* isolates, it could be hypothesized that dogs in the current study aeas were exposed to potentially pathogenic *E. coli* from the contaminated environment such as drinking contaminated water and feeding on dead animals, animal products and garbage.

Conclusions

The overall prevalence (24.2%) of *E. coli* infection dogs in the study area is moderately high. Study towns and study *Kebeles* are the important predictors of *E. coli* infection in the study area. Most of the *E. coli* isolates from dogs are susceptible to many of the drugs used in both human and veterinary medicine. However, some of the isolates have developed multidrug resistance and might be a potential source of the spread of antimicrobial-resistant *E. coli* from dogs to humans and other animals. Further, large-scale epidemiological studies including the contribution of dogs in the transmission to humans, serotyping

and virulence gene detection of *E. coli*, frequent monitoring of antimicrobial drug resistance and responsible dog ownership, and improved hygienic management of dogs are recommended.

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Bovine brucellosis: Seroepidemiology and herder's knowledge, attitude and practices in Bench Maji zone, southern Ethiopia

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Abstract

Brucellosis is a major public and animal health problem in many parts of the world, particularly in pastoral settings where livestock is a major livelihood and food sources. Effective prevention and control of brucellosis depends on knowledge, attitude and practices of the community. This cross-sectional study was conducted between November 2018 and April 2019 in Bench Maji zone, with the objectives of investigating the prevalence and associated risk factors of bovine brucellosis, and assessing the knowledge and practices of herders. A questionnaire survey (n=300) and collection of blood samples (n=772) were carried out. The sera samples were screened using Rose Bengal Plate Test (RBPT) and positive ones were further confirmed by using Complement Fixation Test (CFT). Results showed that 25 (3.24%) and 15 (1.94%) of the 772 animals were positive for RBPT and CFT. This shows an overall sero-prevalence of 1.94%, (95% CI: 0.97- 0.2.92%) bovine brucellosis in the study area. The highest prevalence, 2.74%, was recorded at Menitshasha district while no positive case was recorded at Menitgoldiya districts. Large herd size (OR=4.7) and migration (OR=3.52) showed association with seropositivity. Cows with abortion history had higher likelihood of seropositive than other groups. Majority of the pastoralists (72.7%) did not have information and basic knowledge about brucellosis, whereas about 27.3% of them heard about it and very small proportion (3.3%) knew its zoonotic importance. All of the respondents were practicing high risk behavior of acquiring the infection. Demographic factors such as education, family size and age of herders were found to have effects on knowledge of herders. Low level of knowledge, high-risk practices and willingness of herders to know more about brucellosis call for improving public awareness on zoonotic significance of the diseases through integrating animal health and public health extension services.

Keywords: Attitude, Bovine; Brucellosis; Ethiopia, Knowledge; Practices; Prevalence; Risk factors

Introduction

Brucellosis is perhaps one of the most widespread and economically important diseases in tropical and subtropical countries such as Ethiopia. The disease can cause substantial economic losses at household and national levels. The direct loss of meat (because of abortion, infertility and weight loss) in infected herds of cattle was estimated to be 15% and for milk (reduction in milk production) 20% per infected cow (Mangen *et al*., 2002; Nicoletti, 2010).

Brucellosis is a sub-acute or chronic disease which may affect wild and domestic animals such as cattle, sheep, goats, camels, equines and pigs with infection localizing in the reproductive system and causing abortion in the pregnant animals (Radostits *et al*., 2007). Clinically, infection is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis. Abortions are more prevalent and numbers of organisms shed are much greater in unvaccinated animals (OIE, 2018).

The potential economic and public health impacts of brucellosis are overwhelmingly masked by many more priority diseases in developing countries with limited resources, including Ethiopia. Thus, the disease has not yet get full attention and preventions programs featuring any aspects of brucellosis interventions were not instituted (FAO, 2011). The epidemiology and economic impacts and cost of effective prevention measures of the disease in livestock and humans are not well studied in extensive production system. Brucellosis is known to cause abortion in livestock with the subsequent excretion of a large number of organisms which are easily acquired by other animals. The disease remains endemic and continues to pose public and animal health risks in sub-Saharan Africa of the world (Mangen *et al*., 2002).

Brucella infection is readily transmissible to humans, causing acute febrile illness – undulant fever – which may progress to a more chronic form involving musculo-skeletal, cardiovascular, and central nervous systems complication (OIE, 2018). In particular, brucellosis constitutes significant public health importance for a pastoral community where there is prevailing close contact with animals, raw milk and whole blood consumption practices and low awareness on the disease thereby leading to zoonotic transmission of the disease. Milk is

a major staple food, consumed raw by almost all the pastoral community and be a source of infection with milk-borne zoonosis such as brucellosis.

In Ethiopia, although information on how and when brucellosis was introduced to the country is not established, the disease remains endemic. Several serological surveys have showed bovine brucellosis is an endemic and widespread disease in urban, per-urban, highland and lowland, extensive and intensive farming, smallholder farms and ranches of the country (Dinka and Chala, 2009, Jergefa *et al.,* 2009; Mekonnen *et al*., 2011; Degefu *et al.,* 2011; Asmare *et al.,* 2013). Most of studies so far conducted on cattle brucellosis have been concentrated in central and northern Ethiopia, and do not provide an adequate epidemiological picture of the disease in different agro-ecological zones and livestock production systems of the country (Megersa *et al*., 2011).

Pastoralists or agro-pastoral communities have greater vulnerability to brucellosis because of close contact with animals during husbandry practices and consumption of animal products in addition to their marginalization from public services and information. On the other hand, these communities have significant contribution to national gross domestic products (GDPs) by making marginal lands more productive (Zinsstag *et al*., 2006). In view of that, understanding the seroepidemiology of bovine brucellosis in the pastoral and agro pastoral system of Bench Maji zone, and the extent of herder's knowledge and practices related to brucellosis would help in developing disease control strategies. Therefore, this study was aimed at estimating the seroprevalence of bovine brucellosis and associated factors and assessing brucellosis related knowledge, attitude and practices of livestock keeper in the study area.

Materials and methods

Description of study Area

This study was carried out between November 2018 and April 2019 in three selected districts of Bench Maji zone of SNNPRs. The capital town of the zone is located about 561 km from Addis Ababa in south western part of the country. The zone is found within latitude and longitude ranges of 34°45' to 36°10' east and 5°40' to 7°40' north. It is bordered with South Sudan Republic in southwest. Based on altitude range, the study areas were broadly classified into the different agro-climatic classifications: 52% lowland "Kola" (<1500 m.a.s.l.); 43% midland "Weynadega" (1500 to 2300 m.a.s.l.) and 5% highland "Dega" (> 2300

m.a.s.l.). The annual average temperature ranges from 15.1°C to 27.5°C, while the annual rainfall ranges from 400 to 2,000 mm (Zone Agricultural Office, 2012). The study districts Maji and Menitgoldiya practice agro-pastoral production whereas Menitshasha practices more of pastoral production system.

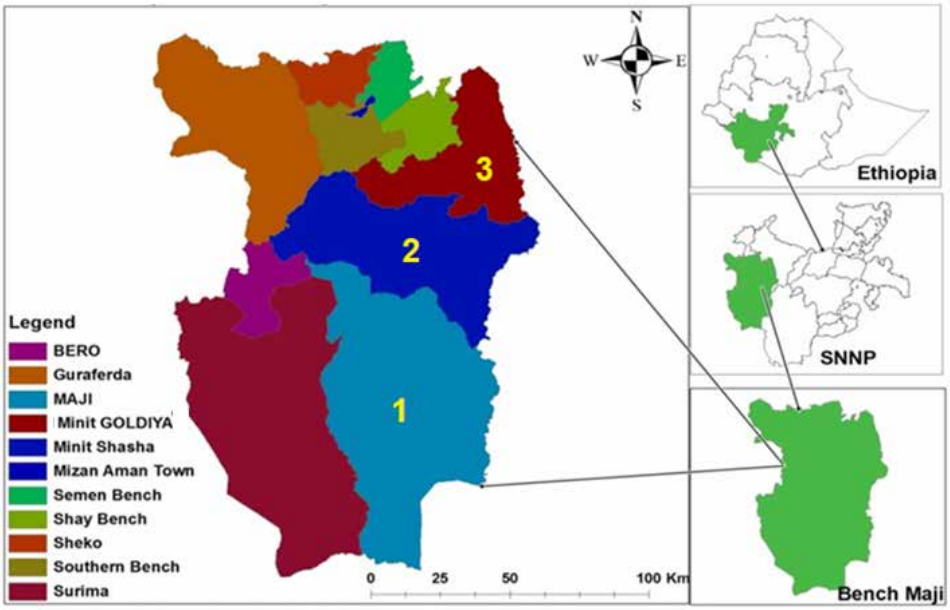

Fig.1: Map of Ethiopia showing the study area (labeled Maji=1, Menitshasha=2, Menitgoldiya=3)

Study population and sampling methods

The target populations for animals were Cattle in Bench Maji zone which are kept under different production system mainly pastoral or agropastoral systems. The study units were comprised of indigenous cattle in three selected districts in Bench Maji zone, which are kept under extensive production system. Both sexes and age groups of more than six months of age were selected for the study. Age were estimated by examining their lower incisor teeth according to Pace and Wakeman (2003) and also by asking the owners, then and categorized as young (< 4 years) and adults (≥ 4 years). Since the study included questionnaire survey, the ethnicity herders in the zone are predominantly *Bench, Menit, Dizi, Sheko, Surma,* and *Zelmamo.*

Animals were selected by multistage sampling methods. Three districts were purposively selected by considering agro-ecology and production system from which kebeles and animals were subsequently selected by random sampling method. The selected districts Menitshasha, Menitgoldia and Maji have 31, 24 and 20 kebeles, from which 4, 3 and 3 kebeles were selected, respectively. Starting from a presumed center of a kebele, we did a transect walk (which is a systematic walk along a specified route (we used the four cardinal directions) across the kebele together with an animal health worker. Then during transect traveling to different parts of the kebeles, encountered households were contacted and their animals were sampled randomly. A lottery system was used to select an individual animal from a herd, by assigning a number 1 (to be selected) or 0 (not to be selected) to an animal. Then those animals that had the chance for a number one were selected for bleeding until the required sample size for each kebele is fulfilled, so that nearly 50% of animals were selected from a herd.

Sample size determination

The sample size for each district was calculated by the formula recommended by Thrusfield and Christley, (2018) by assuming an expected prevalence of 20% and desired precision of 5% with 95% confidence interval which gave 246 animals. Since we used multistage sampling which requires a relatively large sample size, we increased the number of animals by three folds, making a total of 738 cattle. About 5% contingency was added to compensate for any sample losses, mislabeling and discarding. The total sample size of this study was further stratified by each districts taking livestock population proportion of respective district. Finally, a total of 772 serum samples were collected and processed in the laboratory. Subsequently, nearly all animal owners (N=300 respondents) whose cattle sampled were included in the questionnaire survey.

Serum sampling, testing, and questionnaire survey

During blood sample collection factors like age, sex, altitude, parity, abortion/ stillbirth, herd size, distance from water point, and frequency of migration and production system were collected. About 10 ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tubes under aseptic condition. After 24 hours sera were separated and transferred to cryovial tubes, which were labeled and stored in deep freezer until tested. All serum samples were screened using Rose Bengal Plate Test (RBPT) at Mizan Re-

gional Veterinary Diagnostic Laboratory and positive samples were tested at National Veterinary Institute (NVI) using Complement Fixation Test (CFT).
A questionnaire was designed to assess the knowledge, attitude and practice of animal owners (n= 300 participants) on bovine brucellosis. The questionnaire focused on herder's knowledge, attitude and practices related to brucellosis was administered to herders. Firstly, herders were asked 11 questions (mainly yes or no, and sometimes multiple options) related to knowledge of herders on brucellosis, its mode of transmission, zoonotic importance, and prevention means. For example, "*have you ever heard about brucellosis*?" Secondly, they were asked 12 questions related to their attitude or belief such as "*do you think boiling milk prevent brucellosis*?". Then 17 questions related to practices of herders were asked, of which 11 questions were related to exposure (risky) practices i.e "D*o assist delivery with bare hand*?" The remaining 6 questions were related to preventive measures such as "*do you cook meat for consumption*?" Answering, a question properly guaranties the respondent with score of 1 or otherwise 0. For instance, a respondent may obtain score ranging from 0 to 11 for knowledge questions, and score of 0 to 12 for attitude questions. Finally, after completing the questionnaire checking, correction and clarification were mad before leaving the village.

Data management and analysis

The data were entered into Microsoft Excel spread sheet 2010 program and statistical analysis was performed using STATA version 14 (Stata Corp, College Station, Texas). In addition to descriptive analysis, association of *Brucella* sero-positivity with risk factors was assessed using logistic regressions. Variables with p-value <0.2 in univariable analysis were included in multivariate logistic regression. Collinearity among the independent variables were checked using gamma statistics (a measure of rank correlation), and those with gamma coefficient within -0.6 and +0.6 were considered in multivariable logistic regression analysis. We used the Kruskal-Wallis H test (a rank-based nonparametric test) to analyze the effects of socio-demographic factors on knowledge, attitude and practice of respondents' towards bovine brucellosis.

Results

Socio-demography of the Respondents

Out of 300 participants, 82 (27.3%) of them were from Maji, 136 (45.3%) from Menitshasha and 82 (27.3%) were from Menitgoldiya districts. Participants belong to two ethnic groups: 218 (72.7%) of them were Menit while 82 (27.3%) were Dizi. Majority of the participants were agro-pastoralist, 161(53%) were non-educated, while 43(14.3%) of them had attained primary education. About 45% of the participants were between ages 15 to 35 and the rest were above 35 years while 53% were males and 47% were females (Table 1).

Table 1. The socio-demography of study participants

Variable	Category	Frequency	Percent (%)
District	Maji	82	27.3
	Menitshasha	136	45.3
	Menitgoldiya	82	27.3
Agro-ecology	Midland	164	54.7
	Lowland	136	45.3
Sex	Male	159	53.0
	Female	141	47.0
Age	18-35	135	45.0
	35-55	88	29.33
	Above 55	77	25.67
Education	None educated	161	53.67
	Read and write	96	32.0
	Primary school	43	14.33
Ethnic group	Dizi	82	27.33
	Menit	218	72.67
Family size	3–5	137	45.67
	6–9	106	35.33
	> 9 people	57	19.0
Farming system	Sedentary	88	29.33
	Agro-pastoral	143	47.67
	Pastoral	69	23.0

Seroprevalence of bovine brucellosis and associated risk factors

The serological test results show that 25 (3.24%) of the tested animals were found positive by RBPT, which were further subjected to CFT testing. The overall individual animal level seroprevalence based on the confirmatory CFT test was 1.94% (95%CI: 1.0- 2.9). The highest prevalence of 2.74% was recorded at Menitshasha, whereas no reactor was found at Menitgoldiya district.

Table 2 presents prevalence of animal level *Brucella* seropositivity and its association with exposure variables. Animals that kept in large herds were 4.7 times at risk of being seopositive than those from small herd size. Other factors such as altitude, age, sex, distance to water and migration of herds showed marginal association. Seroprevalence was about more than three folds in lowlands compared to highland, and adult animals were two times more likely to be seropositive than young ones. Migrating herds and those with shorter distance to water point had three times more likelihood of being seropositive than their counter parts. Females with history of abortion had higher likelihood of being seropositive than their counterparts.

Table 2. Univariable analysis of potential risk factors associated with *Brucella* seropositivity

Variable Category	No. of cattle tested	No. of cattle positive	Prevalence (%)	Odd Ratio (95% CI)	*p*-value
District*					
Maji	130	3	2.31	-	-
Menitshasha	438	12	2.74	1.19(0.33-4.29)	0.788
Menitgoldiya	204	0	0	-	-
Altitude					
Lowland	438	12	2.74	-	-
Midland	334	3	0.89	0.32(0.09-1.14)	0.081
Age					
< 4years	544	8	1.47	-	-
≥ 4years	228	7	3.07	2.12(0.76-5.92)	0.151
Sex					
Male	280	3	1.07	0.43(0.12-1.55)	0.198
Female	492	12	2.44	-	-
Abortion					
Yes	6	4	66.67	119.75(19.1-750.6)	0.000
No	487	8	1.64	-	-
Herd size					
< 35 cattle	412	3	0.73	-	-
≥ 35 cattle	360	12	3.33	4.70(1.32-16.79)	0.017
Distance to water					
Far	330	3	0.91	-	-
Near	442	12	2.71	3.04(0.85-10.87)	0.087
Migration					
Yes	438	12	2.74	3.10(0.87-11.10)	0.081
No	334	3	0.89	-	-

*Menitgoldiya was excluded from district comparison

Variables such as age, sex, herd size, migration were retained in the final model, while altitude was excluded due to its collinearity with migration. Abortion was also excluded from the multivariable model as it only occurs in breeding females. The multivariable logistic regression model (Table 3) showed that animals kept in large herd sizes were more likely to be exposed to *Brucella* infections (OR = 4.73, 95% CI = 1.32- 16.98) than those maintained in small

herds. Animals in migrating herds also showed marginal significance with three times more likelihood of seropositivity compared to other group.

Table 3. Multivariable logistic regression for selected variables

Variable category	OR	SE	CI (95%)	p-value
Age: (> 4 years)	2.13	1.17	0.73 –6.24	0.167
Sex: (male)	0.49	0.33	0.13 –1.85	0.295
Herd size: (large)	4.73	3.08	1.32 –16.98	0.017*
Migration: (yes)	3.52	2.31	0.97 –12.74	0.055

Respondents' Knowledge, attitude and practices related to bovine brucellosis

Out of 300 participants who were asked about the level of their knowledge of bovine brucellosis, large majority (72.67%) of the herders do not have any information since they did not hear about brucellosis. About 17.3% of the participants responded to one to four knowledge questions, and 14.0% of them answered more than five questions correctly (Table 4). Accordingly, 82 (27.33%) had heard of brucellosis and they mentioned veterinarians 16 (5.33%) and community 66 (22 %) as their information source. For the question stated that, "is brucellosis considered as animal health problem in the area. Only few proportions (4.3%) of the herders responded yes.

Table 4. Respondents' knowledge on different aspects of Bovine Brucellosis (n= 300)

Knowledge of brucellosis	Proportion of affirmative or correct responses (%)	Std. Err.	95% CL	
Have you ever heard about brucellosis?	27.3	2.58	22.3	32.4
Is brucellosis an animal health problem in this area?	4.3	1.18	2.0	6.7
Which livestock species are affected by brucellosis?	27.3	2.58	22.3	32.4
Clinical signs of brucellosis in cattle	20.7	2.34	16.1	25.3
Does it transmit from animal to animal?	16.7	2.16	12.4	20.9
Mode of transmission to animal	16.7	2.16	12.4	20.9
Does it cause illness in human?	3.3	1.04	1.3	5.4
Does it transmit from animal to human?	3.3	1.04	1.3	5.4
Mode of transmission to human	3.3	1.04	1.3	5.4
Clinical signs in human	3.3	1.04	1.3	5.4
Which diseases have similar signs?	3.3	1.04	1.3	5.4

Those who had information about brucellosis further responded to multiple option questions such as which animal species are affected by brucellosis. They responded that cattle 75 (25%), sheep 12 (4%), goats32 (10.67%), equine 15 (5%) and dogs 9(3%). The symptoms reported include retained placenta as most common sign (22.67%), abortion (1.6%) and stillbirth. The reported symptoms in human were sweating and joint pains. The diseases mentioned with similar signs were malaria (13.33%), typhoid (7 %) and common cold (3.67%).

Table 5 presents a Kruskal-Wallis H test result that was conducted to determine if participants' knowledge varied with socio-demographic variables such as district, age, sex, education, family size, ethnicity, farming system and agro ecology. The test showed that there was a statistically significant difference in knowledge between age, education and family size groups, with younger in-

dividuals, people with elementary education and those with small family size having better knowledge than others.

Table 5. Kruskal-Wallis H test of factors affecting knowledge

Variable	Category	No of respondents	Average knowledge score		p –value
District				2.96(2)	0.2274
	Maji	82	2.12		
	Menitshasha	136	1.75		
	Menitgoldiya	82	2.05		
Sex				2.06(1)	0.1512
	Male	159	1.74		
	Female	141	2.08		
Age				45.42(2)	0.0001
	18-35 years	135 |	2.95		
	35-55years	88	1.46		
	Above 55 years	77	0.72		
Education				124.06(2)	0.0001
	Non educated	161	0.36		
	Read and Write	96	2.38		
	Primary school	43	4.49		
Family size	3-5	137	2.98	40.89(2)	0.0001
	6-9	106	1.43		
	above 9	57	0.84		
Farming system				2.55(2)	0.2791
	Mixed	88	1.71		
	Agro-pastoral	143	2.14		
	Pastoral	69	2.12		
Agro-ecology				0.51(1)	0.4764
	Mid land	164	2.12		
	Low land	136	1.85		

Herders had reflected positive attitude towards some questions e.g. “do you think brucellosis is treatable in animals?” in which 83% replied yes, but they thought it is not treatable in human. Similarly, considerable proportions also

responded washing hands after contact with animals (49%) and after assisting delivery (33%) prevent brucellosis. Regarding treatment means of brucellosis they mentioned modern drug 85 (28.33%), herbs 70 (23.33%) and religious practices 95 (31.67%). For the questions of prevention of brucellosis in animals 52 (17.3%) responded brucellosis can be prevented in animals by vaccine and isolation of animals. But all of the respondents thought that boiling milk and cooking meat don't prevent brucellosis transmission in human. Most of the herders (83%) reflected that they need more information on brucellosis.

Table 6. Respondents' attitude (positive attitude) towards brucellosis prevention

Attitude of Respondents'	Yes (%)	No (%)
Do you think		
Boiling milk prevent brucellosis?	0(0)	300 (100)
Cooking meat prevent brucellosis?	0(0)	300 (100)
Hand washing after contact with animals prevent it?	148 (49.33)	152 (50.7)
Hand washing after assisting calving prevent it?	99 (33)	201 (67.0)
You need more information on brucellosis?	249 (83)	51 (17.0)
Brucellosis is treatable in human?	0(0)	300 (100)
Brucellosis is treatable in animal?	250 (83.33)	50 (16.7)
Brucellosis can be prevented in animals?	52(17.33)	248 (82.7)

Most of the respondents of the area indicated that they perform several exposures (risky) practices frequently such as all of them consume raw milk and meat and 39% of drink fresh blood. But they do very few preventive practices such as cooking meat and milk before consumption and proper hand washing after contacts with animals or discharges of potential contaminants (Table 7).

