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Risk factors for reproductive disorders and major infectious causes of abortion in sheep in the highlands of Ethiopia

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ABSTRACT

Community-based sheep breeding programs (CBBPs) were established in Ethiopia since 2010. Improved rams from CBBPs need to be disseminated. However, there is a risk of transmitting reproductive diseases to other community flocks. To investigate this a cross-sectional study was conducted in 2015/16 to determine serological status of major infectious causes of abortion and associated risk factors in sheep in the highlands of Ethiopia. A total of 120 households from three districts (Bonga, Horro and Menz) were enrolled in the study. Per farm, 3–4 samples of animals aged > 2 years were collected. A total of 445 sera were tested for *Chlamydia* spp., *Coxiella burnetti*, Toxoplasma, Brucella spp. and Border disease virus. Poison regression was used to model the number of abortions, number of neonatal lost and number of stillbirths in the flocks. A mixed-effects logistic regression model was fitted to identify covariates associated with prevalence of infectious causes of abortion. The study found that 20% [95% CI 12.74, 27.26] of flocks had recent experience of sheep abortion, lamb losses was at 65% [95% CI 56.34,73.65] and still birth at 10% [95% CI 4.55,15.44]. The incidence risk ratio for abortion was higher in larger flocks. Neonatal losses were higher in *C. burnetii* and *Toxoplasma gondii* positive flocks, in one of the districts, and in flocks with more than 30 animals. The serological analyses revealed the presence of three abortive infections, while none of the samples tested positive for *Brucella spp*. or Border disease virus. Of the 120 flocks tested, 107 (89.17%) were positive for *Chlamydia spp*., 82 (68.33%) for *C. burnetii*, and 85 (70.83%) for *T.gondii*. The results highlight the likely contribution of different infectious agents in the reproductive disorders in sheep production in Ethiopia. The high sero-prevalence of infectious agents, especially *Chlamydia spp*., at both flock and animal level, warrants more in-depth research to attribute reproductive problems to these pathogens.

1. Introduction

Small ruminants are valued for their contributions to the livelihoods of pastoral and smallholder farmers in Ethiopia. Small ruminants in general, and sheep in particular play an important role in food security and food self-sufficiency in the highlands of Ethiopia. Ethiopia has a diverse indigenous sheep population with an estimated number of 30.7 million (CSA, 2017). Sheep are reared mainly for meat, skins and coarse wool production for the cottage industry (Rekik et al., 2015) and kept to meet small and immediate cash needs for farming communities (Alemayehu et al., 2015). However, sheep production for the most part of the country is a low input / low output agricultural activity (Belay and Haile, 2009; Rekik et al., 2015).

Various efforts have been made to improve the productivity of indigenous sheep in Ethiopia. Community-Based Breeding Programs (CBBPs) for local sheep breeds have been considered an option to improve productivity and income of small-scale resource-poor sheep producers by providing access to improved animals, and facilitating targeting of specific market opportunities (Haile et al., 2013; Gizaw et al., 2014; Mueller et al., 2015; Gizaw, 2017)

Community-based breeding programs have resulted in tangible outcomes by increasing the marketing of lambs as a result of more lamb births and heavier animals at market age (Haile et al., 2013). Other complementary interventions led to a general improvement in the

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flocks' management, in particular a reduction in lambs' mortality (Haile et al., 2013; Mueller et al., 2015). In CBBP the approach has been to do selection of breeding rams from the community flocks basing on estimated breeding values and use the selected rams communally. Over the last 8 years there is a genetic progress in the flock. The genetic progress achieved need to be disseminated to other communities to make a change at scale. These could be done through natural mating and/ or artificial insemination. With the dissemination of improved genetics, the risk of spreading infectious diseases comes. This necessitates thorough examination of the reproductive diseases to take precautionary measures. The reproductive performance of sheep kept under different production systems in Ethiopia is generally low (Tsedeke, 2007; Petros et al., 2014). Regardless of these interventions, diseases and poor herd-health management practices remain a bottleneck resulting in low flock productivity.