Table 7. Respondents' Protective and Exposure Practices' towards Brucellosis

Practice of respondents	Frequency	Percentage
Exposure (risky) practices		
Raw milk consumption	300	100
Drinking of milk from aborted cow	40	13.3
Consuming raw meat	300	100
Consuming fresh blood	117	39.0
Assisting delivery with bare hand	211	70.3
Removing placenta by bare hand	75	25.0
Handle aborted fetus with bare hand	141	47.0
Preventive practices		
Cook meat for consumption	26	8.7
Boil milk for consumption	50	16.7
Separate cow during parturition	97	32.3
Separate aborted animals	29	9.7
Properly dispose fetal membrane	0	0.0
Wash hand with soap after delivery	19	6.3

Table 8 shows the mean score estimation of herders' knowledge, positive attitude, and practices (exposure and protective ones) with regard to brucellosis. The mean score of herders' knowledge was moderately low with 2.08 out of 11 scores. Respondents had generally good attitudes (5.57), but performing high level of risky practices (7.52) that can expose them to infection and at the same times they undertake low protective measures (2.48). In general, respondents had low level of information on bovine brucellosis, thus perform high risky practices while taking meager prevention measures.

Table 8. Estimation, of mean score of knowledge (out of maximum score of 11), attitudes (out of maximum score of 12), exposure (out of maximum score of11) and protective (out of maximum score of 6) practices regarding brucellosis

Variable	Mean	SE	95% CI
Knowledge	2.08	0.21	1.66 - 2.50
Good attitude	5.57	0.20	5.18 - 5.97
Risky practices	7.52	0.11	7.31 - 7.73
Protective practices	2.48	0.10	2.48 - 2.89

Discussion

The study showed that seropositivity to *Brucella* infection was detected in two of the three study districts. The overall seroprevalence of 1.96% was comparable to the findings of several other authors in Ethiopia such as 1.66% in Sidama zone (Asmare *et al*., 2010), 1.41% in Jijjiga zone (Degefu *et al*., 2011), 1.97% in East Wollega zone (Yohannes *et al.,* 2012), 2.0% from Debrezeit (Alemu *et al*., 2014). But it is lower than other reports from Ethiopia such as 3.5% from pastoral and mixed farming (Megersa *et al*., 2011), 11.2% from East Showa (Dinka and Chala, 2009), 8.0% from Borana area (Megersa *et al.,* 2012), and 6.1% from Tigray (Mekonnen *et al*., 2011).

Factors such as migration showed marginal association with seropositivity in that herds that migrate at least once a year had three times more likelihood of being seropositive than others. Mobile herds have high chances of coming in contact with other herds and wildlife so that more likely acquire infections than non-migrating ones (Megersa *et al.,* 2011). The observed higher seropositivity in the large herds is in line with previous study findings (Megersa *et al.,* 2011; Mekonnen *et al.,* 2011) and can be attributable to increase in stocking density and frequent contacts among animals in large herds, one of the determinants for exposure to *Brucella* infection especially during the time of abortion or calving (Radostits *et al.,* 2007).

Results of herders' knowledge, attitude and practices about brucellosis showed that most of the livestock keepers didn't have adequate knowledge about brucellosis in general and transmission of brucellosis to human through consumption of raw milk and meat, fresh blood, as well as its prevention measures. Our finding is similar to a previous study report from Borana pastoral communities (Roba, 2017). Another study from Ecuador (Ruano and Aguayo, 2017) also reported that only a small proportion of respondents (0.6% to 30.2%) stated to have knowledge about different aspect of brucellosis, while about 29.8% of the respondents indicated that they knew the clinical signs of the disease in cattle. Low level of knowledge on the disease and wide prevalence of behavioral risk (exposure) such consumptions of raw milk, fresh blood and raw meat, and assisting delivery without proper protection certainly expose pastoralists to brucellosis and clearly show the public health importance of the disease in the study areas.

The information on the zoonotic importance of brucellosis is not known by the community in the present study area, since more than three quarters of the respondents had not heard of Brucellosis. This shows low level of herders knowledge compared to reports from Uganda (Kansime *et al.,* 2014) among pastoral communities living along lake Mburo; in Egypt among cattle and Buffalo farmers in a village in Nile Delta region (Holt *et al*., 2011) and among small ruminant farmers in the peri-urban areas of Dushanbe Tajikistan (Lindahl *et al.,* 2015 in which 99.3%, 83.2% and 57% of the respondents' had heard of Brucellosis and its zoonotic importance. The current finding on the animal species affected by brucellosis was fairly in agreement with a study in Tajikistan (Lindahl *et al.,* 2015) in which two thirds of the livestock keepers mentioned that all animals could be affected.

Analyses on various factors that have potential influence on knowledge of herders showed that age, education and family size were associated with the knowledge of farmers. Younger individuals with age groups of 18 to 35 and those with having smaller family size had better knowledge of brucellosis than their counterparts, which might be explained by high inquisitive nature of younger people and with possibility of possessing information source device such as mobile phone and radio. The effects of educations have been reported by other authors elsewhere such by Ruano and Aguayo, (2017) in which greater knowledge about brucellosis was observed among people with a higher educational level. Similarly, a study by Lindahl *et al.* (2015) also showed that low level of education was significantly associated with poor knowledge of brucellosis.

The main sources of information on brucellosis in this study area were colleagues in community followed by animal health workers. Similarly, a study by Kansiime *et al*., (2014) showed that most of the respondents (91.4%) in Uganda had heard about brucellosis in their area of residence mainly from friends in the community. According to Lindahl *et al.* (2015) respondents in Tajikistan who talked about animal health issues with veterinarians had more likely to hear about brucellosis than those who discuss with family members or friends. This suggests the impact of animal health workers and veterinarians in creating awareness among herders on zoonotic diseases. But our finding of lower proportion information source from animal health workers implies the limited roles of veterinarians in conveying important animal health messages to herders of the area who in most cases do not fairly access basic education and health care services. There is a need for integrating animal health extension into human health extension with regard to zoonotic diseases.

With regards to clinical signs of brucellosis in animals, 26 % respondents mentioned retained placenta and 22.7% abortion as the major clinical sign. This finding is in agreement with a study finding from Kaduna state of Nigeria (Buhari *et al.,* 2015) and the study in Egypt (Holt *et al.,* 2011) in which 94.4% and 59.5% of respondents mentioned abortion as the major clinical sign. Unlike result of the present study, Lindahl *et al.* (2015) reported from Tajikistan only 11% of respondents mentioned abortion as a clinical sign of Brucellosis in the animals.

Regardless of their low level of information on brucellosis, herders had higher positive attitude towards some attitude questions, might be by chance or could be extrapolated from the general knowledge of herders on other livestock diseases. For instance, they might assume that all livestock diseases may have medications and prevention means so that considerable proportion of herders thought brucellosis can be treated or prevented. But since practices of herders depend on their knowledge of the disease, the poor the knowledge the lower the protective practices. Most of the herders consumed unpasteurized dairy products, raw meat and fresh blood more frequently, which are known to be important risk factor for human infections (Lindahl *et al.,* 2015). A majority of the livestock keepers in the current study area did not use protective gloves when assisting delivery and handling cows having an abortion or with aborted materials or did not properly wash their hands similar to observation by Lindahl *et al.* (2015) who reported such practices due to poor knowledge of herders on the zoonotic role of brucellosis.

Conclusions

The observed seroprevalence of 1.94 (95% CL: 1.0- 2.9) at individual animal level in the study area can be regarded as low prevalence but has public health significance. Factors such as herd size and migrating herds seem to be important risk factors that should be considered in disease control. Livestock keepers of the study area had low level of basic knowledge about bovine brucellosis and undertake insufficient disease prevention measures. Enhanced public health education on the cause, symptoms and mode of transmission of brucellosis would be important towards the prevention and control of the disease in the present study area. This can be achieved by creating awareness of the community on control and prevention of zoonotic diseases in the context of "One Health approach".

Acknowledgment

Authors are thankful to Mizan Regional Veterinary Laboratory for providing laboratory facilities and technical assistance during the study. The willingness of livestock keepers to participate in the study and critical comments of the anonymous reviewers are highly appreciated.

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Prevalence of Cystic Echinococcosis in One-Humped Camels Slaughtered at Addis Ababa Municipality Abattoir, Ethiopia

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Abstract

Cystic Echinococcosis (CE) is one of the most important zoonotic and economically important disease prevalent in different parts of Ethiopia and the world at large. This survey aimed to estimate the prevalence, identify associated risk factors, and tissue distribution patterns of CE in camels slaughtered at Addis Ababa municipality abattoir. Out of 416 one-humped camels examined during meat inspection, hydatid cysts were detected in 159 (38.22%) of them. The cyst was detected more frequently in the lungs (37.02%) followed by liver (35.1%), but very few camels had cysts in their heart, spleen, and kidneys. Significantly higher (p <0.05) prevalence of hydatid cyst was observed in female than male camels (48.05% vs 32.4%), in adult camels than young ones (53.1% vs 20.2%), and camels with good (25.0%) and medium body conditions (67.4%) compared to those in poor body condition (7.7%). The study further revealed that out of 768 cysts collected from the different organs, 169 (22%), 215 (28%) and 384 (70.57%) were abscessed/calcified, sterile and fertile cysts, respectively. About 54% (n=235) of the cysts in the lungs and 45% (n=148) in the liver were fertile. Of which, 146 (62.13%) cysts in lungs and 87 (58.78%) cysts in liver were viable. In conclusion, this study revealed a high prevalence of CE with a higher proportion of fertile and viable cysts in the pastoral areas. Therefore, to reduce the wide spread occurrence of CE and hence the presumed public health and socio-economic impacts, extension work on safe disposal of infected offal and dead animals, awareness creation to the community about the disease, construction of abattoirs with appropriate disposal pits and strict meat inspection should be given due consideration.

Keywords: Abattoir; *Camelus dromedarius*; Cystic echinococcosis; Prevalence; Risk factor.

Introduction

The one humped camel (*Camelus dromedarius*), by the virtue of their excellent adaptive behavior, play significant role in the livelihood of pastoralists living in most arid and semi-arid countries of the tropics including Ethiopia. It is almost everything to pastoralistsas source of food and power, cash and prestige, and means of storing wealth (Zeleke and Bekele, 2000). Moreover, quite huge numbers of camels are also slaughtered in the capital city and some towns in the eastern and south eastern part of the country. At the moment camels are also becoming one of the export animals to Arabian Peninsula and Egypt (SOS-Sahel Ethiopia, 2007; Tefera and Abebe, 2012).

Despite their significant socio-economic contributions, camels in the pastoral areas of Ethiopia are still facing shortage of water, feed, housing and health care services (Zeleke and Bekele, 2000; Keskes *et al*., 2013; Regassa *et al*., 2015). Their natural semi-arid to desert habitats, together with the poor husbandry practice, are known to induce severe stress conditions in camels and ultimately make them susceptible to many diseases and ailments (Abbas *et al*., 1993; Volpato *et al*., 2015). Moreover, the broader home range of the camel in pastoral areas, the poor veterinary service and improper disposal of offals and cadavers are known to exposes the camel to various pathogens including eggs of parasites. Specifically, the large number of dogs kept by the pastoralist and the wild carnivores freely roaming in the area can potentially contaminate the communal watering points and the pasture with eggs of *Echinococcus granulosus* (Elham *et al*., 2014)

Cystic echinococcosis (CE), caused by the cestode parasite called *Echinococcus granulosus*, is one of the most important zoonotic and economically important diseases prevalent in different parts of the country and the world at large (Dalimi *et al*., 2002; Ito *et al*., 2003; Latif *et al*., 2010; Ibrahim 2010). Cystic echinococcosis affects most livestock including cattle, sheep, goat, pig and camel and induces significant economic loss through organ condemnation, decreased hide value, carcass weight, and decreased productivity (Oryan*et al*., 1994; Dakkak, 2010). Because of lack of satisfactory test to diagnose CE in living livestock (Craig, 1997; Njoroge *et al*., 2002), the diagnosis is mainly dependent on meat

inspection in the abattoirs (Njoroge *et al*., 2002; Acosta-Jamett *et al*., 2010; Ibrahim, 2010).

Based on several cross-sectional studies conducted in Ethiopia and abroad, cattle, sheep and goats appear to be the most common intermediate hosts for *Echinococcus granulosus*; recent studies in Sudan and Turkana however suggest that camels are equally important intermediate host (Omer *et al*., 2004). In Ethiopia, except the few previous reports (Woldemeskel *et al*., 2001; Muskin *et al*., 2011; Boru *et al*., 2013; Hayer *et al*., 2014; Debela *et al*., 2015; Regassa *et al*., 2015) information available on CE in camels is limited to small part of the country and are fragmented to conclude on the role of camels in the epidemiology of the disease. Therefore, the present study was designed to estimate the prevalence, identify associated risk factors, characterize the cyst and illustrate its tissue distribution in camels slaughtered at Addis Ababa municipality abattoir, Akaki branch.

Material and Methods

Study Area

The study was conducted from October 2018 to May 2019 at Akaki abattoir, which is owned by the Addis Ababa abattoir enterprise and located in Addis Ababa city. The city is located at 9°1'48' North and 38° 44°-24' East at an average altitude of 2,500meters above sea level. Although the camel meat is not widely known in Addis Ababa, camels are slaughtered for the Somali and other Muslim communities who live or stay for short in the city (Salih *et al*., 2011). The camels slaughtered at the abattoir were originated from Borana and Kereyu pastoral areas and Minjar-Shenkora district.

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

Kereyu Pastoral area, circumscribed in Fentale district, is located at about 250 km East of Addis Ababa at an altitude of 930 meters above sea level. The tribes of Kereyu pastoralist occupy the arid lands around the Awash River down in the rift valley for pasture for their cattle, goats and camel (Tefera and Abebe 2012). The area has an average annual rainfall of 504 mm. The mean annual maximum and minimum temperature are 32.40 and 18.5°C, respectively. Pastoralism and agro-pastoralism are the main livelihood systems in the area (Beyene and Gudina 2009).

Minjar-Shenkora is one of the districts in the Amhara Regional state of Ethiopia, located at the southern end of the North Shewa Zone at about 129 km East of Addis Ababa. The district is bordered on the east, south and west by the Oromia Regional state and on the northwest by Hagere Mariam. Its altitude ranges from 1,040 to 2,380 meters above sea level. The average temperature ranges from 14 ºC to 27 ºC while the annual rainfall ranges between 780 and 900 mm. The district is known with its scattered bushes, shrubs and acacia trees (Ferede *et al*., 2014).

West Hararghe zone is located at 7°50'–9°50' N; 40°00'–41°25' E and 1200–3060 meters above sea level. The zone shares boundaries with Afar Regional State, Somali Regional State, as well as the east Hararghe Zone. It has three distinct agro-ecologies that consists of highland (17.5%), mid highland (28.5%), and lowland (54.0%) and have two rainy seasons, the short rainy season and the main rainy season, with a mean annual rainfall ranging from below 700 mm in the lowlands to nearly 1200 mm at higher altitudes (Ketema *et al*., 2018). The farming system is mainly characterized by pastoralism and agro-pastoralism. In addition to other livestock, the zone has high camel breeding potential.

Study population

The study population included the total number of camels presented to and slaughtered in the abattoir. Camels purchased from different markets were transported to the abattoir by trucks and kept at lairage for 3 to 4 days. Camels in the pastoral area (their original sites) browse on bushes and shrubs, but grasses may be consumed rarely when shrubs or trees are not available. The browse species includes the family *Chenopodiaceae*, *Acacia brevispica*, *Opuntia ficus indica*, *Dichrostachys ciniarea* and *Euphorbia tirucalli* (Bekele and Kibebew, 2002). The main sources of water for camels in the areas include wells, ponds and rivers (Wolde, 1991). The watering sites are usually visited

once per week by large numbers of camels and other animals at a time from the surrounding as well as from distant areas. Mostly the pond and river water sources are shared by wild animals too (Mirkena *et al*., 2018). Camels in the pastoral areas are used for packing, transportation, ploughing and traction purposes and as source of cash income, milk and meat (Mehari *et al*., 2007).

Study animals and sample size

The sample size was calculated using the formula given for simple random sampling (Thrusfield and Brown, 2018) with a previous prevalence of 65% (Regassa *et al*., 2015), 95 % confidence level and 5% desired absolute precision. Accordingly, the sample size was determined to be 350. However, 66 more camels were included with the intention of maximizing the sample size for better precision. The sampling procedure was carried out using systematic random sampling (Thrusfield and Brown, 2018), whereby every third camel walking into the lairage was selected and marked.

Study methodology

Antemortem examination

During each regular visit, all the camels brought for slaughter were inspected while entering into the lariage for the presence of any observable abnormality. The general behavior of the animal, body condition, gait, posture, clinical signs suggestive of disease and abnormality of any type were registered (Gracey *et al*., 1999) and judgment was made based on FAO recommendation (Herenda *et al*., 1994).

Data about the age, sex, origin and body condition score of the selected camels were recorded before slaughtering. The age of the camels was estimated using rostral dentition (Bello *et al*., 2013) and then categorized as young (less than 5 years) and adult (≥ 5 years of age) for ease of data analysis. The body condition score of the camels were assessed according to Faye *et al*., (2001) and then grouped as poor (score 1), medium (score 2 and 3) and good (score 4).

Post Mortem Examination

Following slaughter and evisceration, a thorough and systematic inspection of the visceral organs particularly the lungs, liver, spleen, heart and kidneys

were made for presence of hydatid cyst using visual inspection, palpation and multiple incisions, when required. The pathological lesions were differentiated according to guidelines on meat inspection for developing countries (Herenda *et al*., 1994). Cysts of each organ were counted and differentiated as calcified and non-calcified based on their consistency and appearance. The study animals were considered as positive if at least one cyst was found in one or more of the organs examined. All non-calcified hydatid cysts (when the number of cysts in the organ is ≤ 3) and three randomly selected non-calcified hydatid cysts (when their number on the organ is >3) were collected. Briefly, the non-calcified cysts were removed whole and placed in clean polyethene bags, labeled properly and transported to the laboratory of National Artificial Insemination center (at Kaliti, Addis Ababa) for further examination.

Cyst fertility and viability tests

The surface of the cyst was wiped off or blotted with tissue paper and/or gauze and then, to reduce intracystic pressure, part of the fluid was drained with a 21-gauge needle attached with a 12 ml syringe. By cutting the cyst wall with scalpel and scissors, the remaining content of the cyst was poured into a clean petri dish. The fertility of the cyst was determined by considering the presence of protoscolices (as white dots on the germinal epithelium or broad capsule or hydatid sands suspended in the fluid) (Urquhart *et al*., 1996; Bowman, 2014). The cysts which contained no protoscolex as well as suppurative or calcified cyst were considered as non-fertile cyst.

All the fertile cysts were then subjected to viability test. The viability of the protoscolices was assessed by examining them under a microscope (40X) following the application of 0.1% aqueous eosin solution (Smyth and Barrett, 1980). The protoscolices were considered viable when they didn't take the eosin stain.

Statistical analysis

All the collected data were entered into Microsoft Excel spreadsheet coded and then analyzed using STATA statistical software (STATA, 2013; window version 13.1). Association between various risk factors (sex, age, origin and body condition score) and the prevalence of *hydatidosis* was assessed by using chi-square independent test. Moreover, univariable and multivariable logistic regression analyses were carried out to assess the level of significance. In all the analysis, significance was set at $p< 0.05$.

Results

The overall prevalence of CE in camels in the current study was 38.22% (159 out of 416 camels). The hydatid cysts were detected more frequently in the lungs (37.02%) followed by the liver (35.1%), but very few camels had cysts in other organs (heart, spleen and kidney) (Table 1). Majority of the slaughtered camels had hydatid cysts both in their liver and lungs.

Table 1. Prevalence and organ level distribution of hydatid cysts in the examined camels (n = 416).

Tissue /organ	No positive	Prevalence (%)	95% CI
Lung	154	37.02	32.36 – 41.68
Liver	146	35.10	30.49 – 39.70
Kidney	2	0.48	-0.19 – 1.15
Spleen	1	0.24	-0.23 – 0.71
Heart	1	0.24	-0.23 – 0.71
Lung +Liver	143	34.37	29.79 – 38.96
Lung + Kidney	2	0.48	-0.19 – 1.15
Over all	159	38.22	33.53 – 42.91

Relatively higher prevalence and likelihood of occurrence of hydatid cyst were observed in female camels (48.05%, OR 1.93), in old camels (53.07%, OR 4.5), camels originated from Kereyu (43.24%, OR 1.72), and camels with medium body condition (67.4%, OR 22.86) than in the category/ies of the respective risk factors. With the exception of the origin ($p = 0.173$), the difference in the prevalence of hydatidosis between or among the categories of the other considered risk factors (age, body condition score and sex) were statistically significant ($p< 0.05$) (Table 2).

Table 2. Prevalence and logistic regression analysis of hydatid cyst in camel by the putative risk factors.

Variable		№ examined	№ (%) positive	Crude OR (95% CI)	Adjusted OR (95% CI)	*p*- value
Age group	Young	188	38 (20.21)	1	1	
	Adult	228	121 (53.07)	4.5 (2.9 - 6.9)	3.7(2.21 – 6.12)	0.000
BCS	Poor	104	8 (7.69)	1	1	
	Medium	172	116 (67.44)	22.86 (11.29 – 54.69)	35.6(14.4 – 88.02)	0.000
	Good	140	35 (25.00)	4.0 (1.77 – 9.05)	4.7(2.01 – 11.10)	0.000
Sex	Male	262	85 (32.44)	1	1	
	Female	154	74 (48.05)	1.93(1.28 – 2.90)	1.95(1.10 – 3.47)	0.023
Origin	Borana	101	31 (30.69)	1	1	
	Kereyu	111	48 (43.24)	1.72(0.98 – 3.03)	1.64 (0.80 – 3.34)	0.173
	Minjar Shenkora	92	34(36.96)	1.32(0.73 – 2.41)	1.25 (0.60 – 2.60)	0.558
	West Hararghe	112	46 (41.07)	1.57(0.89 – 2.77)	1.32 (0.65 – 2.66)	0.446

BCS = Body Condition Score, OR = Odds Ratio, CI = Confidence Interval

Out of 768 cysts collected from the different organs, 169 (22%), 215 (28%) and 384 (70.57%) were found to be abscessated / calcified, sterile and fertile cysts, respectively. The proportion of fertile cysts was higher in the lungs (54.4%) followed in the liver (45.12%) and kidneys (20%). Of the fertile cysts collected from lungs and liver, 146 (62.13%) and 87 (58.78%) were viable. However, all the cysts collected from kidneys, spleen and heart were non-viable (Table 3).

Table 3. Cyst fertility and viability in different organs of study camels

Organ	No of collected cysts	Sterile cyst No (%)	Calcified cysts No (%)	Fertile cyst No (%)	Viable cysts No (%)
Lung	432	110 (25.46)	87 (20.14)	235 (54.40)	146 (62.13)
Liver	328	102 (31.10)	78 (23.78)	148 (45.12)	87 (58.78)
Kidney	5	1(20)	3 (60)	1(20)	0
Spleen	2	1(50)	1 (50)	0	0
Heart	1	1(100)	0	0	0
Over all	768	215 (28.0)	169 (22.0)	384 (70.57)	231 (60.16)

Discussion

The prevalence of camel hydatidosis recorded in this study (38.22%) is relatively higher than the previous reports 23% by Debela *et al.* (2015), 28.7% by Hayer *et al.* (2014), 22.6% by Muskin *et al.* (2011) and 18.8% by Woldemeskel *et al.* (2001) from different parts of Ethiopia. However, comparably higher prevalence (61.4 to 65%) than the current study was also reported in Ethiopia (Regassa *et al.*, 2015; Boru *et al.*, 2013). Higher prevalence values were also reported from other African countries such as 61.4% from Kenya (Njoroge *et al.*, 2002), 45% from Sudan (Elmahdi *et al.*, 2004) and 44.4% from Nigeria (Okolugbo *et al.*, 2013). The prevalence difference observed between these studies could be partly explained by the difference in the ecology, husbandry, livestock stocking intensity, population of the definitive hosts and the sociocultural practices. Specifically, the most common production practices that may increase the risk of exposure of farm animals to hydatidosis include improper disposal of dead animals, the access of dogs to the offals of slaughtered animals, absence of regular deworming of dogs, communal and mixed-species grazing, and unrestricted use of watering points by camels, stray dogs and other wild canidae (Azlaf and Dakkak, 2006; Christodoulopoulos *et al.*, 2008; Elham *et al.*, 2014).

The prevalence of hydatidosis was significantly higher in female camels (OR 1.95, $p = 0.023$) than the males and in old camels (OR 3.7, $p = 0001$) than young camels. This finding is in line with the reports of Muskin *et al.* (2011), Gizachew *et al.* (2013), Boru *et al.* (2013) and Debela *et al.* (2015) from Ethiopia, Abdul-Salam and Farah (1988) from Kuwait, Ibrahim *et al.* (2011) from Sudan and Elham *et al.* (2014) from Iran. These might be related to the higher chance of direct or indirect contact with freely roaming dogs while the female camels are brought and kept around the homesteads till they get milked. Moreover, as female animals are kept for prolonged years for milk production, the possibility of acquiring and sustaining infections will also increase. Given the high reproductive capacity of *Echinococcus granulosus*, a single infected dog can excrete feces with a large number of parasite's eggs that can contaminate wide range of the foraging areas and watering points (Gemmell, 1990; Parija, 2004).

In the present study, the hydatid cysts were detected more frequently in the lungs (37.02%) followed by the liver (35.1%). Comparable reports were also made previously from Ethiopia (Woldemeskel *et al.*, 2001; Muskin *et al.*, 2011; Gizachew *et al.*, 2013; Debela *et al.*, 2015) and elsewhere (Anwar and Khan,

1998; Ibrahim and Craig, 1998; Sharrif *et al*., 1998; Njoroge *et al*., 2002; Ahmadi, 2005; Okolugbo *et al*., 2013; Elham *et al*., 2014). In contrary, Ibrahim (2010) from Saudi Arabia and Boru *et al*. (2013) and Hayer *et al*. (2014) from Ethiopia reported that the liver is more frequently affected organ than the lungs. In the current study, it is also noted that concurrent infection of both liver and lungs was equally common like infection of either of the organs alone.

The higher frequency of infection in lungs and liver might be due to the fact that the migrating echinococcus oncospheres that get into the subepithelial capillaries of the intestine or the lacteal has to pass first the great capillary bed of the hepatic and pulmonary filtering system before reaching any other organ (Brown *et al*., 2007; Kebede *et al*., 2009). Owing to the largest capillary beds in the lungs, oncospheres entering the vena cava with the lymph will be first filtered out and trapped in the lung and concomitantly forms the cyst than in any other organ (Brown *et al*., 2007).

The infectivity potential of the cysts revealed that 70.57% of the cysts were fertile, of which 60.16% were viable, which is in line with Muskin *et al*. (2011) and Okolugbo *et al*. (2013) who reported 50% and 79% fertility in camels from Ethiopia and Nigeria, respectively. Although not statistically significant, cysts from the lungs were more fertile and viable than the liver. In Ethiopia, Muskin *et al*. (2011), Boru *et al*. (2013) and Hayer *et al*. (2014) also reported relatively more fertile cysts from lungs than the liver. On the contrary, the studies conducted by Elham *et al*. (2014) in Iran and Ibrahim *et al*. (2011) in Sudan showed that hydatid cysts of the liver have higher fertility rate than that of lungs. Our observation can be explained by the relative softer consistency of the lung tissue, compared to liver, which might favor the development of the cyst and hence make them fertile.

Conclusions

The present study disclosed that high prevalence of camel CE with a higher proportion of fertile and viable cysts indicating a serious public health concern particularly in the study areas. Therefore, timely efforts should be made to control the transmission of cystic echinococcosis through extension work on safe disposal of infected offal and dead animals, awareness creation to the community about the disease, construction of abattoirs with appropriate disposal pits and introducing strict meat inspection protocols. Moreover, molecular based study should be conducted to identify the prevailing strains/genotype of *E. granulosus* in the area.

Acknowledgements

We would like to thank workers at Addis Ababa abattoir enterprise Akaki branch and the technical staff of the Parasitology and Pathology laboratory of the Faculty of Veterinary Medicine, Hawassa University for their cooperation during sample collection and support during sample processing, respectively. Special thanks also go to the Addis Ababa abattoir enterprise Akaki branch for allowing us to work in the premises. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no competing interests. This piece of work has not been published previously or not submitted for publication elsewhere. The submission is approved by all authors and all the authors declare that they have no conflict of interest.

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Evaluation of heterosis, maternal and reciprocal effects on different traits of Fayoumi and White Leghorn crossbreeds

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Abstract

Several studies were conducted on evaluation of heterosis, maternal and reciprocal-effects for different chicken breeds. However, there is a limited information on the heterosis, maternal and reciprocal-effect for crossbreed of Fayoumi and White Leghorn. Therefore; this study was designed to evaluate the heterosis, maternal and reciprocal effects on different traits for crossbreed of Fayoumi and White Leghorn from day-old to age at first egg. A total of six hundred chicks were selected,150 from each genotype and evaluated simultaneously under the same management. The specific and general heterosis, maternal, and reciprocal effects were estimated for all traits. The heterosis of body weight ranged from 3.06 to 21.31% for the main and 1.35 to 14.89% the reciprocal crossbred, which is within the recommended range (-6.5-26.2%). The heterosis of weight gain ranged from -0.07 to 33.03%. The F1 of Fayoumi cocks and White Leghorn hens exhibited a higher positive heterotic recorded for the body weight at first egg. The main and reciprocal crossbreds had negative heterotic for age at first egg and lies within the recommended range of (-25 and 11.5 %). The effect of maternity on body weight was found to be more favorable at brooder age for Fayoumi breed while it was at grower age for White leghorn. Negative estimates were observed for maternal and reciprocal effects on body weight at day-old, 20 weeks, and age at first egg; weight gain at 8-12 and 16-20 weeks; feed intake at 0-4 and 12-20 weeks; feed conversion efficiency at 0-8 and 16-20 weeks; and egg weight at first egg. Generally, this study concluded that the crosses between WLH hen and Fayoumi cock produced progenies that are more feed efficient and produced heavier eggs than their main crossbred.

Keywords: Crossbreed; Fayoumi; Heterosis; Maternal; Reciprocal; White Leghorn

Introduction

Crossbreeding is an important instrument for breeders to improve important economic traits in farm animals (Oseni *et al.*, 1997). It combines different characteristics of genetically different animals. The crossbreed offsprings tend to be superior in some quantitative traits to either one or both parental lines. This is often referred to as hybrid vigor or positive heterosis (Baranwal *et al.*, 2012). However, negative heterosis may be desirable for crossbreds, particularly in traits like mortality and disease susceptibility, where merits are associated with lower values (Afolabi *et al.,* 2017). Heterosis for growth traits was found age-dependent (Momoh and Nwosu, 2008). High positive heterosis for body weight at different ages among crossbreds and their reciprocals were obtained in chickens (Mandour *et al*., 1996). Razuki and AL-Shaheen (2011) found the highest positive heterosis that occurred in crosses between Brown line and New Hampshire and New Hampshire and White Leghorn.

Heterosis is influenced by maternal and direct non-additive effects (Lui *et al*., 1995). The common practice in crossbreeding is to select and cross a cock breed of a desirable trait with different hen breeds (Afolabi *et al.,* 2017). However, the choice of a breed to serve as a cock or hen in a crossbreeding program should rather be done objectively to improve the targeted trait as some traits are linked to sex. Khalil *et al*. (1999) and Sabri *et al*. (2000) detected a significant maternal effect on the live weight of offspring at an early age (0-8 weeks). Positive maternal effects were reported for body weight in chicken crosses (Saadey *et al.,* 2008; Lalev *et al.,* 2014). Many literatures noted better overall performance for maternal-side in crossbreds (Khalil *et al.*, 1999; Sabri *et al.,* 2000; Khawaja *et al.,* 2012). The maternal-effects are accounted for the most important reciprocal-effects in chickens (Amira *et al*., 2013). Maternal-effect is useful in defining the extent of genetic dissimilarities between the combining breeds (Viana, 2007) and may also be due to a possible difference in the combining aptitudes between cocks and hens (Keambou *et al*, 2010). Reciprocal-effects were at least as important as heterosis and its magnitude tends to be greater when heterosis is small (Fairfull *et al.,* 1983; Razuki and AL-Shaheen, 2011). Positive reciprocal-effects for body weight in the different genetic groups of diallel crossing of Saso, Italian and Mandarah chickens were estimated for ages of 0-4, 4-8, 8-12 and 0 -12 weeks. Razuki and AL-Shaheen (2011) reported a significant reciprocal-effect on body weight at day-old. On the other hand, Khawaja *et al*. (2012) reported better performance in all traits for crossbreed of Fayoumi cocks and Rode Island Red hens than in its reciprocal crossbreed.

Barbato and Vasilatos-Younken (1991) noted that the maternal-effect in chickens changed with time and its considerable influence is manifested.

As compared to White Leghorn, Fayoumi breed is characterized for its disease resistance, well-suited to hot climates, and surviving normally with farmers as a scavenger in Bangladish (Rajput *et al.,* 2005). However, it is a small-sized, lays smaller eggs, lower carcass yield and lower economic return (Ewonetu, 2017). Even though White Leghorn has a fast-growing performance with higher economic return as layers (Javed *et al.,* 2003), the breed is more susceptible to diseases as evidenced by high mortality rate (12.34 %) during the brooder stage (Ewonetu, 2017). Hence, combining of those two breeds were done with the expectation of complementing the deficient traits of Fayoumi with the best traits of White Leghorn and vice versa. Likewise, the study was conducted by crossing of other different breeds and evaluated for their heterosis, maternal and reciprocal-effects at brooder and grower ages (Yahaya *et al.,* 2009; Keambou *et al.,* 2010). However, there is a limited information on the heterosis, maternal and reciprocal-effect on growth performance and age at first egg for the crossbreeds of White Leghorn and Fayoumi breeds and crossing of those breeds were aimed to produce optimum crossbreds that adapted to semi scavenging and reared in small scale chickens productions. Therefore; this study was designed to evaluate the heterosis, maternal and reciprocal effects on different traits for crossbreed of Fayoumi and White Leghorn.

Materials and methods

Study area

The study was conducted at Haramaya University poultry farm, located at 505 Km east of Addis Ababa and situated at the distance of 5 Km from the nearby town of Haramaya, which is found on the main road connecting the capital, Addis Ababa with the eastern city of Harar. Its geographical location of the research site is at 9° 26' N latitude and 42° 3' E longitudes and an altitude of about 2010 meters above sea level. The area receives an average annual rainfall of 741.6 mm. The mean annual minimum and maximum temperatures of the site are 8.25 °C and 23.4 °C, respectively(quoted by Ewonetu, 2017).

Experimental house and parental flock management

All pens were cleaned and disinfected before start of the actual experiment but drinkers and feeders were washed every day in the morning throughout the study period. A total of three hundred fifty-two chicken (176 from each breed) were were purposively selected as a parental stocks at their peak egg production (34 weeks age) (Table 1). The parental stocks were grouped into four genotypes. Each genotype was reared in a deep litter house covered with *teff* straw and provided the same management. Those parental lines fed the same ration formulated to iso-caloric (2800-2900 Kcal ME/kg DM) and iso-nitrogenous (16-17% crude protein) from ingredients described in Table 2. The parental hens were reared for sixty days in separate pens with cocks in a ratio of one to ten to obtain hatching eggs which were used to produce day-old chicks.

Table 1: Breeding design and number of parent flocks used to produce day-old chicks

Genotypes	Number of parental flocks		
	Males	Females	Total
White Leghorn (female) x White Leghorn (males), purelines	8	80	88
Fayoumi (males) x Fayoumi (females), pure lines	8	80	88
White Leghorn (males) x Fayoumi (females), main cross	8	80	88
Fayoumi (males) x White Leghorn(female), reciprocal cross	8	80	88
Total	32	320	352

Egg collection, selection and incubation

Eggs produced by parental flocks were collected daily at 8:00 AM and 4:00 PM and medium egg size were selected (42.87-55.23g), oval shape, being free of shell cracks, and stored at room temperature for seven days. A total of one thousand eggs, 250 from each genotype was incubated using cabinet incubator (Pas Reform Hatchery technologies; Zeddam, Holland).

Day-old chick's management

All pens, watering and feeding troughs were cleaned, disinfected and the floor was covered using *teff* straw before the chicks were brought to experimental pens. Each pen was installed with two infrared light to provide heat during the brooder age for 24 hrs. A total of six hundred, 150 chicks were selected

from each genotype and each genotype was assigned to three replication, 50 chicks per replication and simultaneously evaluated until age at the first egg under the same environment. Day-old chicks were vaccinated for New Castle Disease and reared in deep litter pens up until age at first egg. The chicks fed on rations that were formulated for respective ages from different ingredients (Table 2). The diets formulated were to meet the nutrient requirements of 2800 kcal ME/kg DM for both age groups while 20 and 16 % CP of chicks (0-8) and growers (8-22), respectively (NRC, 1994).

Table 2. Feed ingredients and nutrient composition (% for DM)

Feed ingredient (%)	Management stages (weeks)		Mean Nutrient composition (% for DM) a					ME kcalkg-1
	Brooder (0-8]	Grower (8-22]						
			DM	CP	EE	Ash	CF	
Ground corn	55.00	45.15	89.00	7.10	5.30	2.30	8.00	3436.88
Soybean meal	12.00	15.00	93.20	38.50	8.90	8.00	9.00	3310.46
Peanut meal	9.00	13.00	94.70	37.30	9.60	6.20	12.00	3155.88
Wheat Short	20.00	25.00	90.30	12.00	3.30	6.80	6.2.00	3303.14
Limestone	3.15	1.00	-	-	-	-	-	-
Vitamin premix	0.50	0.50	-	-	-	-	-	-
Salt	0.35	0.35	-	-	-	-	-	-
Average			91.80	23.73	6.78	5.83	8.80	3301.34

[a] DM = Dry Matter; CP = Crude Protein; EE = Ether Extract; CF = Crude Fiber and ME = Metabolizable Energy.

Cockerels and pullets were reared together until 12 weeks of age and then after only pullets were evaluated for body weight and gain, feed intake and conversion, body weight, egg weight and age at first egg. Besides, heterosis, maternal and reciprocal effects were estimated for each parameters.

Data collection

Body weight and body weight gain

The body weight was taken at hatch and subsequently measured at monthly intervals by weighing chicks in a group and a total weight was divided by total number of chicks in each replication to obtain weight of each chick. The body weight gain of each chicken was calculated by the following formula.

$$\text{Body weight gain (g)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Number of days between two consecutive measurements}}$$

Feed intake and feed conversion ratio

A weighed amount of feed was offered once on the daily basis at 8:00 AM and refusal was collected next day at the same time. Then, the daily feed intake of each chick was estimated by subtracting feed refusal from offered and divided to total number of chicks in each replication. The feed conversion ratio (FCR) was measured by dividing the average daily feed intake of each chick by their live weight gain.

Performance at first egg (age, body weight and egg weight)

Age and body weight at first egg was evaluated for each genotype when their egg production reached 5% (North, 1978; as cited by Shafik *et al.*, 2013). A total of thirty-six eggs, 3 per replication, at first egg was randomly selected and evaluated at the same stage of production.

Statistical methods and experimental design

The effect of specific and general heterosis, maternal and reciprocal was calculated for all traits using the following formulae as cited by Emad and Amin (2015).

$$\text{Specific Heterosis (\%)} = \left[\frac{F1 - \left[\frac{P_1+P_2}{2}\right]}{\frac{P_1+P_2}{2}}\right] \times 100$$

Where:
F1– average values of traits of hybrid lines, $P_{1,2}$ –average values of traits of parents 1 and 2.

$$\text{General Heterosis} = \frac{(\text{Heterosis of Main crossbred}) + (\text{Heterosis of Reciprocal crossb}}{2}$$

Maternal effect = [White Leghorn Cock * Fayoumi hen] – [Fayoumi Cock * White leghorn hen]

$$\text{Reciprocal effect (RE)} = \frac{[PF1(WLm\ X\ Fff) - PF1(Ffm\ X\ WLf)}{2}$$

Where,

PF1 (WL_m X FF_f) - the mean performance of the F1 from a White Leghorn cock and Fayoumi hen crossings,

PF_1 (FF_m **X** WL_f) - the mean performance of the F_1 from a Fayoumi cock and White Leghorn hen crossings

Results

Heterosis Effect

Table 3 describes the heterosis of main and reciprocal progenies of White Leghorn and Fayoumi crosses. The estimates of heterosis percentages of White Leghorn cocks x Fayoumi hens (main cross) and its reciprocal crossbred gave positive heterotic effect for body weight throughout the study period. Therefore, the use of cocks or hens as a sire or dam in crossbreeds of White Leghorn and Fayoumi produces offspring that are heavier than either of their pure lines.

Table 3. Heterosis of different traits at 4 ,8, 12, 16 & 20 weeks of age in cross-breeds

Traits (g)	Heterosis (%)		
	General	Main crossbred	Reciprocal crossbred
Weight at 0 wk	4.44	3.73	5.16
W4	11.70	19.36	4.02
W8	12.09	21.31	2.87
W12	8.85	12.41	5.29
W12*	3.06	3.06	3.06
W16*	3.13	4.91	1.35
W20*	14.72	14.55	14.89
BWG 0-4	18.03	33.03	3.03
BWG4-8	12. 15	21.61	2.68
BWG8-12	12.48	9.78	15.17
BWG12-16*	-3.26	6.44	-0.07
BWG16-20*	23.15	22.87	23.43
Feed Intake at 0-4wk	-3.48	-0.84	6.11
FI4-8	-1.93	3.81	-0.04
FI8-12	-0. 65	3.13	-0.17
FI12-16*	-3.18	2.82	-3.54
FI16-20*	-1.56	-0.40	2.71
Feed Conversion Ratio at 0-4 wk	-20.69	-32.76	-8.62
FCR4-8	-27.78	-33.33	-22.22
FCR8-12	-5.00	0.00	-10.00
FCR12-16*	-10.00	-10.00	-10.00
FCR16-20*	-20.84	-25.00	-16.67
Body Weight at First Egg	11.77	11.45	12.08
AAFEL	-4.49	-2.39	-6.59
EWAFE	-2.64	-2.22	3.06

Means within a row with different lowercase letters are significantly different at P<0.05. W0, 4, 8, 12, 16, 20 = body weight at hatch, 4 ,8, 12, 16 & 20 weeks of age; BWG 0-4, 4-8,8-12,12-16 & 16-20=Body weight gain from 0-4, 4-8,8-12,12-16 &16-20 weeks; FI0-4, 4-8,8-12,12-16,16-20 =Feed Intake from 0-4, 4-8,8-12,12-16 & 16-20 weeks; FCR0-4,4-8,8-12,12-16 & 16-20= FCR with 0-4,4-8,8-12,12-16 & 16-20 weeks; BWAFEL-Body weight at age at first egg lay; *AAFEL-Age at first egg; EWAFE-Egg weight at first egg; Main crossbred-White leghorn cock X Fayoumi Hen*; **Parameters taken only for females.*

The heterotic effect was positive for weight gain in main crossbred throughout the study period while it was positive in reciprocal crossbred during brooder age but it was negative during the grower at the age of 12-16 weeks. Moreover, the general heterosis for body weight gain were positive starting from day-old to 20 weeks of age except at 12-16 weeks in growers. This positive heterosis demonstrated that the tremendous higher mean body weight gain in the crosses than the pure lines. The heterosis for feed intake in the main crossbred was reverse to the reciprocal and the main crossbred was better than their parents by their lower feed intake at brooder age (0-4 week) and at grower age (16-20 week) whereas the reciprocal crossbred was lower in feed consumption than parents from the age of 4-16 weeks. Overall, the general negative heterotic for feed intake demonstrated that the lower feed consumption of the crossbreds than the pure lines. Besides, the general negative heterotic for feed conversion efficiency throughout the study period. Estimates of heterosis percentages in main and reciprocal crossbreds were -2.39 and -6.59% for age at first egg, respectively. While lower heterotic effect was attained for the progeny of White Leghorn hens and Fayoumi cocks. Moreover, the F1 of White leghorn hens and Fayoumi cocks had a higher and positive heterotic for egg weight at first egg than its main crossbred. Hence, the F1 of White leghorn hens and Fayoumi cocks is preferred to reduce the age at first egg and produce heavier egg weight first egg.

Maternal and reciprocal effects

An estimate of maternal and reciprocal effects for body weight, feed intake, feed conversion ratio, body weight gain, age, body weight and egg weight at at first egg are given in Table 4. The results showed that the F1 of White Leghorn cocks and Fayoumi hens achieved positive estimates of maternal and reciprocal effects for body weight at all the studied stages except at day-old and 20th week. Similarly, the main crossbred noted that positive maternal and reciprocal effects for body weight gain except at 8-12 and 16-20 weeks. The negative estimates of maternal and reciprocal effects in this study indicated that the chicks mothered by White Leghorn are preferred for body weight and weight gain compared to chicks mothered by Fayoumi. Thus, it is recommended to use White leghorn as a hens in crossbreeding programs to improve body weight and weight gain. The results showed that the F1 of Fayoumi hens and White Leghorn cocks had a positive and higher maternal and reciprocal effects for feed intake at brooder ages, except within 0-4 weeks while its reciprocal was positive at the grower stage within the age of 12-20 weeks. This demonstrated

that chicks mothered by the Fayoumi hens consumed more feed during brooder age after 4th weeks onward whereas White Leghorn mothered chicks consumed more during the brooder stage. The main crossbred exhibited all negative results of maternal effect for feed conversion ratio except for 8-16 weeks while the reciprocal effect was all negative except at 12-16 weeks. Chicks mothered by Fayoumi demonstrated that more feed efficient than chicks mothered by White Leghorn. The positive maternal and reciprocal effects for age and egg weight at first egg were indicated that chicks from White Leghorn hens started egg production at an earlier age with heavier eggs than chicks mothered by Fayoumi.