Sheep often encounter complex disorders that hinder optimum off-spring production. Pregnancy wastage in the form of abortion, stillbirth and perinatal lamb mortality remain significant issues to the sheep industry worldwide, and are recognized as the single largest contributor to economic losses in most sheep enterprises (Huffman et al., 1985; Banai, 2002; Mearns, 2007; Menzies, 2007; Fragkou et al., 2010; van der Linden, 2013; Kardjadj et al., 2016). Determinants associated with neonatal lamb survival are lamb birthweight, single vs. multiple lamb¸ age of dam, breed, ewe/lamb behavior, season of lambing and parity of the dam (Huffman et al., 1985; Banai, 2002; Holmøy et al., 2012; Holmøya et al., 2014). In addition, inclement weather conditions before, during, or after birth can dramatically reduce neonatal lamb survival (Darwisha and Ashmawy, 2011; Holmøy et al., 2012; Robertsona et al., 2017).

There are several potential factors underlying the causes of abortions that can be broadly categorized into infectious and non-infectious. According to available research evidence, infectious agents are the most plausible causes of abortion in sheep and goats as compared to non-infectious agents and many of which pose serious zoonotic risks (Buxton and Henderson, 1999; Menzies, 2007, 2011).

The main infectious causes of abortion in sheep are bacterial, such as *Chlamydia* species causing Ovine chlamydiosis, also called enzootic abortion of ewes (EAE) or ovine enzootic abortion, *Campylobac*ter spp, *Listeria* spp, *Leptospira* spp, *Coxiella burnetii* causing Q-Fever, and *Brucella* spp. Further protozoa *Toxoplasma gondii* and *Neospora caninum* and the Border disease virus (BVD) are common pathogens implicated in reproductive failure (Chanton-Greutmann et al., 2002; van den Brom et al., 2012; Moeller, 2001; Martins et al., 2012; Hazlett et al., 2013).

It was reported by Gebremedhin et al. (2013a) that 57.5%, 28.9% and 47.9% of farmers in central Ethiopia observed abortions, stillbirths and neonatal losses in their flocks, respectively. A study undertaken reviewing livestock production systems of Ethiopia reported mean annual abortion/ stillbirth rate and lamb mortality rate ranged 7–36.4% and 14.9–36%, respectively (Fentie, 2016). However, the causes of these reproductive failures remained undiagnosed and the contribution of sexually transmitted diseases unknown. To improve the reproductive performance of sheep, implementation of an active strategy to improve animal health in order to reduce reproductive diseases is crucial. To build such intervention strategy, information on magnitude of reproductive failures and their possible causes is needed. The present study attempted to investigate and estimate for various Ethiopian sheep breeds enrolled in community-based breeding programs (CBBP), the prevalence of reproductive failures and correlate its possible association with infectious agents and other risk factors.

2. Material and methods

2.1. Description of the study area

The study was carried out in six villages in three districts namely Adiyo Kaka (Bonga), Horro and Menz Gera. These areas are representative of 3 main Ethiopian local sheep breeds, Bonga, Horro and Menz, respectively. In these districts, community-based sheep breeding programmes have been set up with the purpose to develop breeding schemes that suit the communities' conditions and farmers' production objectives (Mueller et al., 2015). Adiyo Kaka is located in 36° 47′E longitude and 7° 26′N latitude which consists of 20.45% *dega* (highland > 2300 m.a.s.l), 61.53% *Woinadega* (intermediate highland 1500–2300 m.a.s.l) and 18.02% (lowland < 1500 m.a.s.l). perennial crop system is the most predominant farming system in the area. Horro is located at about 315 km from Addis Ababa in 9° 34′N latitude and 37° 06′E longitude. The major soil type in the district is red soil. Agro-ecologically, *dega* (highland), *woinadega* (mid-highland) and *kola* (lowland) accounted for about 43% (33,525 ha), 56% (43,661 ha) and 1% (7767 ha) of the total land areas of Horro district. Clay and sandy soils are the major soil types of the district. Mixed crop–livestock systems is the main farming system in the district which integrate crops with livestock husbandry. Menz Gera district is located about 280 kms north of Addis Ababa with an altitude of 3037–3117 m.a.s.l. and 9.783 °N, 7.533 °E (latitude and longitude).Sub-moist/dry, sub-alpine highlands is main agro-ecological setup in the district. Temperature is the main factor determining productivity in the highland sheep–barley production system. At times, night temperatures fall below 0 °C and frosty nights are common, particularly between October and January. Cropping intensity in these areas is generally low. Sheep are the dominant livestock species. The main feed resource-base includes wasteland grazing, stubble and sometimes straw.