Table 4. Maternal and reciprocal-effects on the performance of Fayoumi and White Leghorn crossbreeds

Traits	Maternal Effects		
	WLH cock x Fayoumi Hen	WLH Hen x Fayoumi cock	Reciprocal Effect
BW0	-0.42	0.42	-0.21
BW4	9.69	-9.69	4.85
BW8	84.66	-84.66	42.33
BW12	31.54	-31.54	15.77
BW12*	0.00	0.00	0.00
BW16*	31.45	-31.45	15.73
BW20*	-3.25	3.25	-1.63
BWG0-4	74.97	-74.97	37.49
BWG4-8	85.08	-85.08	42.54
BWG8-12	-21.58	21.58	-10.79
BWG12-16	31.45	-31.45	15.73
BWG16-20	-3.16	3.16	-1.58
FI0-4	-0.83	0.83	-0.425
FI4- 8	1.69	-1.69	0.85
FI 8-12	1.35	-1.35	0.68
FI8- 12*	2.98	-2.98	1.49
FI 12-16*	-2.05	2.05	-1.03
FI 16-20*	-1.47	1.47	-0.74
FCR0-4	-0.14	0.14	-0.07
FCR4-8	-0.01	0.01	-0.01
FCR8-12	0.01	-0.01	-0.005
FCR12-16*	0.00	0.00	0.00
FCR16-20*	-0.01	0.01	-0.01
BWAFEL	-7.67	7.67	-3.50
AAFEL	7.00	-7.00	3.50
EWAFE	-2.00	2.00	-1.00

Means within a row with different lowercase letters are significantly different at P<0.05. W0, 4, 8, 12, 16, 20 = body weight at hatch, 4 ,8, 12, 16 & 20 weeks of age; BWG0-4, 4-8,8-12,12-16 & 16-20=Body weight gain from 0-4, 4-8,8-12,12-16 &16-20 weeks; FI0-4, 4-8,8-12,12-16,16-20 =Feed Intake from 0-4, 4-8,8-12,12-16 & 16-20 weeks; FCR0-4,4-8,8-12,12-16 & 16-20= FCR with 0-4,4-8,8-12,12-16 & 16-20 weeks; BWAFEL-Body weight at age at first egg lay; AAFEL-Age at first egg; EWAFE-Egg weight at first egg; Main crossbred-White leghorn cock X Fayoumi Hen; *Parameters taken only for females.

Discussion

Specific and general heterosis

The positive heterotic observed for body weight at different ages (Table 4) which was in line with reports of many scholars (Iraqi *et al.*, 2002; Saadey *et al.,* 2008; Razuki and AL-Shaheen, 2011). On the contrary, Nawar *et al.* (2003) reported negative heterosis for body weight at different ages. The heterosis for body weight was ranged from 3.06 to 21.31% and 1.35 to 14.89% for main and reciprocal crossbreds, respectively that fall within the range -6.5-26.2% that reported by Afifi *et al.* (2002). The positive heterotic effects during the grower ages (12-20 weeks) implied the betterment of crossbreds over their parents in body weight. There was no definite heterosis trend observed for body weight and it was varied with age which is in agreement to Lamont and Deeb (2001). The positive heterotic for body weight at all age groups indicated the compatibility of the two parental breeds which is in line with Iraqi *et al.* (2002).

The higher heterosis for body weight gain was observed in F1 of Fayoumi hens and WLH cocks as compared to the reciprocal from hatch to 8 weeks (Table 4). Likewise, Jeremiah *et al.* (2017) reported a higher heterotic-effect for weight gain in main cross (57.37%) than reciprocal cross (45.39%). The heterosis of weight gain in this study was ranged from 0.07 to 33.03%. The negative heterotic-effect could be due to the higher weight gain of parents than their crossbreds. Similarily, Keambou *et al*. (2010) reported positive (67.2) and negative (-14.9) heterosis for weigh gain in main and reciprocal crossbreds, respectively. The positive heterotic-effects for body weight gain suggested that the superiority of the crossbreds over the parents being a good indicator of faster growth rate in F1 generation through the positive or negative heterosis can biologically be the phenotype that is preferred depending on the trait interested in.

The positive heterosis for body weight at first egg seen in crossbred of Fayoumi cock and White Leghorn hen (Table 3) which was in line with the study reported by Iraqi (2002) and Lalev *et al.* (2014). On contrary, negative heterosis of age at first egg for the main and reciprocal crossbreds were reported at different age groups (Table 3) though it was within the recommended range (-25 to 11.5 %) as reported by Williams *et al.* (2002). The negative heterotic indicated the improved hybrid vigor concerning the age of sexual maturity for the crossbred. The variation was due to heavier body weight and the maternal-effect of White Leghorn which is in line with report of Lalev *et al*. (2014). The negative

heterotic for age at sexual maturity observed for the F1 of WLH and Fayoumi chickens , this implied the desirable direction for age at first egg.

The White Leghorn hen and Fayoumi cock crossbred revealed positive heterosis for egg weight at age at first egg. This is inconsistent with the study of Saadey *et al.* (2008) who reported positive heterosis for egg weight produced by main and reciprocal crossbreds. Besides, Yahaya *et al.* (2009) reported positive heterosis of egg weight for the main (2.06 to 5.60%) and reciprocal (0.64 to 1.95%) crosses. This study indicated that the cross between WLH hen and Fayoumi cock produced progenies that have the potential to yield heavier eggs than their main crossbred.

Negative and positive heterotic was noted for feed intake in the main and reciprocal crossbreds, respectively (Table 3). Likely, the negative heterotic effect was reported in Mandarah x El-salam cross for feed intake (Taha and Abdei, 2013). The main crossbred was better than their parents by their lower feed intake at brooder ages (day-old to end of 4^{th} weeks) and at grower ages (16-20^{th} weeks) whereas the reciprocal crossbred was better than parents from the age of 4-16^{th} weeks. From this result, it can be concluded that the main crossbred revealed an inverse feed intake to its reciprocal crossbred at different ages. The negative heterotic estimates for the progeny of reciprocal crossbred was observed for FCR at all developmental ages (Table 4). The better efficiency in feed utilization of reciprocal progenies could be due to the inheritance of paternal genetic effect. The negative values of heterosis in either of the main or reciprocal crossbred implied the superiority of parents in efficient feed utilization than F1.

Maternal effect

The positive maternal-effect of body weight for Fayoumi hen and WLH cock crossbreds (Table 4) indicated their heavier body weight. Similarly, positive maternal-effects for body weight were reported by many researchers (Saadey *et al.,* 2008; Lalev *et al.,* 2014). On the contrary, Khalil *et al.* (1999) found that maternal-effects were in favor of White Leghorn for body weight in crossed between Saudi chickens and White Leghorn. A significant maternal effect on the live weight of offspring at an early age (0-8 weeks) was reported by Sabri *et al.* (2000). The F1 of main crossbreed resulted higher positive maternal-effects for weight gain at 0-8 and 12-16 weeks whereas reciprocal crossbred demonstrated positive maternal-effects at 8-12 and 16-20 weeks. The maternal-effect

was more favored the Fayoumi hens during the brooder age but favored the WLH hens during the grower period. This finding disagrees with Taha and Abdei (2013) who reported low and neglected maternal-effects in hybrid chickens for body weight gain. However, Ouyed and Brun (2008) reported negative maternal genetic-effect of daily weight gain. The negative maternal effect for body weight gain indicated that the reciprocal crossbreed progenies performed lower than main crossbred.

A positive and higher heterotic for feed consumption at (4-12 weeks) for F1 of main crossbred which is consistent with Taha and Abdei (2013). The study exhibited that the negative maternal-effect was observed for feed intake which was in line with the report of Emad and Amin (2015). An estimated result of feed conversion ratio was positive maternal influence at all ages except at grower (8-12) weeks of age for F1 of reciprocal crossbred (Table 5). This indicated that the use of WLH as hens and Fayoumi as cocks in crossing would produced the progenies that are less efficient in feed conversion at the respective ages. The negative maternal-effects in chickens for FCR were also reported by Taha and Abdei (2013). The F1 of White Leghorn as hen and Fayoumi as cocks had negative maternal-effect at age at first egg in Table 4. This indicated that the age at first egg was advanced over the pure line genetic groups and this has shown the evidence of maternal influence on age at first egg. Many scholars also found that the evidence of maternal-effects on traits of age at first egg (-1.9; Nawar and Abdou, 1999) and (-8.5; Iraqi *et al.,* 2007) in Dandarawi chickens. The body weight at the first egg for F1 of reciprocal crossbreed had positive maternal-effects than its reciprocal (Table 5).

This was consistent with the finding of Saadey *et al.* (2008) who reported superior maternal-effects for bodyweight at the first egg for Baladi Saudi and White Leghorn cross but Nawar and Abdou (1999) found a negative maternal-effect for body weight at first egg (-4.36) in the crossbreed progenies of White Leghorn and Baladi Saudi chicken. Further, Iraqi *et al.* (2007) reported negative maternal effect for the body weight at first egg (-6.3) in Rhode Island Red in Egypt. The positive heterotic effect of egg weight in main crossbred was superior performance than their parents. A positive maternal-effect for egg weight was also reported by Saadey *et al.* (2008). Besides, Sola-Ojo (2011) observed positive maternal-effect in egg weight for the cross between exotic egg-type strain Dominant Black and Fulani ecotype chickens.

Reciprocal effect

The negative reciprocal-effects were observed (RE) for body weight at day-old, 20th week and age at first egg (Table 4). The use of WLH as a cocks and Fayoumi as a hens would resulted higher body weight from the 4th to the end of 16th weeks. Consistently, Waleed *et al.* (2011) reported positive RE for body weight at hatch. The negative RE for body weight gain at 8-12 and 16-20 weeks was revealed lower weight gain in F1 of WLH as cocks and Fayoumi as hens. On the contrary, the positive and negative reciprocal-effects were reported for body weight gain at different ages in diallel crosses of Saso, Italian and Mandarah chickens at 0-4, 4-8, 8-12 and 0-12 weeks (Emad and Amin, 2015).

The positive RE was observed for feed intake at age of 4-12 weeks for F1 of WLH cocks and Fayoumi hens (Table 4). Consistently, a positive reciprocal effect also noted for feed intake by Jeremiah *et al.* (2017) for progienies of naked neck and Frizzle crossbreed. Furthermore, many scholars observed positive RE for feed intake at various ages (Nwachukwu *et al.,* 2006; Laxmi *et al.,* 2009; Razuki and Al-Shaheen, 2011). However, the negative RE was observed for feed conversion ratio the crossbreed of WLH cocks and Fayoumi hens except at 8-12 and 12-16 weeks of age, this implies that the F1 of White Leghorn as cocks and Fayoumi as hens generate offsprings with better FCR than reciprocal crossbreed. The negative RE was observed when WHL as hens and Fayoumi as cocks crossbreed for age at first egg and this indicate that the earlier maturity of reciprocal crossbred than the progenies obtained from main cross. Similarly, the negative RE for age at sexual maturity also reported by (Bahie *et al.,* 1998). The positive and higher RE was illustrated for body weight and egg weight at the first egg of WLH as a hens and Fayoumi as a cock crossbreed. This implied that the crossbreed progenies were higher in body weight and produced heavier eggs than pure lines at age at first egg.

Conclusions

In conclusions, Fayoumi hens crossbreed with White Leghorn cocks progenies were the highest body weight; where it's reciprocal crossbreed progenies were the most feed efficient. The progenies obtained from White Leghorn hens crossed with Fayoumi cocks had negative reciprocal effect on age at first egg and it had positive and higher reciprocal effect for body weight at first egg. This study was conducted from hatch to age at first egg and it recommends the crossbred of Fayoumi cocks and White Leghorn hens should be used for their earlier sexual maturity.

Acknowledgment

The authors are greatly thankful and appreciate the staffs of Poultry farm who assisted during the study. Moreover, the authors also thanks to Haramaya University for research grant.

Conflict of interest

No potential conflict of interest among the authors.

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Small Ruminant *Brucella* Sero-prevalence and potential risk factor at Dallo-Manna and Haranna-Bulluk Districts of Bale Zone, Oromia regional state, Ethiopia

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Abstract

A cross-sectional study was carried out on randomly sampled 384 animals to assess the occurrence of small ruminant brucellosis and risk factors contributing for the zoonotic potential of the disease at Dallo-Manna and Haranna-Bulluk districts of Bale Zone. Rose Bengal plate test (RBPT) and complement fixation test (CFT) were used serially. All collected serum samples were subjected to RBPT first and then positive sera with RBPT were further tested for confirmation using CFT. Animal level prevalence of 6.5% and 2.9%, and flock level prevalence of 50% and 22% were recorded by RBPT and CFT respectively. Flock level prevalence at Dallo-Manna is 3.8-fold (95% OR CI = 1.17-12.19) than at Haranna-Bulluk (95% OR CI = 0.32-3.31) but no statistical significant difference ($p>0.05$). The Chi-square ($\chi 2$) statistical analysis indicated that age ($\chi 2=6.18$; $p<0.05$), parity ($\chi 2=0.57$; $p<0.05$), retained fetal membrane ($\chi 2=35.5$; $p<0.001$) and abortion history ($\chi 2=45.1$; $p<0.001$) were associated with *Brucella* sero-reactors in study areas. Small ruminant with history of retained fetal membrane (OR=3, CI: 3.52- 27) and small ruminant with abortion history (OR=32, CI: 2.26-462.8) were also found significantly associated with seropostiveity. Questioner survey revealed only 30% of the respondents were aware of the small ruminant brucellosis. Most of them (84%) handle aborted materials with bare hand, 94% of the respondents mix sheep and goat at grazing field and watering point. Traditionally the habit of raw milk consumption is com-

mon (100%). In conclusion, the result of this study demonstrated the presence of *Brucella* sero-reactors at moderate level in small ruminants and identified certain predictors of the infection. Therefore, based on the findings, authors suggest the need for further investigation on the disease-causing agent to take proactive control intervention measures. Meanwhile, actors need to work on raising public awareness to prevent the risk of public health hazard due to *Brucella* infection.

Key words:- Brucellosis; CFT; RBPT; Small ruminant; Zoonosis

Introduction

Brucellosis is known by different names in different host species including Melitococcosis, undulant fever, Malta fever, Mediterranean fever (in man); contagious abortion, infectious abortion, epizootic abortion (in animals); Bang's disease specifically in bovine (WHO, 2001). The disease is a highly infectious zoonotic. Wide species of domestic and wild animals suffer from the diseases worldwide, particularly in developing countries. Brucellosis is caused by facultative, intracellular and Gram- negative bacteria called *Brucella* (Pal *et al.,* 2013, Adem and Duguma, 2020). Based on the differences in host preference and biochemical properties, *Brucella* genus classified into six (6) recognized/ classical species (Osterman and Moriyon, 2006), that is *B. abortus* (cattle), *B. melitensis* (sheep and goats), *B. suis* (pigs), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (wood desert rats). Recent isolates from human (*B. inopinata*), from aquatic mammals (*B. pinnipedialis* and *B. ceti*), and from common vole (*B. microti*) are recognized as new species (Paul *et al.,* 2015). To date, 12 different *Brucella* species have been described including two most recently described species, *B. papinios* isolated from retained placenta of baboons (Whatmore *et al.,* 2014), and *B. vulpis* isolated from the mandibular lymph nodes of red foxes in Australia (Scholz *et al.,* 2016).

Cross transmission of brucellosis can occur between cattle, sheep, goats, camels, equines and other domestic and wildlife (Dawood, 2008). Small ruminant brucellosis is caused by *B.ovis* (for sheep) and *B. melitensis* (mainly for goats), the latter one is the most virulent species of the *Brucella* genus (Pal, 2007). The disease in naturally infected sheep and goats is characterized by abortion in the last trimester of pregnancy, stillbirth and birth of weak offspring in females, and acute orchitis and epididymitis in males (Corbel, 2006). Transmission of Brucellosis in human occurs through breaks in the skin, following direct

contact with infected tissues, blood, urine, vaginal discharges, aborted materials (fetuses or placentas), and food-borne infection occurs following ingestion of raw milk and other milk products from infected animals, but rarely from eating raw or undercooked meat of infected animals and accidental inoculation of live vaccine and occupational exposure of infection also occur in human (Gameel *et al.,* 1993).

Complex nature of brucellosis makes it harder to treat effectively, but, long-term treatment with a combination of an antibiotic is thought to be beneficial. However, the state of the disease still does not lose its importance (Moon, 2014). To control the disease in human, prevention of the disease in reservoir host is important, so test and slaughter followed by proper disposal of seropositive animals to decrease the incidence of infection and effective vaccination and hygienic practices would reduce the disease spreading in/from endemic regions (Li *et al.,* 2017). The obvious way to do this elimination of the disease from animals is often beyond the financial and human resources of many developing countries. In many situations there is little alternative but to attempt to minimize impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene (WHO, 2001).

Brucellosis is often persisting in the poorest and most vulnerable populations (FAO, 2003). In Africa and Central Asia where the disease is still endemic, the incidence of brucellosis is generally considered higher in livestock raised in pastoral production systems (McDermott and Arimi, 2002). In these settings, where the disease is still endemic, the prevalence of human and animal brucellosis may remain increasing, and factors such as low awareness, poor understanding of brucellosis and absence of control policies along with limited resources could be the main reasons (Ismail *et al.,* 2019). Even though, there was no published data on small ruminant brucellosis in the study area, existing of risk factors to *Brucella* infection are not uncommon in pastoral and agro- pastoral areas of Ethiopia (Anteneh, 2014), and the previous studies in different geographical areas of Ethiopia shows the sero-positivity of small ruminants brucellosis (Abegaz and Yimar, 2018; Haile *et al.,* 2018; Lakew *et al.,* 2019; Yeshibelay and Teferi, 2019). Like other developing countries, in pastoral and agro-pastoral areas of Ethiopia, there is a limited information on prevalence of the disease and knowledge, Attitude, practices (KAP) of the communities about brucellosis (Tilahun *et al.,* 2013; Legesse *et al.,* 2018).

The importance of doing such research on Small Ruminant *Brucella* Sero-prevalence and potential risk factor at Dallo-Manna and Haranna-Bulluk districts of Bale Zone, has two major benefits. Frist, as it has been discussed in the problem statement part, no research has been done so far to estimate sero-prevalence of small ruminant brucellosis in the areas and hence the finding of this study serves as a good basis for forthcoming researchers who have a strong desire to carry out a research on this or related topics in Bale Zone, or elsewhere. Second, this study will also assess the knowledge, attitudes and practices associated with small ruminant brucellosis in Dallo-Manna and Haranna-Bulluk agro-pastoral districts of Bale zone in order to determine the risk factors that contribute to spread of the disease and to gain evidence-based information geared towards prevention and control of brucellosis both in animals and humans in the future. Therefore, the objectives of the study were to estimate the status of small ruminant brucellosis and risk factors contributed to the disease in livestock and human in study areas

Materials and methods

Description of study Area

The study area falls within two districts of the Bale zone, namely Dallo-Manna and Haranna-Bulluk. The districts have been formed in 2005 through the division of Manna-Angetu district and located at about 540 km southeast from the capital city, Addis Ababa. The study districts are lies between 39°15'0"-40°15'0" E Longitude and 6°17'30"-6°45'0" N Latitude (Flintan *et al.*, 2017). The annual rainfall pattern in the area is the bi-modal type, i.e., March through April (short rain season) and August through October (long rain season). Mean annual rainfall in the area actually varies from around 700 to over 1200 mm and the mean annual temperature is 18^0C (Tadesse and Feyera, 2008). Even though, the exact figure is difficult to know, unpublished data from Bale zone pastoral development office in 2019 shows that the livestock populations of both districts are composed of cattle 499,403, goats 235,661, sheep 69,901, donkeys 27,524, camels 43,573, horse 8,716 and mule 4,438.

Study designs and study animals.

A cross-sectional multistage sampling technique was involved to estimate the sero-prevalence and associated risk factors of small ruminant brucellosis. The study was carried out on local breeds of sheep and goats kept under extensive

type of management system in the study areas were considered as a study population. Sheep and goats which were above 6 months of age and apparently healthy were included in the study. In this report, the term flock refers to a number of domestic animals, especially sheep, goats, or geese that are kept together.

Sampling procedure and sample size determination

Two districts namely Dallo-Manna and Haranna-Bulluk were selected purposively based on accessibility, the number of small ruminant population and willingness of the community in the districts. Both districts have 14 peasant associations (kebeles) each. Selection of 4 out of 14 kebeles from each district and 24 villages from a total of 84 villages in both districts was based on random sampling. Sampling of individual animals were applied randomly on the flocks of small ruminants found in selected villages, after relevant individual animal level information were recorded.

The study sample size was determined according to (Thrusfield, 2018) formula for a large population with 95% confidence level, 5% desired absolute precision by considering an expected prevalence of 8.1% (Wubishet *et al.,* 2018) in Yabello districts of Borena Zone and 6.2% (Wubishet *et al.,* 2017) in Liban District of Guji Zone. Therefore, taking an average prevalence of two areas as an expected prevalence, calculated sample size was 107. However, to increase precision, the sample size was increased by 3.59-fold. Accordingly, 384 small ruminants were sampled based on proportional allocations of the sample size for each Kebele.

For questionnaire survey sample size was calculated using the formula given by (Arsham, 2005); N = 0.25/SE2, where: N = sample size, SE (standard error) = 5%. Thus, the required sample size for the questionnaire survey was 100. However, only 50 volunteers were included.

Sample collection

Questionnaire survey

Semi-structured questionnaire was administered to 50 flock owners, one (1) owner per flock of Small ruminants by the local language during taking blood sampling from the animals.

The questionnaire was designed for a survey of the potential risk factors associated with zoonotic brucellosis in sheep and goat flocks. Thus, the relevant information such as overall small ruminant flocks management practices, occurrence of abortion and presence of retained fetal membrane, knowledge about zoonotic diseases, habit of consuming raw milk and meat, handlings of aborted fetuses and contaminated materials.

Serological survey

Approximately about 5 ml of whole blood sample was collected from the jugular vein of each small ruminants included in the study using plain vacutainer tubes and needles. Each sample tube was labeled using codes specific to the individual sample. Collected samples were kept in a slanting position overnight at room temperature to separate the serum and the clotted red blood cells (OIE, 2009). Then sera were gently transferred into sterile screw cupped Nunc tubes, labeled and transported in cold chain to Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu and stored at -20°C until screened and tested for antibodies against natural *Brucella* exposure analysis using Rose Bengal Plate Test (RBPT) (Radostits *et al.,* 2007). RBPT was done at Addis Ababa University, College of Veterinary Medicine and Agriculture, by using, *B.abortus* antigen and all serum samples collected were screened, according to the procedures described by (Alton, 1990; OIE, 2009). The presence of agglutination was considered positive reaction while the absence of agglutination was considered negative. Positive sera with RBPT were further tested with Compliment Fixation Test (CFT) for confirmation using standard *B.abortus* antigen at National Veterinary Institute (NVI). The preparation of reagents and CFT procedures were performed according to the protocols of the Federal Institute for Consumer Protection and Veterinary Medicine Service Laboratory, Berlin, Germany (OIE, 2009). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above or absence of sedimentation of Red blood cells were considered as positive and lack of fixation/ complete hemolysis or the presence of sedimentation of Red blood cells were considered as negative.

Data Analysis

The data obtained from both serological tests and questioner surveys were entered into a computer on a Microsoft Excel spreadsheet. Descriptive and analytic statistics were computed using software SPSS® Version 20.0. The Chi-square ($\chi 2$) and logistic regression tests were employed to identify possible association between risk factors and reproductive characteristics with seropositive to *Brucella* infection. The degree of association was considered significant when a p-value of less than 0.05 is obtained or when the 95% confidence intervals (95% CI) in the logistic regression analysis doesn't include one or if odds ratio (OR) is different from one (Thrusfield, 2018).

Results

Association of risk factors with Brucellosis at individual animal level

For an individual sero-prevalence, among 384 small ruminant, 25 (6.5%) tested positive by RBPT. From these, 11 (2.9%) animals were confirmed positive by CFT. Associations of the putative risk factors were computed by Pearson's Chi-square test and the sero-prevalence of small ruminant brucellosis in abortion history, history of retained fetal membrane, parity and age were all statistically significant ($p<0.05$).

All significant variables in Pearson's Chi-square test were also showed statistically significant ($p<0.05$) with sero-prevalence of small ruminant brucellosis in the univariable logistic regression analysis (Table 1).

Table 1. Effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis using CFT.

Risk factors		Complement fixation test		
Variable	**Category**	**OR**	**95%CI**	**p-value**
Parity	No-parity *	2	1.43-8.14	0.038
	Primiparous			
	Pluriparous			
Flock size	<15	1	0.47-5.67	0.436
	>15			
District	Haranna-Bulluk	1	0.56-6.80	0.643
	Dallo-Manna			
Species	Sheep	1	0.29-2.32	0.522
	Goats			
Sex	Male	1	0.34-4.12	0.369
	Female			
Age	Young*	9	1.12-69.74	0.013
	Mature			
History of Abortion	Absent*	71	8.23-603.9	0.000
	Present			
History of RFM	Absent*	32	6.04-169.9	0.000
	Present			

*Reference category; OR: Odds ratio; CI: Confidence interval.

The result of multivariate logistic regression model indicated that animals with history of retained fetal membrane (OR=3, CI: 3.52- 27) and animals with history of abortion (OR=32, CI: 2.26-462.8) were also found evident in multivariable logistic regression analysis (Table 2).

Table 2. Multivariable logistic regression analysis of risk factors and small ruminant brucellosis

Variables	Complement Fixation test		
	OR	**95% CI**	**p-value**
History of abortion	32	2.26-462.8	0.000
Retained fetal membrane	3	3.52-27	0.014

Sero-positivity of Small-ruminant brucellosis at flock level

Out of 50 flocks included in the study half of them (50%) were positive using RBPT and 11 flocks (22%) was positive using CFT. Using RBPT, prevalence in Dallo-Manna is 3.8 fold (95% OR CI=1.17-12.19) than Haranna-Bulluk showing not statistically significant association with p-value >0.05 (Table 3), the difference may be due to, many large flock size population of small ruminant (≥15) were sampled from Dallo-Manna (17 large flocks), while only 9 large flocks were from Haranna-Bulluk. But it was similar at Dallo-Manna (25%) and Haranna-Bulluk (19%) using CFT.

Table 3. Flock level Sero-prevalence of small ruminant brucellosis

Study districts	№ of Examined Flocks	RBPT			CFT		
		№ (%)	OR	95% CI	№. (%)	OR	95% CI
Dallo-Manna	24	16 (67)	3.8	1.17-12.19	6 (25)	1	0.37-5.37
Haranna-Bulluk	26	9 (35)	1	0.32-3.31	5 (19)	1	0.25-3.97
Total	50	25(50)			11(22)		

Knowledge of brucellosis

A total of 50 respondents, (66% male and 34% female), were interviewed to assess their knowledge, attitude and practices towards brucellosis in both districts (24 from Dallo-Manna and 26 from Haranna-Bulluk). Little difference was observed on awareness of Brucellosis between study districts. The awareness on the zoonotic importance of the small ruminant Brucellosis in the study districts were increasing with age of the respondents. Out of all respondents 30% were aware on the zoonotic importance of Brucellosis (Table 4).

Table 4. Participants awareness on zoonotic Brucellosis in study area (n = 50)

Parameters of Study		№ of Respondents	№ (%) of respondents with awareness
Districts	Dallo-Manna	24	6 (25)
	Haranna-Bulluk	26	9 (35)
Sex	Male	33	11 (33)
	Female	17	4 (24)
Age	Young	14	6 (43)
	Adult	30	8 (27)
	Old	6	1 (17)
Total		50	15 (30)

Most of the respondents (84%) handle aborted materials with bare hand without protecting themselves. Mixing of shoats (sheep and goat) at day time was practiced by 47 (94%) owners. Most of the flocks 43 (86%) recognized the chance of contact with other flocks at grazing and watering. The habit of drinking raw milk was present all (100%) of the respondents but almost all 48 (96%) with no habit of consuming raw meat. Children are the most responsible personnel for rearing of the flocks 48 (96%), while house wife share the remaining responsibility in rearing/herding. The majorities of milking (78%) were practiced by women while 22% by children (Table 5).

Table 5. Small ruminant management practices and utilization of products associated to transmission of *Brucella* to human and animals

Study parameters	Categories	Probability of contracting the disease № (%)
Removal of aborted materials	Bare hand	42 (84)
	Protected hand	8 (16)
Management way of aborted materials	Feed Dogs	14 (28)
	On field and tree	35 (70)
	Burying	1 (2)
Raw milk consumption	Yes	50 (100)
	No	0 (0)
Raw meat consumption	Yes	2 (4)
	No	48 (96)
Keeping of sheep and goat at day time	Mixed	37 (74)
	Separated	13 (26)
Keeping of sheep and goat at night time	Mixed	3 (6)
	Separated	47 (94)
Contact with other flock at watering and grazing	Present	43 (86)
	Absent	7 (14)
Responsible person for rearing/herding	Children	48 (96)
	Husband	0 (0)
	Wife	2 (4)
Responsible person for milking	Wife	39 (78)
	Children	11 (22)
	Husband	0 (0)
Occurrence of abortion in female animals	Present	27 (54)
	Absent	23 (46)
Human clinical signs (headache, fever, back pain and night sweeting)	Present	36 (72)
	Absent	14 (28)
Sick person visits clinic or hospital	Yes	18 (36)
	No	32(64)

Discussion

In the present study the overall sero-prevalence of small ruminant brucellosis using CFT was 2.9%. The difference in prevalence between the districts was not

statistically significant. It could be due to the similarity in the agro-ecological conditions and livestock management system in the area. This finding is fairly in agreement with some recent studies conducted in selected pastoral and agro- pastoral low lands of Ethiopia (Sintayehu *et al.,* 2015), Tselemti districts of Tigray region (Kelkay *et al,* 2017), southern zone of Tigray region (Teklue *et al.,* 2013). Selected Settlements of Dire Dawa Administrative Council Area, Eastern Ethiopia (Haile *et al.,* 2018), in Werer Agricultural Research Center, Afar Region, North East Ethiopia (Bezabih and Bulto, 2015) with prevalence of 1.9%, 1.8%, 3.5%, 2.6% and 2.3% respectively. This could be attributed to the similarity in agro-ecological conditions and livestock management system in the areas (Teshale *et al.,* 2006). However, the result of this study is lower than the observations recorded in Tallalak district of Afar region, Ethiopia (Tadeg *et al.,* 2015), in Chifra and Ewa districts of Afar Region (Tegegn *et al.,* 2016), in Yabello districts of Borena Zone Oromia Regional State, Southern Ethiopia (Wubishet *et al.,* 2018), in selected Kebeles of Amibara district of afar region (Muluken, 2016) with sero- prevalence of 13.7%, 12.4%, 8.1% and 7.5% respectively. This difference might be due to the diagnostic test used, the differences in breeding, animal management, production systems and husbandry practices (Teshale, 2006). In contrast, the observation of current study is higher than sero-prevalence rates of 0.8% reported in Babile Woreda, Eastern Hararghe, Ethiopia (Yeshibelay and Teferi 2019), 0.4% in and around Bahir Dar, North West Ethiopia (Ferede *et al.,* 2011), 0.7% in and around Kombolcha, Amhara Regional State, North-Eastern Ethiopia (Tewodros and Dawit, 2015) and this variation in sero-prevalence could be due to difference in management system, the difference in sample size used and agro-ecology.

Brucellosis is considered as disease of flock importance, in this study flock level Sero-positivity of 22% was found lower when we compared to (Adugna *et al.,* 2013) (50.5%) and (Anteneh, 2014) (57.8%) in Afar. This could be due to the small number of sample size in the present study in each study districts. The flock level prevalence is higher than individual animal level and this characterizes the nature and importance of the disease in the large flock size. This concept coincides with the current study that the sero-prevalence of brucellosis between the categorized flock sizes (<15 and ≥15) showed higher sero-prevalence recorded in the large flock sizes (3.5%) than that of small flock sizes (2.2%) of small ruminants. This result was in agreement with the previous reports in Afar region (Adugna *et al.,* 2013).

The higher sero-prevalence in goats (3.4%) than in sheep (2.3%) in this study was in consistent with that of (Adugna *et al.,* 2013; Bezabih and Bulto, 2015; Lakew *et al.,* 2019). The difference in sero-prevalence between species may be in part due to the greater susceptibility of goats to *Brucella* infection than sheep and partly it may be due to the fact that sheep unlike goats do not excrete the *Brucella* organisms for longer periods of time. This can reduce the potential of the spread of the disease among sheep flock (Radostitis *et al.,* 2007).

The study revealed that there was statistically significant difference ($p<0.05$) in sero-prevalence of brucellosis between the young and mature age groups, higher sero-prevalence was found in mature animals (4.8%) than young animals (0.6%). This finding was in line with the study conducted in Werer Agricultural Research Center of Afar Region, with sero-prevalence of 2.7% in mature and 0% in young animals (Bezabih and Bulto, 2015). Sexually matured animals are more prone to *Brucella* infection than sexually immatured animals of either sex (Radostits *et al.,* 2007). This might be due to the fact that as sex hormones and erythritol tend to increase in concentration with age and sexual maturity and favor growth and multiplication of *Brucella* organisms (Radostits *et al.,* 2007). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection (Gyles and Prescott, 2004) although latent infections can occur (Walker, 1999).

There was statistically significant association ($p<0.05$) among parities and the sero-prevalence of the disease. The sero-positivity of female sheep and goats with the history of no parity, Primiparous and Pluriparous were 0 (0%), 1(1%) and 7(6%) respectively. This is therefore, in consistent with the previous study (Yohannes et al., 2013; Anteneh, 2014), this might be due to repeated exposure of the female animals to parturition and other physiological stress increases the probability of acquiring *Brucella* infection.

The analysis result also revealed that the prevalence of brucellosis between sexes did not show significant association ($p>0.05$). The prevalence was higher in females (3.5%) compared to prevalence in male (1.9%). The present finding was in agreement with the records obtained from (Mengistu, 2007) who was report brucellosis in females (3.2%) and males (1.2%) in Adamitulu-Jido Kombolcha District, Oromia Regional State, Ethiopia. Higher susceptibility of female animals could be due to the fact that they have more physiological stresses than the males (Walker, 1999). In addition, male animals are less susceptible to *Brucella* infection due to the absence of erythritol (Hirsh and Zee, 1999). It

may be due to supply of male to markets immediately upon maturation than female or shorter exposure period, while female serve as a source of milk (longer exposure period). The results obtained in this study revealed that, abortion and retained fetal membrane appears to be major risk factors for brucellosis compared with other risk factors (p=0.000). This result supports the truth that reproductive problems like abortion and retained fetal membrane in small ruminant can be caused by brucellosis (Walker, 1999).

Brucellosis is transmissible from animals to humans through contaminated milk, raw milk products, and direct contact with infected animals. In the current questionnaire survey, most of the respondents (54%) recognize the occurrence of abortion in their small ruminant flocks, but only 30% of them aware of the brucellosis and most of the respondents (84%) were used to handle retained fetal membranes and assist delivery with unprotected hand, which have risk of transmission while contact (Radostits *et al.,* 2007). All (100%) of the participants in the interview consume raw milk which is one of the sources of human Brucellosis (OIE, 2009; Radostits *et al.,* 2007), in contrast almost all (96%) of the respondents practiced consumption of cooked meat, which reduces the risk of getting infected with *Brucella*. Although, 94% of the respondents in the studied community keeps their animal separately at night time, 74% of them mix their sheep and goats at day time and 86% of them use communal grazing land with neighbor flocks. This may facilitate the transmission of the disease from infected flocks to disease free flocks, through contact during grazing and watering. Intermixing of different species and flocks of livestock occur creating a potential risk factor for interspecies and inter flock's disease transmission. Overall, mixing of different flocks and different species of animals in the study districts; lack of community awareness about brucellosis; and the habit of raw milk consumption might greatly contribute for further spread of brucellosis (Muluken *et al.,* 2017; Lakew *et al.,* 2019).

Conclusions

The sero-prevalence described in this study shows that brucellosis is a widespread and well-established infection between small ruminants and there were risk factors to the occurrence of the disease in livestock and human across the study districts of Bale zone. However, as the diagnostic tests used were serological there is a need for further investigation to look for circulating *Brucella* biotype so as to identify the target species for control intervention. Meanwhile,

brucellosis being a disease of economic and public health significant, there is a need for intervention through creation of public awareness.

Acknowledgements

This Study was financially supported by thematic research project entitled "Epidemiological and bacteriological studies of Major livestock disease of economic and zoonotic significance in Ethiopia" funded by Addis Ababa University. We would like to acknowledge Mr. Muluken Tekle for his Laboratory assistance at college of Veterinary medicine and agriculture of Addis Ababa University, Bishoftu. Dr. Sufian Abdo, W/ro Rukia Hussein Kubo and Mr. Gazzali Muhammad and Animal owners are highly acknowledged for their assisting during sample collection in Dallo-Manna and Haranna- Bulluk districts.

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Effects of Neem (*Azadirachta indica)* and Pumpkin *(Cucurbita maxima)* Seeds and their Combination as Feed Additive on Intake, Muscle chemical composition, Sensory Quality and Hematology of Broilers

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Abstract

A study was conducted to evaluate the efficacy of feeding neem (***Azadirachta indica***) and pumpkin (***Cucurbita maxima***) seed as natural feed additive for broiler chicks on dry matter intake, mortality, meat quality and blood parameters of broilers. One hundred ninety two day-old Cobb 500 chicks distributed to four treatments with three replications in a completely randomized design. Feed offered and refusals were recorded and Dry matter intake was calculated as the difference between the two on dry matter basis. At the end of the trial, four broilers were randomly picked up from each replication and slaughtered for carcass evaluation and the treatment used were ration that contain only commercial broiler diet (0 kg Neem and pumpkin seed (0NS-PS)), 1kg neem seed on 100kg commercial broiler diet (1NS), 1kg pumpkin seed on 100kg commercial broiler diet (1PS) and 1kg of neem and pumpkin seed combination on 100kg of commercial broiler diet (1NS-PS) stands for Treatment1, Treatment 2, Treatment 3 and Treatment 4 respectively. The average daily dry matter intake during the entire experimental period was 106, 111, 114 and 117 g/bird for 0NS-PS, 1NS, 1PS and 1NS-PS respectively, and it was significantly higher ($p<0.05$) for 1NS-PS as compared to 0NS-PS, 1NS and 1PS. The serum cholesterol and white blood cell of broilers in the experimental period were significantly ($p<0.05$) decreased but total blood protein was significantly ($p<0.05$) increased among treatment. Crude protein content of breast and thigh meat was significantly high for 1NS-PS. It is concluded that neem and pumpkin seed can be a good feed additive for broiler production aside its nutritional importance.

Key words: Broiler; Feed additive; Hematology; Neem and Pumpkin seed.

Introduction

Chicken meat and eggs have been recommended to bridge the protein gap more than other species of livestock because of short generation interval, high rate of productivity, quick turnover rate, higher feed efficiency, small land requirement and relatively low capital investment (Ani and Okeke, 2011).

Feed additives are plant-derived products incorporated into diets to improve the productivity of livestock through improvement of feed properties, promotion of the animals' production performance, and improving the quality of food derived from those animals (Windisch *et al*., 2008). The objective of feed additives is maintaining greater livability and lowered mortality in poultry chickens. These feed additives are termed as growth promoters and also stimulate feed intake (Ihsan, 2017). Modern intensive poultry production has achieved great gains in the production of high-quality safe chicken meat and eggs. Biologically active constituents of herbs possess many beneficial properties. Many synthetic drugs and growth promoters are supplemented to broilers to effect rapid growth, but their use have shown many disadvantages like high cost and adverse side effect on health of broilers and human, human becoming resistant to the antibiotic and residual effect. There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics and herbs (Banerjee, 2006). The use of medicinal plants is gradually gaining importance as natural products have a medicinal value against various diseases and have no residue in tissues and eggs. One of the plants is *Azadirachta indica*, commonly known as "Neem", and contains various active substances such as azadiractin, nimbin, nimbindin, quercetin and others which have antioxidant, antibacterial, antifungal, anthelmintic and antiprotozoal properties beside immuno-stimulatory effects the finding of Jawad *et al*. (2013); Ahsan *et al*. (1999) observed that the beneficial influence of neem leaves on antigen in broiler chickens.

Similarly, pumpkin (*Cucurbita maxima*) seed is one of the alternatives that have the potential to serve as protein source in the poultry industry because of its high crude protein content (Wafar *et al*., 2017). Besides its use as hypoglycemia agent pumpkin seed applications use for the treatment of parasites and hypercholesterolemia (Kerise *et al*., 2008). The pumpkin seed contains a substance called cucurbitin that treats worms and parasites (Bauri *et al*., 2015; Acorda *et al*., 2019). However, there is no information available about the integral utilization (combined effect) of Neem (*Azadirachta indica*) and pumpkin

(*Cucurbita maxima*) seed combination in broiler feeding. Therefore, the current experiment was conducted to evaluate the effect of feeding neem (*Azadirachta indica*) and pumpkin (*Cucurbita maxima*) seed as natural feed additive on intake, meat sensory characteristics, hematology and serum biochemical aspects of broilers.

Materials and methods

Experimental site

The experiment was conducted in Poultry Farm at Haramaya University, which is located at 42° 3' east longitude, 9° 26'north latitude, at an altitude of 1980 meter above sea level and 515 km east of Addis Ababa, the capital city of Ethiopia. The mean annual rainfall of the area amounts to 780 mm and the average minimum and maximum temperatures are 8.5°C and 23.4°C, respectively (Mishra *et al*., 2004).

Feed additives and rations

The starter and finisher commercial broiler diets were purchased from Debre Zeit Alema Koudijs farm. Dietary ingredients used for this study were maize grain, wheat short, soybean meal, Noug seed cake, salt, vitamin premix, limestone and dicalcium phosphate. Dried neem seed was collected from Dire Dawa town and pumpkin seed was purchased from Harar. The Dried neem and pumpkin seed were ground in the size of adjusted hammer mill at 5 mm by at Haramaya University feed processing plant. The obtained powder was used as feed additive in the broiler ration. The starter phase was until 3 weeks of age. The finisher phase was offered from 3 weeks up to slaughtering (42 days).

Experimental design and treatments

Completely Randomized Design (CRD) with four treatments and three replications were used in the study. One hundred ninety two day-old chicks were randomly allotted in to four treatments. The treatments were only commercial broiler diet without neem seed powder and pumpkin seed powder (control), 1kg of neem seed powder on 100kg commercial broiler diet, 1kg of pumpkin seed powder on 100kg commercial broiler diet and 1kg of neem seed powder and pumpkin seed powder combination on 100kg commercial broiler diet which were termed as 0NS-PS, 1NS, 1PS and 1NS-PS, respectively.

Experimental chicken management

The experimental houses were cleaned and disinfected two weeks before of the arrival of the chicks. The pens were washed with water and detergent then disinfected by HI-7(1L/330L water) and the chicks were kept on floor covered with wood shaving litter material of about 6.5cm depth. The feeding and drinking troughs were properly cleaned, dried and disinfected before chicks' arrival. Experimental chicks (Cobb500) were purchased from Debre Zeit Alema Koudijs farm. Each pen was also equipped with a 250-watt infrared heat bulb. The chicks were fed *ad libitum* in groups in feeder throughout the experimental period. The neem and pumpkin seed powder added at rate of 1kg/100kg diet alone and their combination of 0.5kg/100kg each added to diet for treatment. Water was available at all times, and provided in plastic fountains, as well as the watering troughs. The experiment was conducted for a total of 42 days. The chicks were vaccinated against Newcastle Disease on day 7 (HB1) by ocular route and on day 21 (Lasota) was given through by drinking water. The study animals were also vaccinated for Gumboro on day 14 and 28 through drinking water. Other health precautions and disease control measures were taken throughout the study period. Vitamins were given to chicks through drinking water to recover from stress of transportation and early age acclimatization problems according to the manufacturer's recommendation.

Measurements

Dry matter intake

The amount of feed offered and refused per pen was recorded daily. The amount of feed consumed was determined as the difference between the feed offered and refused on DM basis.

Chick mortality

Daily monitoring of the chickens was followed as a routine activity to check the health status and to record deaths. Mortality was recorded as it occurred and was determined for each treatment as a percentage of the total mortality at the end of the whole experiment. Calculation of mortality percentage /MP/ is expressed as percentage as follows:

$$\text{Mortality percentage(MP)} = \frac{\text{Number of dead chicks}}{\text{Number of total chicks}} * 100$$

Hematological and serum biochemical parameters

Blood samples were collected at slaughter randomly from the jugular vein of 4 chicks (5 ml) from each replicate A sample of 2.5 ml of blood was drawn into EDTA (ethylene diamine tetraacetic acid) tube in order to prevent coagulation while the remaining 2.5 ml was drawn in plain tube and left to coagulate. RBC and WBC were determined by manual methods by using improved neubauer-hemocytometer chamber (Bernard *et al*., 2000). Hemoglobin concentration was determined by using acid hematin or Sahli's methods. The packed cell volume (PCV) was determined by microhematocrit method after the blood in EDTA was centrifuged at 3000 rpm for 5 minutes. Finally, Serum was harvested from blood collected in plain tube to Endorphin tube and stored at -20°C and analyzed for serum chemistry parameters with an automated chemistry analyzer (Doumas *et al*., 1981).