2.2. Study population

Participating flock owners were selected among smallholder farmers, including members and non-members of the CBBP. The target animal population was represented by sheep flocks in mixed crop livestock systems under extensive management. Studied animals were allowed to graze freely on pastures during daytime and kept in barn constructed adjoining to human house or sheep house separately built during the night, and all day-to-day management decisions were made by the owners. Some flocks were supplemented with home prepared concentrate, hay and crop residue during morning and night. Local enumerators were recruited for each community to help the research team in animal identification and recording.

2.3. Study design and sample size

A cross-sectional study was carried out to determine the magnitude of reproductive disorders and sero-prevalence of selected abortion causing pathogens in sheep and to investigate potential associated risk factors. In order to incorporate more farms (clusters), the number of animals per farm was limited to a maximum of four animals. A total of 120 households/farms distributed across the six villages were selected for the study. From each flock, blood was randomly taken from 3 to 4 animals. Accordingly, a total of 445 serum samples were collected and tested for five major (*Chlamydia* spp., *Coxiella burnetii*, Toxoplasma, *Brucella* spp. and border disease virus) infectious causes of abortion.

2.4. Blood sample collection and serum separation

Blood samples were taken from 127 male animals aged above six months of age and 318 female animals aged two years and above. Whole blood was collected from the jugular veins into 10 ml sterile vacutainer tubes and stored overnight at room temperature for serum collection. The serum was transferred into a sterile cryovial tube bearing the identification number, location, breed and village then transported on ice to the laboratory where it was stored at −20 °C until analyzed in the National Animal Health Diagnostic and Investigation Center (NAHDIC) in Sebeta.

2.5. Laboratory analyses

For brucellosis, Rose Bengal Precipitation Test (RBPT) was performed and for the other pathogens, commercial indirect ELISAs were conducted following the protocols provided by the test manufacturers. For Chlamydia, the *Chlamydophila abortus* Antibody Test Kit (IDEXX® Switzerland AG, 3097 Liebefeld-Bern Switzerland), for Q-Fever (*Coxiella burnetti*) the Antibody Test Kit, (IDEXX® Switzerland AG, CH-3097 Liebefeld-Bern Switzerland), and for Toxoplasma the *Toxoplasma gondii* Antibody Test Kit, (IDEXX® Switzerland AG, 3097 Liebefeld-Bern Switzerland) were used. For Border Disease Virus (BVD), the Bovine Virus Diarrhoea Virus P80 Antibody Test from Boehringer lngelheim® Svanova, Sweden was used.

2.6. Questionnaire survey

Using a standardized questionnaire, data from 120(79 CBBP members and 41 non-members) sheep flock owners from six villages was collected by face-to-face personal interview. Heads of household were interviewed about history of reproductive failure (abortion, stillbirth, neonatal loss and dystocia) in the flock during the last 12 months (between January 2015 to December 2016), flock size and flock management risk factor attributes, grazing system, presence of pets (dog and cat), feeding practices, ownership of other livestock species such as cattle and goats. Animal level risk factors such as breed, sex, age and body condition were noted during blood samples collection. Age was determined by dentition and body condition scoring was done by body parts examination according to the method described by Abebe and Yami (2008).