Chemical composition of meat

Chemical composition of the meat samples was analyzed following the procedure (AOAC, 1995). Samples of breast and thigh muscles were minced, dried and ground with 1mm size by mechanical miller then analyzed for the content of dry matter, crude protein, ether extract and ash. Dry matter was determined by drying 6 g of ground meat samples in a draft oven at 105°C for 24 hrs. Nitrogen (N) was determined according to Kjeldahl procedure and crude protein content of the sample was calculated as N*6.25 (AOAC, 1995). Total lipid (ether extract) content of the muscles determined following the standard procedure (AOAC, 1995). Total mineral content was determined by burning 6 g of the samples in a muffle furnace at 550°C for 3 hours.

Sensory evaluation of meat

The sensory parameters determined were juiciness, tenderness, flavor and overall acceptance. Skinless breast and thigh muscle samples were frozen until cooking, the pieces were thawed at room temperature minced and cut into 2.5 cm cubes by using knife. Breast meat was cooked for 15 min on a pan by vegetable oil, but without salt. Thigh cooking required 2 min more than the breast. After cooking, the pieces were cooled to room temperature. The breast and thigh meats were evaluated following the sensory profile procedure (ISO, 2003). The panel consist 20 trained graduating classes of undergraduate and postgraduate students of Food Science Department of Haramaya University. Panelists were instructed to chew and taste the meat, drink water and rinse

their mouth with bottled drinking water of room temperature between each sample and pause for 20 seconds before tasting the next sample.

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2002) version 9.2. When the analysis of variance revealed the existence of significant differences, differences among treatment means were compared using least significant difference (LSD) test.

The model used for data analysis is;
$Yij = \mu + Ti + eij$
Where:
Yij = the response variable (is an observation (experimental unit),
μ = Over all mean
Ti = Treatments effect
eij = Random error term

Results

Dry matter intake

The average daily and total dry matter intake (DMI) during the starter and finisher phases as well as for the whole experimental period was significantly affected ($P<0.05$) by treatment (Table 1). Accordingly, addition of neem seed, pumpkin seed and their combination to the diet has improved DMI as compared to the control during the entire period. DMI increased with addition of neem seed and pumpkin seed alone and their combination.

Table 1. Effect of feeding neem and pumpkin seed powder and their combination on dry matter intake of broilers

Parameter	Treatments				SEM	*p*-value
	0NS-PS	1NS	1PS	1NS-PS		
Starter phase						
Total DMI (g)	1022[a]	944[b]	1009[a]	1033[a]	12.4	0.013
Daily DMI (g/bird/day)	49[a]	44[b]	48[a]	49[a]	0.59	0.013
Finisher phase						
Total DMI (g)	3323[b]	3505[a]	3669[a]	3729[a]	54.6	0.007
Daily DMI (g/bird/day)	166[b]	179[a]	183[a]	186[a]	2.73	0.007
Entire period						
Total DMI (g)	4394[c]	4565[b]	4701[ab]	4810[a]	56.6	0.015
Daily DMI (g/bird/day)	106[c]	111[b]	114[ab]	117[a]	1.38	0.015

[abc]Means within a row with different superscript letters are significantly different; (P<0.05); SEM=Standard error of the mean; DMI=dry matter intake.

Hematological and serum biochemical parameters

Results in Table 2 refer to presence of significant differences (P <0.05) between the control group and experimental group in total protein (TP) and serum cholesterol concentration. Total protein recorded was 3.46 g/dl in 0NS-PS compared with 1NS, 1PS and 1NS-PS which was 4.24, 4.4 and 5.1 g/dl, respectively.

Table 2. Effect of adding neem seed powder, pumpkin seed powder and their combination on some hematological indicators and serum biochemistry of broiler chicks

Parameters	Treatments				SEM	*p*-value
	0NS-PS	1NS	1PS	1NS-PS		
Packed cell volume (%)	33.68	38.98	36.75	38.04	1.096	0.3847
Red blood cell (x10^6/mm)	3.633	4.033	3.55	3.70	0.093	0.2993
White blood cell (x10^3/mm)	66.08[a]	63.27[b]	62.1[bc]	59.34[c]	0.875	0.0032
Hemoglobin (g/dl)	11.35	12.27	10.77	11.30	0.293	0.3761
Serum cholesterol (mg/dl)	106.54[a]	95.50[b]	87.46[c]	85.25[c]	2.668	0.0004
Total blood protein (g/dl)	3.458[c]	4.235[b]	4.40[b]	5.10[a]	0.188	0.0005

[abc]Means within a row with different superscript letters are significantly different; (P<0.05); SEM=Standard error of the mean.

Chick mortality percentage

The results showed that the mortality rate of the broilers (Table 3) was statistically not significant (p>0.05).

Table 3. Effect of adding the neem seed powder, pumpkin seed powder and their combination on mortality percentage of broilers

Parameters (%)	Treatments					
	0NS-PS	NS	PS	INS-PS	SEM	*p-value*
Mortality starter phase	6.25	2.08	2.08	4.16	0.93	0.3630
Mortality finisher phase	4.44	4.30	2.08	4.30	0.96	0.8399
Mortality entire period	10.42	6.25	4.16	8.33	1.29	0.3999

[abc]Means within a row with different superscript letters are significantly different; (P<0.05); SEM=Standard error of the mean.

Sensory evaluation of meat

The panel members did not find differences in the sensorial quality of the breast and the thigh meat (Table 4), which proves that the neem seed powder and pumpkin seed powder alone and their combination did not significantly *(p>0.05*) affect the juiciness, tenderness, flavor, and overall acceptance of the meat from the breast and thigh of broiler chicken.

Table 4. Score of the effect of adding neem seed powder, pumpkin seed powder and their combination on meat sensory characteristics of broilers

Parameters	Treatments					
Juiciness	**0NS-PS**	**1NS**	**1PS**	**1NS-PS**	**SEM**	***p*-value**
Breast	3.90	3.90	3.90	3.70	0.036	0.0951
Thigh	3.50	3.80	3.65	3.65	0.043	0.0799
Tenderness						
Breast	3.75	3.65	3.75	3.85	0.030	0.1189
Thigh	3.55	3.65	3.75	3.53	0.038	0.1457
Flavor						
Breast	3.65	3.80	3.70	3.85	0.054	0.6075
Thigh	3.80	3.70	3.60	3.90	0.051	0.1927
Overall acceptance						
Breast	3.70	3.90	3.70	3.70	0.034	0.0519
Thigh	3.45	3.75	3.80	3.75	0.058	0.1043

[abc]Means within a row with different superscript letters are significantly different; (P<0.05); SEM=Standard error of the mean. Sensory scale = Five-point sensory scale for juiciness, flavor, tenderness and overall acceptance; Juiciness (5 = very juicy, 4 = juicy, 3 = moderately juicy, 2 = dry and 1 = very dry); Tenderness (5 = very tender, 4 = tender, 3 = moderately tender, 2 = tough and 1 = very tough); Flavor (5 = excellent, 4 = good, 3 = accepted, 2 = poor and 1 = extremely poor); Overall acceptance (5 = excellent, 4 = good, 3 = accepted, 2 = poor and 1 = extremely poor).

Chemical composition of meat

The proximate chemical composition of breast and thigh meat of broiler was indicated on Table 5. In this study, 1NS-PS was higher in CP% compared with control and other treatments groups.

Table 5. Effect of adding neem seed powder, pumpkin seed powder and their combination on meat chemical composition of broilers

Parameters (%)	Treatments					
	0NS-PS	1NS	1PS	1NS-PS	SEM	*p*-value
Moisture						
Breast	75.51	75.52	75.59	75.64	0.021	0.0682
Thigh	73.66	73.75	73.70	73.74	0.022	0.4717
Crude protein						
Breast	21.63^{d}	27.32^{c}	28.06^{b}	29.66^{a}	0.914	<.0001
Thigh	20.52^{d}	23.32^{c}	23.37^{b}	24.08^{a}	0.411	<.0001
Ether extract						
Breast	6.85	6.78	6.83	7.18	0.117	0.6715
Thigh	8.29	8.48	7.92	8.66	0.109	0.0641
Ash						
Breast	4.88	5.05	5.09	4.95	0.043	0.3564
Thigh	3.78	3.88	3.79	4.09	0.063	0.3063

^{abc}Means within a row with different superscript letters are significantly different; (P<0.05); SEM=Standard error of the mean.

Discussion

Lower dry matter intake during starter period at inclusion level 1NS than other treatments this may be chickens doesn't adapt to additive at early phase. Whereas, dry matter intake during finisher and entire experiment period were higher in 1NS-PS as compare to control and 1NS. The study of Ogbuemu *et al*. (2011) who reported that neem seed has natural substances that stimulate the appetite for feed intake. Pumpkin seed is used to stimulate animal digestive system by enhance the production of digestive enzymes and improve utilization of digested nutrients through enhanced liver functions (Ihsan, 2017). The effect addition of aromatic plants and their extracts on feeds and drinking water has positive effect on feed intake, feed conversion ratio, growth performance and carcass yield (Alcicek *et al*., 2003).

The result obtained for 1NS-PS was superior to other treatments on DMI, total blood protein and crude protein content of breast as well as thigh muscle. Ihsan (2017) also reported that addition of neem powder in broiler diets significantly higher total protein value. Significantly (p<0.05) lower serum cholesterol concentration was recorded in 1PS and 1NS-PS compared with 0NS-PS and 1NS. The reason for the reduction was polyunsaturated fat in pumpkin seed specifically omega-3 and omega-6 fatty acids (Sirelkhatim and Asha, 2014). Similar research has shown that these essential fatty acids can lower bad cholesterol and prevent cardiovascular disease. Pumpkin seed has been reported to possess cholesterol lowering effect and these results are in agreement with the observations of previous researchers (Meineri *et al*., 2018; Fruhwirth and Hermetter, 2007). This is similar with the finding of Perez-Gutierrez (2016) who reported that Glycemia and cholesterol levels were significantly (p<0.05) lower in the serum of chicken fed pumpkin seed supplemented diets than in those fed diet without pumpkin seed supplementation. Similarly, the study of Ihsan (2017) who reported that low cholesterol concentration in treatment diet with neem than without neem. The current study result was similar to finding of Bonsu *et al*. (2012) who reported that inclusion of 1% neem leaf meal lower the number of WBC when compared with the control.

The higher WBC in the control indicates a probable higher resistance which might have stimulated the production of WBC to fight against the potential causative agent. The relatively lower WBC of the chicken fed the neem seed could be attributed to no fight against potential disease threats before the body's system could be stimulated to produce WBC. This is in agreement with the findings of Fajinmi *et al*. (1990) who reported that neem seeds are valuable materials and not inimical to human and livestock health. The values obtained for all hematological parameters were within the normal range (Douglas *et al*., 2010).

Mortality in this study was not severe because pumpkin seed addition decreases mortality; this can be related to pumpkins' vitamin C content as a natural antioxidant and a reducer of heat stress. Furthermore, pumpkin has been considered beneficial to health because it contains various biologically active components such as polysaccharides, p-amino benzoic acid, fixed oils, sterols, proteins and peptides (Yadav *et al*., 2010). Several *in vitro* studies reported significant antibacterial and antifungal properties of pumpkin seed oil (Xiong, 2000; Ng *et al*., 2002). The current finding agreed with the study result of Imran *et al*. (2014) who reported addition of 1% neem seed cake on the feed of

broiler which lower mortality and parasitic count. The present findings showed that addition of neem seed powder and pumpkin seed powder may even have some medicinal, nutritional and health benefit and they can be used as a feed ingredient in broiler production without compromising the hematological parameters, carcass characteristics and meat taste at the present addition rate. This may be due to the synergetic effect of the bioactive compounds in herbal seed which have beneficial effect on performance (Brenes and Roura, 2010).

The sensory evaluation results were similar to study reported by Katiyar *et al*. (1996) who indicated no significant differences (p>0.05) in the qualitative and quantitative characteristics of the meat of chickens fed a diet containing 20% neem kernel cake. The sensory quality of breast and thighs was not affected by the addition of pumpkin seed of 1kg on 100kg of feed and also similar result was obtained by Wafar *et al*. (2017) inclusion of pumpkin seed meal up to 20% on the broiler chicken feed. Generally, the present findings show that addition of neem seed powder and pumpkin seed powder did not affect the juiciness, tenderness, flavor, and overall acceptance of the meat from the breast and thigh of broiler chicken and the meat were acceptable for the human consumption.

Significant difference in CP content of meat clearly showed that blood protein content is directly related to the muscle protein content. Similar results have been reported by Ihsan (2017) and these authors indicated addition of neem powder in broiler diets significantly (p<0.05) higher crude protein value of the meat. The Moisture, Ether extract and Ash content of the breast and thigh meat of the broiler was not affected by the treatments. The chemical compositions of breast and thigh muscles recorded in the present experiment are within the range reported for broiler meat (Abdullah *et al*., 2010).

Conclusion

Based on results of the study neem and pumpkin seed has health improvement potential as a feed additive in broiler chicken ration without compromising meat sensory quality. The study results suggest that the addition of neem and pumpkin seed combination (0.5kg NS and 0.5kg PS) on 100 kg broiler diets could be regarded as natural feed additives and environment safe and sound diets as alternatives to banned and hazardous synthetic antibiotics.

Acknowledgements

The authors are grateful to Haramaya University Research and Extension Affairs Office for funding the work.

Competing interests

The authors declare that they have no competing interests.

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Prevalence and associated economic loss of fetal wastage in small ruminants slaughtered at Addis Ababa municipality abattoir, Ethiopia

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Abstract

The study examined prevalence and economic implications of abattoir pregnancy/ fetal wastage in small ruminants. Survey was conducted on ewes (n=201) and does (n=183) slaughtered at Addis Ababa municipality abattoirs, during December 2017 and January 2018. Female reproductive tracts were examined for presence and types of pregnancy and/ or gross disorders. Net economic loss and net economic risk due to abattoir fetal wastage were calculated for each species after accounting for naturally expected abortion and neonatal losses, and domestic net market values. One hundred forty-two (37 %) animals were pregnant 131 (34.1 %) with single and 11 (2.9 %) with twin fetus. A total of 153 fetuses were recovered giving a fetal wastage prevalence of 39.8 %. More animals in the second trimester (25.5 %) were slaughtered than those in first (8.3 %) or third (3.1 %) trimesters ($p < 0.05$). Prevalence of pregnancy was 32.8 % in does and 40.8 % in ewes ($p = 0.104$). Prevalence of fetal wastage was 37.2 % (68 fetuses) and 42.3 % (85 fetuses) in goats and sheep, respectively ($p > 0.05$). Abattoir pregnancy prevalence showed variations relative to slaughter month in goat ($p < 0.01$) and body condition in sheep ($p < 0.05$). Observed abattoir pregnancy prevalence levels incurred net economic loss of 313.55 USD in sheep (per 201 ewes) and 315.4 USD in goats (per 108 doe). This translated to net economic risk of 1.7 USD per mature doe or ewe slaughtered for meat. Nine (4.9 %) does and 1 (0.5 %) ewe showed gross reproductive tract disorders ($p < 0.05$). Frequent female slaughter without efficient ante-mortem pregnancy screening predisposed significant proportion of small ruminants to pregnancy/ fetal wastage. Deeper investigations are needed to understand reasons behind pregnant small ruminant slaughter and to mitigate its negative impacts on sustainability of animal production. Evaluating and capacity building on alternative small ruminant pregnancy diagnosis methods requires due attention.

Key words: Doe; Pregnancy; Reproductive disorders; Ewe; Fetal wastage; Economic implication; Abattoir

Introduction

Ethiopia owns 30.7 million sheep and 30.2 million goats (CSA, 2017). Small ruminants account for a quarter of domestic annual meat consumption as well as over 90 % of live animals and meat exported annually from the country. However, national off-take rate, carcass yield and per-capita consumption for mutton (33-40 %, 10 kg and 1.3 kg) and goat meat (27-35 %, 8 kg goat, and 1.3 kg), respectively, are very low even by sub-Saharan Africa standards. In absence of tangible productivity gains, rapidly growing local demands for meat are driving sustained increase of annual small ruminant slaughter volumes in Ethiopia (Legese and Fadiga, 2014; Shapiro *et al.,* 2017; Eshetie *et al.,* 2018).

Across sub-Saharan Africa, similar pressure to meet rising human demand for meat has been linked to significant pregnant livestock slaughter and fetal wastage which seriously undermine sustainable animal protein production. In part, later trends reflected effects of farmers economic and husbandry limitations and weak slaughter regulation (Abassa, 1995; Tizhe *et al.,* 2010; Fayemi and Muchenje, 2013). There are several methods of small ruminant pregnancy diagnosis with choices depending on gestation stages and level of expertise and technical inputs available. Real time ultrasonography is by far the most reliable method followed by plasma or milk progesterone analysis. More advanced accurate pregnancy detection methods are often used in modern small ruminant operations of developed regions but remain inaccessible in most tropic extensive farming systems (Noakes *et al.,* 2001; Tamassia, 2007; Ptaszynska, 2009). Hence, unknowing farmer dispatch of pregnant stock to slaughter could be substantially lower in developed regions.

Considerable risk of small ruminant fetal wastage is highly likely in Ethiopian contexts. Annual domestic female small ruminant (mainly replacement stocks) slaughter shares increased from 26.6 % (1.26 million) to 29.1 % (1.92 million) in past decade alone (CSA, 2008; CSA, 2017). Ethiopia's Meat Inspection Regulation (Section 2 Article 10.3.) indicated recommends that pregnant animals should be withheld from slaughter at ante-mortem examination (MoA, 1993). Still, uncontrolled small ruminant breeding (Abebe, 2008) and slaughter (Legese and Fadiga, 2014; Mummad and Webb, 2015) systems typical across the

country make it doubtful that females destined for meat undergo formidable pregnancy screening. Accordingly, high levels of abattoir pregnancy and fetal wastage were reported in sheep (> 70 %) and goats (47 %) from the central highlands (Mukasa-Mugerwa and Tekelye, 2003; Tamirat *et al.,* 2015). However, situation of small ruminant abattoir fetal wastage at Addis Ababa, probably the largest urban meat consumer market in Ethiopia, is unknown. Therefore, this study explored prevalence pregnancy/ fetal wastage and associated economic losses in doe and ewes slaughtered at Addis Ababa municipality abattoir. Further interest was on describing type and prevalence of gross female reproductive disorders.

Materials and methods

Study area

Addis Ababa is the capital and administrative center of Federal Democratic Republic of Ethiopia. The city is located on 9°1'48' N latitude, 38° 44' 24` E longitude and average altitude of 2,400 meters above sea level. Addis Ababa city has an estimated land cover of 530.14 square kilometers and population density of 5,165.1 inhabitants per square kilometer. It has a cool humid climate with bimodal annual rainfall averaging 1800 mm and average daily temperature ranging from 10.7 to 25.6 °C (Beshada, 2012; Assefa *et al.,* 2017).

Study population

The study population comprised of mature does and ewes slaughtered at Addis Ababa municipality abattoir. This facility is over 60 years old and was reported to slaughter 36,000 sheep and 18,000 goats in a year (Assefa *et al.,* 2017). Recent slaughter statistics stratified by sex and age proved difficult to access. Slaughter animals are supplied to Addis Ababa city from different parts of Ethiopia including; Northwest, west, Northeast, east and south west in decreasing order of shares (Beshada, 2012). This represents diverse highland and lowland small ruminant production systems in the country.

Study design and variables

Cross-sectional abattoir survey was conducted to explore pregnancy, fetal wastage and gross reproductive disorders in mature does and ewes slaughtered at

Addis Ababa municipality abattoir during December 2017 and January 2018. Outcomes of interest were abattoir pregnancy/ fetal wastage prevalence (number of pregnant females/ fetuses divided by total female examined x 100), and associated net economic losses (NEL) and net economic risks (NER) as well as prevalence (affected/ examined females x 100) and types of gross female reproductive disorders. The independent variables included animal species, body condition and study months.

Sampling method and sample size

Thirty abattoir survey dates were selected by random lottery method in December 2017 (16) and January 2018 (14). Abattoir was visited from 5 PM to 10 PM and all mature does and ewes slaughtered at this time were sampled. Sample size (n) was calculated using the formula by Thrusfield (2015) for simple random samples with 50 % expected prevalence (Pexp), 5 % desired precision level (d) and 95 % confidence level (α= 0.05). Accordingly, 384 study animals were sampled including 183 does and 201 ewes. Average daily frequency of study animals was 12 during December 2017 (6 doe and 6 ewes) and 14 in January 2018 (8 doe and 6 ewes).

Abattoir study methods

Ante-mortem examination of small ruminant at the study abattoir was limited to inspection for physical problems and no reliable pregnancy screening system was in place. On slaughter floor, female small ruminants were checked for maturity based on presence of more than 2 pairs of permanent incisors. Body condition of study animals was subjectively classified as thin, medium and fat according to ESGPIP (ESGPIP, Technical Bulletin No. 8). Post mortem, female reproductive tracts were harvested intact and examined by inspection, palpation and uterine incision to detect pregnancy and/ or gross disorders (e.g. pyometra denotes uterine lumen distended with pus and imminent abortion referred to abnormal appearance of fetal fluids, fetus and placenta indicative of pregnancy disruption). In pregnant females, liter size was recorded and crown - rump length (CRL) of largest fetus was measured (cm) using measuring tape or ruler as previously described (Tamirat *et al.,* 2015). Fetal age (FA) or gestation length (GL) was calculated (in days) from CRL measurement using recommended formulas (Sivachelvan *et al.,* 1996; Hussein, 2008). Based on the estimated FA/ GL, stage of pregnancy was classified in to 1st (< 50 days), 2nd (50 to 100 days) and 3rd (> 100 days) trimester.

Sheep GL/ FA (in days) = 2.1 (CRL + 17) &
Goat GL/ FA (in days) = (2.74 x CRL) + 30.15

In pregnant small ruminants, month of fertile mating was approximated by counting back GL/ FA days from date of abattoir detection. Corresponding parturition months were estimated by counting forward from abattoir detection date the difference between average small ruminant gestation length (150 days) and calculated GL/ FA (n days).

Economic analysis

Economic impact of fetal wastage reflects financial value of potential offspring's forgone due to slaughter of pregnant female animals for human meat consumption. This study drew on abattoir fetal wastage economic loss estimation methods that account for naturally expected abortion/ stillbirth and neonatal mortality losses described by other studies (Tamirat *et al.,* 2015; Kashoma and Melkiory, 2017). The approach was modified by considering net market return values (*gross value – rearing costs*) of sheep and goats in Ethiopia so as to estimate net economic loss (NEL) due to pregnancy/ fetal wastage for total animals examined as outlined below.

NEL (Birr) = NMRV (Birr/ Animal) x VOW (n Animals) where;

NMRV stands for net market return value of sheep (223.0) and goats (267.3) in Ethiopia calculated by averaging values reported by Shapiro *et al.* (2017).
VOW denotes net quantity of viable offspring's wasted due to observed abattoir pregnant slaughters. This was calculated by deducting naturally expected abortion/ stillbirth and neonatal mortality losses from observed abattoir pregnancy/ fetal wastage levels in each small ruminant species as outlined below.

VOW (n) = Live Born Offspring (n) – Premature Offspring Mortality (n)

Live born offspring (n) = (n) Pregnant females (Total– Abortion/ Still birth) X Average liter size (n)

Abortion/ Still birth (n) = Total pregnant females (n) X Expected abortion/ still birth prevalence (%)

Expected abortion/ still birth prevalence (ExpASbP) was approximated by averaging previous national estimates (Fentie *et al.,* 2016) for goats (16.8 %) and sheep (14.7 %)

Premature offspring mortality (n) = Live born offspring (n) X Expected offspring mortality prevalence (%)

Expected offspring mortality prevalence (ExpOMP) was approximated to 18.8 % for both goats and sheep by averaging national estimates reported by Fentie *et al.* (2016) and CSA (2017)

To appreciate current NEL projections on larger slaughter volumes, the study calculated net economic risk (NER) which reflects pregnancy/ fetal wastage related financial loss risk expected when 1 mature doe or ewe is slaughtered for human consumption. For each small ruminant species, NER (Birr or USD/ mature female slaughtered) was calculated by dividing NEL with total number of females examined (n).

Data analysis

Study months, animal species and body condition, gross reproductive disorder (presence and type) and pregnancy (presence, liter size and CRL (cm), FA/ GL (days), gestation stage) observations were recorded and/ or calculated on Microsoft Excel Sheet. Economic loss (Birr/USD) calculations were done on a separate Microsoft Excel sheet using built in arithmetic functions on combination of observed and referred input variables. Further statistical analysis was carried out on SPSS version 16 software. Categorical variables were summarized in frequency (n (%)) tables and frequency distribution of fertile mating months was contrasted using line graphs with 95 % confidence interval (CI). Numerical variables (CRL and FA/ GL) were summarized using mean ± standard error. Association between categorical factors and outcomes was analyzed using Chi-square and Fisher exact tests. Relationship of numerical and categorical variables was evaluated by comparison of means using 95 % confidence interval (CI). Statistical significance was set at $p < 0.05$.

Results

Pre-slaughter 17 (4.4 %), 170 (44.3 %) and 197 (51.3 %) animals exhibited thin, medium and good body conditions, respectively (p <0.05). Post-mortem reproductive examination revealed that 142 (37 %) female small ruminants were pregnant with single (34.1 %) and twin (2.9 %) fetus (p < 0.05). A total of 153 fetuses were recovered giving an overall abattoir fetal wastage prevalence of 39.8 %. Prevalence of pregnant slaughter was 32.8 % in does and 40.8 % in ewes (Chi-square = 2.64, p = 0.104). Twin pregnancy was higher (Chi-square = 4.54, p = 0.033) in does (4.4 %) than in ewes (1.5 %). Overall prevalence of fetal wastage was 37.2 % (68 fetuses) in goat and 42.3 % (85 fetuses) in sheep (Chi-square = 1.53, p = 0.216).

Fetal CRL and calculated FA/ GL varied widely amongst pregnant small ruminants but showed limited average variation in relation to animal species (Table 1). More pregnant small ruminants were in second trimester (25.5 %) compared to counterparts in first (8.3 %) and third (3.1 %) trimesters (p < 0.05). Likewise, more fetuses were lost from second trimester pregnancy (27.1 %) than either first (9.4 %) or third (3.4 %) trimester pregnancies (p < 0.05). Prevalence patterns of pregnancy and fetal wastage at different trimesters were consistent in goats and sheep (Table 1)

Table 1. Fetal length, age, gestation stage in pregnant small ruminants slaughtered at Addis Ababa Municipal abattoir

Variable	Statistics/ Categories	Total (N=140)	Goat (N=60)	Sheep (N=82)
CRL (cm)	Min-Max	3 - 35	3 – 34	4 – 35
	Mean ± SE	15.34 ± 0.70	13.4 ± 1.05	16.75 ± 0.91
	95 % CI; LB-UB	13.96 – 16.72	11.29 – 15.51	14.95 – 18.56
FA / GL (Days)	Min-Max	42 – 109.2	42 – 107.1	44.1-109.2
	Mean ± SE	67.91 ± 1.5	63.8 ± 2.21	70.89 ± 1.91
	95 % CI; LB-UB	65.01 – 70.81	59.42 – 68.26	67.09 – 74.68
Pregnancy n (%)	1st Trimester	32 (8.3)	17 (9.3)[b]	15 (7.5)[b]
	2nd Trimester	98 (25.5)	39 (21.3)[a]	59 (29.4)[a]
	3rd Trimester	12 (3.1)	4 (2.2)[c]	8 (4)[c]
Fetal Wastage n (%)	1st Trimester	36 (9.4)	20 (10.9) [b]	16 (8) [b]
	2nd Trimester	104 (27.1)	44 (24) [a]	60 (29.9) [a]
	3rd Trimester	13 (3.4)	4 (2.2) [c]	9 (4.5) [c]

Superscripts [a, b & c] INDICATE FREQUENCY VARIATIONS OF DIFFERENT CATEGORICAL OUTCOME LEVELS IN SPECIFIC SPECIES AT P < 0.05

Approximated fertile mating (conception) time of pregnant small ruminants was higher ($p < 0.05$) around October (38.7 %) and November (38 %) than in September (6.5 %) and December (2.1 %). Corresponding expected parturition (lambing/ kidding) dates concentrated ($p < 0.05$) during March (46.5 %) and April (34.5 %) than in February (4.7 %) and May (2.3 %). Respective temporal reproductive variations were consistent in pregnant does and ewes (Figure 1 a, b).

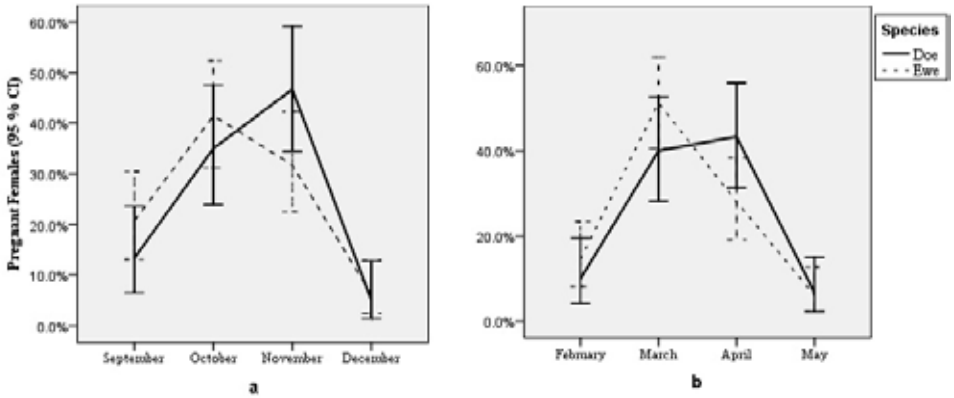

Figure 1. Fertile mating (a) and parturition (b) months relative to species of pregnant animals

On the other hand, 10 (2.6 %) female small ruminants exhibited gross reproductive disorders including pyometra (1.6 %) and signs of abortion (*hemorrhagic turbid fetal fluid and/ or placental lesions*) in progress (1 %). A single case of pyometra was detected in ewes (0.5 %) whereas remaining disorders were observed in does i.e. pyometra (2.7 %) and abortion (2.2 %). Total prevalence of reproductive disorders was higher in does (4.9 %) than in ewes (0.5 %) (Chi-square = 7.38, $p = 0.007$).

Overall, prevalence of small ruminant pregnancy and fetal wastage (Chi square = 5.67, $p = 0.017$) were higher during January 2018 than December 2017 whereas gross reproductive disorders showed limited temporal variation. Body condition was not significantly associated to prevalence of pregnancy (Chi square = 3.77, $p = 0.104$), fetal wastage (Chi square = 4.47, $p = 0.107$) and reproductive disorders (Chi square = 2.9, $p = 0.235$). Species wise, association of study months to prevalence of pregnancy (Chi square = 12.4, $p = 0.000$) and fetal wastage (*Chi square = 11.95,* $p = 0.001$) were significant in goats. In

sheep, prevalence of pregnancy (Chi square = 8.4, $p = 0.015$) and fetal wastage (Chi square = 8.3, $p = 0.016$) were higher in fat than thin body condition groups (Table 2).

Table 2. Association Prevalence of pregnancy, fetal wastage, and reproductive disorders with study month and body condition of slaughtered small ruminants

Animals	Factors	Categories	Examined (N)	Pregnant N (%)	Fetal Wastage N (%)	Reproductive Disorders N (%)
Total	Study month	Dec 2017	190	59 (31.1)	64 (33.7)	4 (2.1)
		Jan 2018	194	83 (42.8) *	89 (45.9) *	6 (3.1)
	Animal Body Condition	Thin	17	3 (17.6)	3 (17.6)	-
		Medium	170	60 (35.3)	64 (37.6)	7 (4.1)
		Fat	197	79 (40.1)	86 (43.7)	3 (1.5)
Goats	Study Period	Dec 2017	101	22 (21.8)	26 (25.7)	4 (4)
		Jan 2018	82	38 (46.3) *	42 (51.2) *	5 (6.1)
	Animal Body Condition	Thin	-	-	-	-
		Medium	77	27 (35.1)	29 (37.7)	6 (7.8)
		Fat	106	33 (31.1)	39 (36.8)	3 (2.8)
Sheep	Study Period	Dec 2017	89	37 (41.6)	38 (42.7)	
		Jan 2018	112	45 (40.2)	47 (42)	1 (0.9)
	Animal Body Condition	Thin	17	3 (17.6) b	3 (17.6)b	
		Medium	93	33 (35.5)	35 (37.6)	1)1.1)
		Fat	91	46 (50.5) a	47 (51.6)a	

Superscript * and a & b indicates variations of specific column variable in different categorical factor levels at $p < 0.05$ level (2 sided)

After accounting for pregnancy and neonatal losses expected under local conditions, abattoir small ruminant pregnant slaughters incurred net loss of 105 viable offspring (59 sheep and 46 goats) per 384 mature females (201 ewes and 183 doe) slaughtered. This amounted to NEL of 25,415.7 birr (628.95 USD at December 12 2020 exchange rates of 1 USD = 38.82 birr). Projected NEL estimates amounted to 12,243.6 birr (315.4 USD) in goats and 13,172.2 birr (313.55 USD) in sheep. These NEL estimates translate to NER of 66.9 birr (1.72 USD) and 65.5 (1.69 USD) per mature doe and ewe slaughtered at the abattoir, respectively (Table 3).

Table 3. Abattoir pregnancy/ fetal wastage related economic impact calculations and estimates

ID	Input Variables	Goats	Sheep
A	Females Examined (n)	183	201
B	Females Pregnant (n)	60	82
C	Total Fetus in Pregnant Females (n)	68	85
D	Average Fetus per Pregnant Females (n) = C/ B	1.13	1.04
E	Pregnant Females' Abortion-stillbirth (n) = ExpASbP *(*Fentie *et al., 2016) x B*	10 (16.8 % X 60)	12 (14.7 % x 82)
F	Pregnant Females Giving Birth (n) = B - E	50 (60 -10)	70 (82 – 12)
G	Live Born Offspring's (n) = F x D	56 (50 x 1.13)	73 (70 x (1.04)
H	Pre-mature Offspring Mortality (n) = ExpOMP (Fentie *et al., 2016 & CSA 2016/17*) *x G*	10 (18.8 % x 57)	14 (18.8 % x 73)
H	Viable Offspring's Wasted (n) = G - H	46 (57 – 11)	59 (73 – 14)
I	Average NMRV (Birr/Animal) *(Shapiro et al., 2017)*	267.3	223.0
Ja	NEL (Birr for total females examined) = I x H	12,243.6 (267.3 x 46)	12,172.2 (223 x 59)
Jb	NEL (USD) = Ja/ 38.82	315.4 (12,179.7/ 38.82)	313.55(13,128.9/ 38.82)
Ka	NER (Birr per 1 mature female) = Ja/ A	66.9 (12,179.7/ 183)	65.5 (13,128.9/ 201)
Kb	*NER (USD) = Jb/A*	1.72 (315.4/ 183)	1/69 (313.55/201)

Discussion

Postmortem gross reproductive examination of 384 female small ruminants at Addis Ababa abattoir revealed substantial prevalence of pregnancy (37 %) and fetal wastage (39.8 %) accompanied by occasional pyometra (1.6 %) and imminent abortion (1 %). Majority of female small ruminants slaughtered at the abattoir seem to have normal breeding potential as evidenced by rare genital disorders and frequent pregnancy postmortem. In sub-Saharan Africa, pregnant livestock slaughter has been linked to rising unmet domestic demands for meat, farmer economic and husbandry limitations, weak slaughter regulation and other (biological, social and climatic etc.) influences (Abassa, 1995; Tizhe *et al.,* 2010; Atawalna *et al.,* 2013; Mshelia *et al.,* 2015). Ethiopian statistics indicate 1.92 million female small ruminants were slaughtered for domestic consumption in 2017 (CSA, 2017). Previous figure shows a jump of 0.66 million from values a decade back (CSA, 2007) and this was probably driven by growing unmet domestic meat demands (Shapiro *et al.,* 2017). Majority of local small ruminant slaughters occur in unregulated backyards (Legese and Fadiga, 2014) and low-capacity public abattoirs (Mummed and Webb, 2015). Such scenarios reflect risks of broader indiscriminate pregnant livestock slaughter in the country. By contrast, pregnant maternal slaughter in developed regions was often tied to economic reasons like harvesting medicinal pregnancy hormones, salvaging expenses when meat prices drop, etc (Fayemi and Muchenje, 2013).

Pregnant small ruminants slaughtered at Addis Ababa abattoir were between 42 and 109 days of gestation. Majority of pregnant animals were in 2nd trimester (69 %) followed by 1st (22.5 %) and 3rd (8.5 %) trimester stages in decreasing order ($p < 0.05$). Producers appear to verify small ruminant pregnancy by inspecting for gross physical changes like abdominal and mammary enlargement apparent after 100 (often later) days i.e., small percentage of potential pregnant animals targeted for meat. Absence of formidable pregnant small ruminant screening and/ or slaughter regulation at abattoir level appears to allow considerable risk of fetal wastage. If done by experienced examiner, abdominal palpation/ ballottement for fetal masses can detect small ruminant pregnancy after 70 (preferably 90) days post service. Using real time B mode ultrasonography, it is possible to identify pregnancies as early as 27 (rectal probe) to 40 (trans-abdominal probes) day post-service. The technique offers further benefits of determining litter size as well as age, sex and viability of fetuses. Plasma or milk progesterone levels of 2.5-4 ng/ml 18 – 23 days post-

breeding offers a reliable (80-84 %) indirect indicator of pregnancy whereas 1ng/ml and lower levels at same intervals confirms (100 %) absence of pregnancy (Noakes *et al.,* 2001; Tamassia, 2007; Ptaszynska, 2009).

Postmortem small ruminant pregnancy was currently higher in January (42.8 %) compared to December (31.1 %). Based on pregnancy detection month and stage, conceptions peaked during October and November months. January represents middle of long dry season wherein pasture feed resources is declining as well as a period of high meat demand by Orthodox Christians before stating two months long fast (Seleshe *et al.,* 2014). Small ruminant producers trying to avert risks posed by escalating feed scarcity and attracted by favorable prices may unknowingly sell grossly unapparent pregnant stocks for meat in later month. Conception dates of corresponding gestations coincide with relatively good pasture feed availability at end of major rains reflecting nutrition modulated seasonal fertility fluctuations. Tropical small ruminant flocks exhibit limited breeding seasonality (Ptaszynska, 2009; Petrovic *et al.,* 2012) evidenced by fertile mating peaks around the major and/ or short rains both in Ethiopia and sub-Saharan Africa (Otte and Chilonda, 2002; Abebe, 2008). Brief duration of current abattoir study, could not give full picture of seasonal breeding trends for concerned national small ruminant flocks. A related drawback was inability to evaluate seasonal reproductive trends according to verified geographical origin of study animals.

Currently, abattoir pregnancy and fetal prevalence in ewes (40.8 % and 42.8 %) and doe (32.8 % and 37.2 %) were comparable but twining was lower ($p < 0.05$) in former (1.5 %) compared to later (4.4 %) species. The two species showed consistent frequency of different gestation stages ($2^{nd} > 1^{st} > 3^{rd}$ trimesters) and overlapping peak fertile mating periods (October and November). Hence conditions influencing reproductive activity and slaughter fate of doe and ewes appear similar. Previous Ethiopian studies had signaled alarming abattoir pregnancy levels including 71.7 % (24 % twins) in central highland sheep (Mukasa-Mugerwa and Tekelye, 2003) as well as 74.4 % (45.7 % twins) in sheep and 47.6 % (60 % twins) in goats at Asella town (Tamirat *et al.,* 2015). Conversely, a recent undergraduate study at Jigjiga abattoir (Yikeber, 2018) reported lower postmortem pregnancy prevalence in goats (22.9 % (1.8 % twins) and sheep (31.5 % (2.7 % twins)). In parallel with Ethiopian trends, widely varying abattoir small ruminant pregnancy and fetal wastage levels have been reported from other African countries. Main examples include 38.6 to 57.7 % (2.2 % multiple fetuses) in sheep and 40 to 51.8 % (3.5 - 33 % multiple

fetuses) in goats from Tanzania (Swai *et al.,* 2015; Kashoma and Melkiory, 2017; Kilumbi and Nonga, 2018); 15.7 to 47.7 % (25.1 % multiple fetuses) in sheep and 19 to 59 % (17.3 - 66.3 % multiple fetuses) in goats from Nigeria (Addass *et al.,* 2010; Tizhe *et al.,* 2010; Mshelia *et al.,* 2015; Okorie-Kanu *et al.,* 2018); and 50.6 % (50.4 % multiple fetuses) in sheep and 20.4 % (77.35 % multiple fetuses) in goats from Ghana (Tasiame *et al.,* 2016). Most Ethiopian and African studies had observed higher fetal wastage in 1st and/ or 2nd trimesters suggesting drawbacks of gross physical pregnancy screening approaches adopted across the region.

Discrepancy of abattoir fetal wastage across and within geographic regions has been attributed to variable climatic, husbandry and genetic influences on fertility (Addass *et al.,* 2010; Tizhe *et al.,* 2010; Fayemi and Muchenje, 2013). Ethiopian studies reflect higher risk of pregnant slaughter in sheep but higher frequency of twining in goats with effect of evening out total species fetal wastage volumes. Fetal wastage in both species also tended to be higher in highland compared to lowland abattoirs. Ethiopian highland sheep tend to have short heavy body frame with coarse wavy wool that conceals abdomen whereas local goats often show lean body frames with short smooth hair coats (FARM Africa, 1996; Awgichew and Abegaz, 2008). Such differences could render gross detection of late pregnancies more difficult in ewes compared to doe resulting in parallel pregnant slaughter discrepancies. Greater pastoralist milk supply function of goats (Awgichew and Abegaz, 2008; Legesse and Fadiga, 2014) could further discourage slaughter of goats with suspected pregnancy. Relatively better pasture and crop byproduct availability in highland compared to lowland areas (Gizaw *et al.,* 2010; Sheriff and Alemayehu, 2018) could reflect fertility effects corresponding to abattoir pregnancy variations. Twining rate of indigenous sheep and goat's shows wide variability (2 – 51 %) reflecting genetic and nutritional influences on ovulation rates (Sheriff and Alemayehu, 2018). Broader feeding range of goats compared to sheep may allow higher ovulation rates in former species under pasture deficient conditions. Further, Somali and Borana pastoralists were suggested to deliberately cull twin bearing doe for slaughter so as to improve kid survival and family milk supply (FARM-Africa, 1996).

Economic cost of abattoir small ruminant fetal wastage was currently estimated employing species-specific approach that accounted for average expected pregnancy and offspring losses as well as net financial values of national flocks. Gross fetal wastage levels observed at Addis Ababa abattoir were pro-

jected to result in net loss of 105 potential viable offspring's (46 goat and 59 sheep) amounting to NEL 313.55 USD in sheep and 315.4 USD in goats. Later estimates reflect NER of around 1.7 USD attributable to fetal wastage for each mature doe and ewe slaughtered at the abattoir. If 50 % of the 36,000 sheep and 18,000 goats reported to be annually slaughtered at the abattoir (Assefa *et al.,* 2017) were in active breeding state, current NER estimates would result in NEL of 45,900 USD per year. If same NER level was extended to half of annual domestic female goat (875, 406) and sheep (1,049, 052) slaughters (CSA, 2017), Ethiopia stands to lose around 3.3 million USD due to abattoir fetal wastage. Previous studies had reported higher abattoir small ruminant fetal wastage related to annual economic losses of 158,560.0 USD in Ethiopia (Tamirat *et al.,* 2015) and 464,940.0 - 774,900.0 USD in Tanzania (Kashoma and Melkiory, 2017). Larger annual female slaughter volumes; lower expected pregnancy (14 %) and neonatal (19 %) loss risks; and higher gross small ruminant market values used towards later projections could explain corresponding inflated projections. Meanwhile, annual economic losses calculated based on gross market values of small ruminant newborns (15 – 20 USD) was estimated between 228, 240.0 and 380,400.0 USD at two Tanzanian abattoirs (Kilumbi and Nonga, 2018) and around 46,480 USD in Nigerian goats (Okorie-Kanu *et al.,* 2018). Apart from considering larger annual female slaughter volumes and net animal financial values, later studies ignored risks of heavy pre-weaning mortality expected under tropical small ruminant farming systems (Abassa, 1995; Otte and Chilonda, 2002; Fentie *et al.,* 2016).

Causes of reproductive failure (infertility) in small ruminants are diverse involving failure to mate; failure to conceive; embryo or fetal loss; and neonatal mortality (Ptaszynska, 2009). Research on epidemiology of small ruminant infertility is patchy in Ethiopia (Abebe, 2008). This study noted higher prevalence of imminent abortion (2.2 %) and pyometra (2.7 %) in goats as compared to only 0.5 % pyometra in ewes. Abortion is a major cause of small ruminant infertility associated to specific or non-specific genital infections, environmental stress and/ or maternal factors (Ptaszynska, 2009; Pugh, 2016). Previous small ruminant abortion prevalence estimates in Ethiopia varied from 2.4 to 45.4 % (Abassa, 1995; Fentie *et al.,* 2016). The etiological and predisposing factors behind such discrepancy are not well known. Non specific genital infections have been given limited attention in small ruminants compared to larger livestock. Yet, predisposing risk factors such as abortion, genital prolapse, dystocia and placenta retention are common in small ruminant suggesting potential importance of non-specific genital infections in these species.

Conclusions

Despite limited temporal coverage and sample size, this study tried to offer useful insights on epidemiology and financial impacts of pregnant doe and ewe slaughtered (fetal wastage) at Addis Ababa abattoir. Regular slaughter of mature female animals coupled with lack of robust ante-mortem pregnancy screening system appears to contribute to substantial fetal wastage in physically inconspicuous gestational stages. This trend could undermine sustainable livestock meat productivity and consumption. Hence, further studies are needed to deepen knowledge on epidemiology and impacts of abattoir pregnancy/fetal wastage at broader scale including other food animal species. Responsible bodies need to pay attention to this wasteful practice and enact proper regulations by evaluating feasibility of accurate ante-mortem pregnancy screening primarily by ultrasound scanning systems.