2.7. Data analysis

Data was analyzed using STATA v.14.0 (STATACORP, College Station, Texas). Cross tabulations and frequency tables were made and differences in the proportion of reproductive failures between districts were analyzed using Chi-square test. Outcomes of interest were abortion, stillbirth, lamb losses and dystocia. A flock is considered positive if there was at least one animal with the respective condition reported. Similarly, the flock was considered as positive for a disease if at least one animal was seropositive for the tested pathogen. Univariate logistic regression analysis was used to quantify the association between flock level reproductive failures (i.e. history of abortion, lamb losses) and associated potential risk factors. Furthermore, standard poisson and Zero-inflated poisson regression was fitted to predict the number of abortions and number of neonatal losses in the flocks, respectively. Vuong test was performed to evaluate the model's goodness of fit. A mixed-effects logistic regression model was fitted to identify covariates associated with prevalence of infectious causes of abortion using flock as random effect and breed (Bonga, Horro and Menz) sex (male and female), age (lamb, young, adult and old), body condition score (thin, medium and fat) and flock size (< 15, $\geq 15 < 30$ and ≥ 30) as fixed effects. Variables with a p-value of $\leq$ 0.25 in univariable analysis were included for fitting the mixed effect multivariable logistic regression model at animal level. Stepwise backward elimination was performed until all the variables included were significant at a P-value ≤ 0.05.

3. Results

3.1. Overall prevalence of reproductive failures in the flock

Irrespective of district and breed, the overall flock level rate of abortion, stillbirth, dystocia, and neonatal losses per year were found to be 20%, 10%, 5% and 65%, respectively. From 120 flocks surveyed, higher ($P < 0.001$) frequency of abortions at flock level was recorded in Horro district compared to Menz and Bonga (Table 1). However, no differences were observed between districts for the occurrence of stillbirth, dystocia and neonatal losses.

Table 1
Flock level prevalence of reproductive failures in the different districts.

Reproductive failures: n (%)	Menz Gera (n = 41)	Horro (n = 39)	Bonga (n = 40)	Total (n = 120)	P-value
Abortion	5 (12.20)	16 (41.03)	3 (7.5)	24 (20)	0.000
Stillbirth	1 (2.44)	7 (17.95)	4 (10)	12 (10)	0.069
Dystocia	2 (4.88)	4 (10.26)	0 (0.0)	6 (5)	0.112
Neonatal losses	27 (65.85)	29 (74.36)	22 (55)	78 (65)	0.195

3.2. Factors associated with abortion and neonatal loss

Given the low number of cases for dystocia and stillbirth, univariate analysis was conducted only for occurrence of abortion and lamb losses. Due to multicollinearity between district and breed, breed was removed from analyses. Occurrence of abortion was higher ($P < 0.05$) in Horro than Menz district; in the presence of dogs, in flocks grazing in communal pastures compared to private grazing, and in flocks housed in sheep house compared to adjoining the house. The occurrence of lamb losses was significantly associated with *C. burnetti* sero-positivity (Table 2).

3.3. Poisson regression analysis of number of abortions

A total of 33 abortion cases were recorded in the 120 flocks surveyed with mean number of abortions within aborted flocks of 0.28 (33/120) per flock. The annual number of abortions per flock ranged from 0 to 3. The number of abortions in the flocks was higher in Horro district (IRR = 4.55, 95% CI: 1.85, 11.18; $P < 0.001$) as compared to Menz Gera District. Univariate Poisson regression showed that the number of abortions at flock level increased as flock size increased ($P < 0.001$). The incidence risk ratio for abortion was higher in feed supplemented flock compared to un-supplemented flocks (IRR = 2.82, 95% CI: 1.22, 6.50; P = 0.02) (Table 3).

The final Poisson regression model revealed district and flock size as independently associated explanatory variables with number of abortions ($P < 0.05$)(Table 4). The number of abortions was higher in Horro district (IRR = 3.32, 95% CI: 1.08, 10.21; P = 0.04) than Menz Gera district. Similarly, number of abortions was higher in flock size 15–30 animals (IRR = 0.34, 95% CI: 0.14,0.82; P = 0.02) and above 30 animals (IRR = 0.27, 95% CI: 0.11-0.64; P = 0.00) (Fig. 1)