Conflict of interests

The authors have not declared any competing of interests

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Application of Geographical Information System in Animal Disease Surveillance and Control: A Review

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Summary

Animal disease patterns are changing because of climate change and there is a continuous occurrence of re-emerging and emerging types of diseases. So, new and modern tools are essential for monitoring and surveillance of these diseases. This review was done to give an insight on the applications of GIS in animal disease surveillance, reporting and control. Geographic information systems (GIS) provide the easy access, utilization and manipulation of geospatial information. The advantage of GIS is mapping the many different locations of farms and other facilities with animals on a single map which helps in better monitoring and surveillance. GIS also provides detailed information on disease forecasting, prediction of outbreaks, identification of disease clusters or hotspot, creation of buffer zones and to evaluate different strategies to prevent the spread of infectious diseases. Moreover, it provides an ideal condition for the collection of disease related data and their analyses in relation to population distribution, surrounding social and health services and the natural environmental conditions.

Keywords: Animal diseases; Control; GIS; Surveillance

Introduction

A GIS is a computerized information system in which user can capture, store, manipulate, analyse, manage, present, retrieve and share all types of spatial or geographic data. GIS is more user-friendly computer software which can show many different kinds of data on one map and enables users to analyse and interpret data on different locations plotted on map to understand relationships, patterns and trends (Bhatt and Janak, 2012). GIS has emerged as a tool with a multidisciplinary field with practical potential to be applied for any discipline handling data related to geographical locations (Norstrom, 2001). As

GIS can map a variety of epidemiological information like morbidity, mortality, prevalence and incidence and geographical distribution of the diseases, it is very important in the veterinary field. GIS was first applied in veterinary medicine in 1994 for foot and-mouth disease epidemic (Sanson et al., 1994). GIS helps the epidemiologists and public health professionals in the veterinary sector in analyzing associations between various locations, environment and disease pattern by providing different types of maps particularly for the spatial analysis (Cringoli *et al*., 2007; Sadkowska-Todys and Kucharczyk, 2012). GIS was applied for assessing the risk and the spatio-temporal distribution of plague in India (Rahelinirina *et al*., 2010) and other vector and waterborne diseases (Gubbels *et al*., 2012) in different countries.

Control of animal diseases requires epidemiological data about that particular disease. These data can be gathered using different systems like by using surveillance. Animal health surveillance is an essential tool to detect disease or infection, to monitor disease trends, to support claims for freedom from disease or infection, to provide data for use in risk analysis, and to substantiate the rationale for sanitary measures. Animal disease surveillance and monitoring are very important for improving disease analysis, early warning and controls the spread of diseases (Kshirsagar et al., 2013).

Molecular biology and genomics have given several sophisticated tools for rapid and confirmatory diagnosis of a particular disease. Disease surveillance and monitoring are also important to assess the status of a disease in a specific area and to implement effective prevention and control strategies (Deb and Chakraborty, 2012). GIS is a system used to display the information from research spatially for easy understanding (Deb *et al*., 2013; Dhama *et al*., 2013).

GIS is used as a powerful tool for displaying the regions of high disease prevalence and keeping an attention on control programs being carried out. GIS and Global Positioning System (GPS) provide an integrated approach enhancing the quality of data analysis and decision making to control the disease and its prevalence at regional or national level. Even if it has a number of uses there are a number of constraints to apply GIS easily. Although there are some reviews about the general use of GIS there is still a lack of organized information on the application of GIS in veterinary medicine and its challenges. Therefore, the objectives of this review are to indicate the applications of GIS in animal disease surveillance, reporting and control and its challenges.

Geographic Information System

GIS is a potent tool for retrieval, interrogation, transformation and display of spatial data obtained from worldwide sources. GIS can act as a decision support system that involves the integration of all kinds of geo-referenced data (Burrough and McDonnell, 1998). GIS is used to map, analyze and interpret data related to some particular geographical location and disease distribution (Maguire, 1991; Alizadeha and Moghaddam, 2012). It has a range of powerful functions in addition to simple mapping, which include graphical analysis based on spatial location, statistical analysis and modeling. GIS has functions for solving problems (Cowen, 1988) such as the issue of emerging infections, to map and spatially analyze disease occurrences and distribution (Maguire, 1991).

GIS has an efficient capability of integrating different data in different format acquired from a wide range of data sources in to compatible format (Margonari *et al*., 2006). For example, the data to be used in a GIS may be available in the form of paper maps, tables of attributes, electronic files of maps and associated attribute data, aerial photos, satellite images and other sources in digital format (Margonari *et al*., 2006). These types of data can be displayed spatially using different tools on the GIS software. For example, using table join tool we can join and display spatially a disease incidence data stored in excel without geometry with other data that have a shape file. The commonly used GIS software's in veterinary medicine includes quantum GIS (QGIS) and arc GIS. The output of GIS provides a way to see the data or information in the form of maps, tables, diagrams, and so on (Aronoff, 1989). In veterinary epidemiology GIS can be applied for buffer generation, overlay operation, neighborhood analysis and spatial analysis (Bhatt and Janak, 2012; Margonari *et al*., 2006).

Overlay operations is operation in GIS that allow us to combine information from different vector files into a new file that is more tailored to our needs. By applying an overlay operation, specific changes occur at the spatial level as well as on the attribute level. Buffer generation calculates the distance from each cell to its nearest source. It is frequently used for applications, such as finding the nearest hospital, to calculate the distances from site of outbreak, to calculate the distances to major roads (Keith, 1997).

Data Sources of GIS

The data for GIS can be derived from paper map, remote sensing and Global Positioning System (GPS).

Paper map

It is one of the most known sources of GIS, in which the information is plotted within a coordinate system that allows us to find its location. Mapping is a common technique of displaying the geographical distribution of disease and associated risk factors with the aid of digitizing maps (Jacoby *et al*., 2002). To digitize paper maps into a digital format, first, we must convert data from analog to digital format. Then, convert digital map into a scanned document and finally transform the digitized map from a source coordinate system to the geographic coordinate system using tics marks (Kumar, 2018).

Remote sensing

Remote sensing is the science and art of obtaining information about an item, area, or phenomenon through the analysis of data obtained by a device that isn't in physical contact with the object, area, or phenomenon under investigation. In such conditions, information is gathered in the form of digital photos of the earth's surface from airborne or satellite platforms and transforming them into maps (Lillesand *et al*., 2000). Usually, sensor devices are mounted on satellites or aircraft, or are installed at fixed coastal locations, that measure the electromagnetic radiation (EMR) that is emitted or reflected by features of the earth's surface, and which then convert the EMR into a signal that can be recorded and displayed as either numerical data or as an image (Lillesand *et al*., 2000).

Global positioning system

GPS is a satellite-based navigation system made of a network of twenty-four satellites placed into orbit that transmits precise microwave signals. The microwave signals latter allow the GPS receiver to determine its location, direction and time. Data from GPS can be utilized in association with existing spatial databases for a range of applications in spatial decision making (Jebara, 2007).

The Link of spatial data and GIS

Spatial data are the backbone of GIS. In GIS the use of geo-coded data with co-ordinates is being promoted. The geo-referenced data are used as theme layers. Theme layers are spatial representation of analyzed data of elements of the same type. Moreover, they can be displayed singly or as overlay one above the other. Such data include an overhead projector that requires a geo-relational database and each of its features has linkage of attributed data for storage in a table and joining with the geographical data via a common identifier (ID). Therefore, each spatial data can be easily depicted as a map using GIS (Bhatt and Janak, 2012).

Applications of GIS in Veterinary Medicine

GIS has a wide-range of applications in veterinary medicine, such as outbreak notification, prevention and eradication of disease, disease surveillance, understanding and explaining disease dynamics and spreading patterns and correlation of disease trends with climate (Kuldeep *et al*., 2013).

Application of GIS in Animal Disease Surveillance

For a control strategy or to eradicate a disease, the exact disease status in that community is required to be known (Verma et al., 2008). GIS is one of the best tools used in various disease monitoring and surveillance programs today. GIS is being used to visualize disease foci, monitor newly infected or re-infected villages, and identify populations at risk, target cost-effective interventions, and monitor eradication efforts. GIS have been used in territorial cross-sectional and longitudinal parasitological surveys in order to experiment new applications to plan sampling protocols and to display the spatial distribution of infectious disease data to understand natural habitat and pattern of disease caused by infectious agents to animals (Rinaldi et al., 2004). GIS can be used to combine the information of computer maps with geographical data in order to support the spatial relationships along with patterns and trends in predicting future health status that need to be explored. Previously GIS was used to display the distribution of brown ear ticks in southern Africa, retrospectively comparing the eco-climatic favorability of particular locations for Rhipicephalus appendiculatus with the occurrence of East Coast fever (Lawrence, 1991).

Formats of Disease Occurrence Data on a Map Using GIS

The representation of disease incidence data can vary from simple point maps for cases and pictorial representation of counts (Lawson *et al*., 1999). The pattern and the presentation of spatial disease distribution can be divided into dot, diagram, choropleth and flow maps (Kistemann *et al*., 2002). Dot maps are able to show each health event with the resolution of a pair of coordinates, x (longitude) and y (latitude). Choropleth maps are used to display mortality or morbidity rates for defined geographical units by coloring, shading or hatching, whereas flow maps are able to show the distribution dynamics of health events in time and space, and diagram maps provide added value to the presentation of quantitative data within a map (Kistemann *et al*., 2002).

The choice of map color is of great importance as it helps to transform numerical information into an informative map. It is also important to make a decision on the number of categories and the choice of cut-off points. In some cases, the primary aim of classification is to provide the reader the maximum available information, and the choice will depend on whether or not the scale is data-dependent. A clear distinction between different parts of the map should be appreciated by the reader (Smans and Estéve, 1992).

Recording and reporting disease information using GIS

GIS can be used to produce maps of disease incidence, prevalence, mortality, and morbidity on farm, region, or national levels. The information is more easily understood when visualized on a map. If the information is mapped at the farm level, value of data is maintained, and also small parts of a region can be visualized at the same time. The GIS was also incorporated in outbreak notification, for example in an eradication program of the Aujeszky's disease in North Carolina (McGinn et al., 1997). Geographical and disease incidence data were used as an input to notify the community for the occurrence of a particular disease in a specific area. GIS is one of the best tools for study and application of the Global Early Warning System (GLEWS) that formally brings together human and veterinary public health systems and application of environmental data for study of zoonotic and vector borne diseases (Kshirsagar et al., 2013).

Temporal distribution: Epidemic notification

In case of an outbreak of an infectious disease, GIS can provide an excellent tool for identifying the location of the infected farms and at-risk farms within a specified area of the outbreak (Musekene and Tessema, 2009; Schimmer *et al*., 2010). It has been used to strengthen data collection, management, and analysis, develop early warning systems, plan and monitor response programs, and communicate large volumes of complex information in a simple and effective way to decision makers and epidemiologists. After the infection sources are known buffer zones can be drawn around those farms and with a link to tables of the addresses of the farms at risk; the farms can be informed within a short time after a notified outbreak. Then at-risk farms will apply the appropriate preventive option. Buffer zones can also be generated around other risk areas or point sources, such as roads where infected cattle have been driven or around market places to limit spreading of the outbreak. Further, the maps can assist the field veterinarians to plan their work related to the current situation, and the veterinary authorities in how to handle a potential outbreak (Sanson *et al*., 1994, Schimmer *et al*., 2010). GIS can be used to display the magnitude and distribution of a specific disease within the different seasons of the year. This helps to differentiate risky seasons and to apply strategies that mitigate the distribution of the disease.

Depicting the Spread of a Disease

GIS has been extensively used in veterinary epidemiology for the study of different diseases, their etiology, association with ecology, transmission patterns, disease forecasting as well as the role of soil, vegetation types and other environmental factors in disease occurrence. Several viral, bacterial, parasitic and protozoal diseases have been studied to identify their spatial distribution, characteristics, and risk factors such as temperature, soil type, elevation, slope and land use. For example, Aujeszky's disease in US, fascioliasis in Brazil, bovine tuberculosis in New Zealand and UK, FMD in France, UK, Brazil and New Zealand; Campylobacteriosis in Sweden; Rift valley fever in US (Sorensen *et al*., 2000; Nygard *et al*., 2004; Musella *et al*., 2011; Konrad *et al*., 2012; Martins *et al*., 2012) spread were mapped using GIS. In Ethiopia, Yilma and Malone (1998) applied GIS to forecast model for strategic control of fasciolosis.

Integration of epidemiological data with the spatial and ecologic data plays important roles in analysis of variables responsible for disease transmission

(Konrad *et al*., 2012). Spatial analysis involves three basic steps; the preparation of an appropriate model, its proper visualization, and an exploratory data analysis, which range from simple map overlay to statistical models (Law *et al*., 2004). Spatial analysis interprets and predicts population and inanimate objects movement from one place to another (Ord and Getis, 1995). For example, the movement of animals between wild and domestic areas is a form of spatial interaction, which has a crucial role in disease transmission. By accurately projecting these movements, high risk areas for disease transmission can be identified well in advance and thus intervention efforts can be planned and implemented accordingly.

Disease Mapping and Geographical Information System

One of the most useful functions of GIS in epidemiology is its use in disease mapping. When data are collected either routinely or through purposely-designed surveys, they are presented in tabular forms, which can be exploited for analytical usage. However, the reading and interpretation of such tabular data is often a laborious and time-consuming task and does not permit easy decision-making (Paolino et al., 2005). However, if the collected data is depicted on the map using GIS it will be easily understood by the readers.

Disease mapping methods were first used for communicable diseases in an attempt to identify the sources of infection and to describe the rate of spreading of disease (Paolino *et al*., 2005). Mapping of chronic diseases started with the recognition that environmental factors play an essential role in their etiology. Geographical epidemiological studies, in which health and environmental exposure data are analyzed in fine geographical detail, represent an important new approach (Paweska *et al*., 2008). The aims and purposes of disease mapping are: to describe the spatial variation in disease incidence for the formulation of etiological hypotheses; to identify areas of unusually high risk in order to take preventive action; to provide a reliable map of disease risk in a region to allow better resource allocation and risk assessment (Pfeiffer, 2002; Rinaldi *et al*., 2005).

Geographical Information System for Planning of disease Control Strategies

GIS technology has many features which make it ideal for use in animal disease control, including the ability to store information relating to demographic

and causal factors and disease incidence on a geographical background, and a variety of spatial analysis functions. The neighborhood analysis function can be used to identify all adjacent farms to an infected farm. It is a function that identifies all adjacent features with a certain criterion to a particular feature. Contact patterns such as common use of grasslands, watering points or sources of purchasing etc. could be visualized with a so-called spider diagram. This could provide insight into the possibility of transmission of infectious diseases between herds. In the planning of eradication of diseases, GIS has the capability to perform superimpose analysis to find high or low risk areas for diseases which depend on geographical features or conditions related to the geography (Kamiya, 2007). For example, previous studies on trypanosomiasis (Rogers, 1991); theileriosis (Lessard et al., 1990) and dengue fever (Alzahrani et al., 2013), shows how to use GIS to plan eradication of diseases depending on habitats of vectors or wild animal population.

Emerging and re-emerging diseases pose a major threat in various parts of the world, partly due to climatic changes, as well as the recent spread of several contagious and vector-borne diseases into new or previously controlled areas (Rogers and Randolph, 2006). The current capabilities of GIS (especially collection of satellite data with respect to spatio-temporal and spectral resolution) make it appropriate for epidemiological research (Abdullayev et al., 2012) and mapping vector-borne re-emerging diseases (Bergquist, 2011), including schistosomiasis (Yang et al., 2006), malaria as well as leishmaniasis and dirofilariasis (Genchi et al., 2009). The GIS also helped researchers to identify areas having high prevalence and risk groups apart from identifying areas having shortage of resources to make decisions to allocate resources in case of vector borne diseases. As a result, based on the information obtained any responsible authority can plan the best control option.

Challenges for the application of GIS

GIS can be used in different studies including marketing studies, telecommunications, and location of restaurants, museums and hospitals; in establishing maps of animal population density by species or maps of vegetation coverage change; in locating forests, rivers, and mountains; indicating disease outbreak sites (Jebara, 2007). However, the application of GIS in the routine activities of the majority of developing countries including Ethiopia is not optimal. This is mainly due to lack of awareness by decision-makers; low stock of base data; uncertain data discovery, access and exchange mechanisms; and insufficient

human and technical resources (Melese, 2002). One of the challenges in GIS science and many other fields is the efficient and economical processing of massive data sets (Babalobi *et al*., 2005).

Limitations and strength of GIS

The strength of GIS relies on its ability to merge geographic information with the information of veterinary medicine. GIS can allow viewing, questioning, understanding, visualizing and interpreting the data into numbers of ways which will reveal relationships, trends and patterns in the form of globes, maps, charts and reports (Babalobi *et al*., 2005). Many factors undermine the ability of a developing country to use GIS effctively. However, the efforts applied to solve these challenges leads the creation of options, collectively known as spatial data infrastructures (SDI), which flourishes a country's ability to use geo-information effectively (Bolstad, 2016).

Although GIS has several advantages, it has its own limitations. For example, it is expensive and requires enormous data inputs amount that are needed to be practical for some other tasks. GIS layers might lead to some costly mistakes once the property agents are to interpret the GIS map or the design of the engineer around the utility lines of the GIS. There might be failures initiating additional efforts in order to fully implement the GIS but there might be large benefits to anticipate as well. A GIS system stores extremely large amounts of data at any given time. This may create problems when it comes to analysis due to the complexity of the data and the risk of generalization. GIS data require complex overlay operations that are difficult to achieve especially when the personnel involved are not properly trained (Babalobi, 2007).

Geographical Information System in Ethiopia

Even if GIS has paramount importance in the surveillance, monitoring, control and eradication of particular diseases from a certain locality; still it is not well used in Ethiopia. This is mainly due to a lack of training for personnel, the difficulty of accessing good quality data, and cost indicated to produce quality data (Mesele, 2002). Only some researchers used GIS to show the spatio-temporal distribution of some diseases. For example, Fentie *et al*. (2017) used GIS to show the spatio- temporal distribution of sheep and got pox outbreaks in Amhara region during the year 2010-2014. Molla *et al*. (2017) also used GIS to show both the spatial and temporal distribution of Lumpy skin disease in

cattle in Ethiopia. The global risk of the major arboviral diseases transmitted by *Aedes aegypti* and *Aedes albopictus* were mapped by Leta *et al*. (2017) by identifying areas where the diseases are reported, either through active transmission or travel-related outbreaks. GIS was also applied in Ethiopia for strategic control of fasciolosis, malaria management, and abattoir construction site selection (Yilma and Malone, 1998; Craig *et al.,* 1999). The Spatial distribution of incidence of foot and mouth disease outbreaks in Amhara region of Ethiopia in the period 1999 to 2016 was mapped by Aman *et al.* (2020). Kumsa (2015) used GIS, remote sensing and GPS to show land use/ land cover change of Jarmet wetland (western Ethiopia) and its surrounding environment over years as a response to population growth. GIS were also applied on climate change and desertification, land use planning and monitoring, municipal application, hydrology surveys, soil surveys, geological surveys, and demography (Melese, 2002).

In Ethiopia there are many economically important diseases that are reportable to the OIE. In order to apply strategies that limit the spreading of these diseases and to create disease free compartments, application of GIS is very important.

Conclusions and recommendations

GIS is competent technology for collecting, processing and presenting data pertaining to disease incidences, to help design control and preventive approaches. GIS can add a significant value to epidemiological data that lacks a spatial component. It adds value to enhance the usefulness of displayed information to make better informed decision. GIS is believed to play an increasingly crucial role to survey, monitor and assess infectious diseases at national or international levels and to aid in rapid controlling of economically important diseases. Moreover, GIS has the ability to link spatial and non-spatial data which facilitates powerful analysis of spatial and temporal disease distribution and related issues. In veterinary science using GIS it is possible and easy to draw the maps and visualize possible temporal and spatial risk factors, outbreak distribution and areas at risk of developing the outbreak. Using GIS, the surveillance and monitoring system can be strengthened and the collection, storage, management and reporting of data can be improved to enable policy makers take better informed decision. In the developed world, the application of GIS in the field of veterinary epidemiology is increasing recently, but its application is at low level in developing countries that needs to be strengthened. Thus,

training of veterinary epidemiologists and other veterinary experts about application of GIS for disease surveillance and monitoring programs should be considered for the optimal use of this technology.

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Erratum

In the article "**Trypanocidal drug utilization practices in tsetse suppression and non-suppression areas of South Omo Zone, Southwestern Ethiopia",** by Tesfaye et al., 2020 (*Ethiop. Vet. J.,* 2020, 24 (2), 90-111), the following sentences in the abstract (Page 90) was mistakenly written. The original sentence reads "Sixty (60) of the cattle owners were from suppression area and 124 from tsetse non-suppression area." The amended sentence should read "***One hundred twenty-four (124) of the cattle owners were from suppression area and sixty (60) from tsetse non-suppression area***." The Ethiopian Veterinary Journal editorial office and the authors are kindly apologies for the error.

Ethiopian Veterinary Journal

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Ethiop. Vet. J.,
ISSN: 1683-6324

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The second page should provide an abstract of not more than 300 words summarizing the background, objective, materials and methods, major findings and their significance, and conclusions. Avoid the use of undefined abbreviations.

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Conclusion

This section should be separately presented with supporting evidences based on the major findings of the study. Appropriate recommendations can be made if necessary

Acknowledgements

Acknowledgments should be briefly stated after the Conclusions. Under this, technical, financial and material support can be mentioned.

Conflict of interest

Authors should declare that they have no conflict of interest.

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Jobre, Y., Malone, J. B., McCarroll, J. C., Erko, B., Mukaratirwa, S. and Xinyu, Z., 2001. Satellite climatology and the environmental risk of Schistosoma mansoni in Ethiopia and east Africa. *Acta Trop.*, (*In press*).

C) Papers in proceedings

Tegegne, A., Wirtu, G., Mukasa-Mugerwa, E. and Kassa, T., 1994. Oestrus phenomenon and oestrus detection efficiency using androgenized cows and entire bulls in Boran and Boran x Friesian crossbred cows. In: Proceedings, Advances in Tropical Agriculture in the 20th Century and Prospectus for the 21st: TA 2000, 4-9 September 1994, Port-of- Spain, Trinidad.

D) Books or chapters in books:

Thrusfield, M., 1995. Veterinary Epidemiology, 2nd edition, Blackwell Science Ltd, Oxford, UK.

Malone, J. B and Jobre, Y., 1999. Predicting outbreaks of fasciolosis from Ollernshaw to satellites. In: Dalton, J.P. (Ed.), Fasciolosis. CBA International Publications, Cambridge, Pp. 151-183.

E) Organizations as author:

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