3.4. Zero inflated Poisson regression analysis of number of neonatal losses

A total of 258 annual neonatal losses were recorded from 120 flocks surveyed in the three districts. The mean number of lamb losses within flocks was 2.15 (258/ 120). The annual number of lamb loss per flock ranged from 0 to 30. Univariate zero inflated poisson regression showed that the IRR of neonatal loss in Horro district was 1.83 times that in Menz Gera (IRR = 1.83; 95% CI: 1.06, 3.15;P = 0.03). Number of neonatal loss at flock level was higher in *C. burnetii* positive flocks (IRR = 11.65, 95% CI: 1.14,2.39; P = 0.01). Similarly, the number of neonatal losses was higher in flock size above 30 animals (IRR = 1.78, 95% CI:, 1.32, 2.41; $P < 0.001$) as compared to flock size less than 15 animals. No significant association ($P > 0.05$) was found between number of neonatal loss and membership of a CBBP, presence of pets (cats and dogs), presence of other livestock (goat and cattle), grazing system, feed supplementation, and *Chlamydia* spp or and *T. gondii* serostatus (Table 5).

The final Zero inflated Poisson regression model revealed that district, *C. burnetii* and *T. gondii* serostatus and flock size as independently associated explanatory variables with number of neonatal death ($P < 0.05$). The risk of neonatal loss in Horro flocks is increased by 2.17 times compared to Menz flocks (IRR = 2.17, 95% CI: 1.53, 3.08;

Table 2
Risk factors associated with flock level abortion and neonatal loss univariable logistic regression analysis.

Variables	Categories	No. of flocks sampled	No. of flocks with abortion(%)	P-value	No. of flocks with neonatal loss (%)	P-value
District	Menz	41	5(12.20)		27(65.85)	
	Horro	39	16(41.03)	0.005	29(74.36)	
	Bonga	40	3(7.50)	0.483	22(55)	0.195
Flock size	< 15	53	10(18.87)		34(64.15)	
	≥ 15, < 30	45	8(17.78)	0.890	27(60)	0.374
	≥ 30	22	6(27.27)	0.421	17(77.27)	
CBBP	No	41	8(19.51)		28(68.29)	0.586
	Yes	79	16(20.25)	0.923	50(63.29)	
T. gondii positive	No	35	5(14.29)		25(71.43)	0.343
	Yes	85	19(22.35)	0.315	53(62.35)	
C. burnetii positive	No	38	5(13.16)		19(50)	0.019
	Yes	82	19(23.17)	0.202	59(71.95)	
Chlamydia spp. positive	No	13	1(7.69)		11(84.62)	0.116
	Yes	107	23(21.50)	0.240	67(62.62)	
Presence of cats	No	67	12(17.91)		44(65.67)	0.862
	Yes	53	12(22.64)	0.520	34(64.15)	
Presence of dog	No	46	5(10.87)		30(65.22)	0.969
	Yes	74	19(25.68)	0.049	48(64.86)	
Communal grazing	No	30	1(3.33)		15(50)	
	Yes	90	23(25.56)	0.008	63(70)	0.050
Supplementation	No	43	7(16.28)		29(67.44)	0.675
	Yes	77	17(22.08)	0.446	49(63.64)	
Housing	adjoining house	38	3(7.89)		24(63.16)	0.773
	Sheep house	82	21(25.61)	0.024	54(65.85)	
Presence of goat	No	98	21(21.43)		64(65.31)	0.882
	Yes	22	3(13.64)	0.409	14(63.64)	
Presence of cattle	No	15	3(20.0)		4(73.33)	0.469
	Yes	105	21(20.0)	1.00	38(63.81)	

Table 3
Risk factors associated with flock level number of abortions using univariable Poisson regression analyses.

Variables	Categories	Univariate IRR	(95%CI)	P-value
District	Menz Gera		1	
	Horro	4.55	1.85, 11.18	0.00
	Bonga	1.36	0.38, 4.81	0.64
Flock size	< 15		1	
	≥ 15, < 30	0.27	0.12, 0.64	0.00
	≥ 30	0.26	0.11, 0.59	0.00
CBBP	No		1	
	Yes	0.96	0.46, 2.01	0.91
T. gondii positive	No			
	Yes	1.86	0.72,4.83	0.20
C. burnetii positive	No		1	
	Yes	1.05	0.44, 2.55	0.91
Chlamydia spp. positive	No		1	
	Yes	3.76	0.51, 27.54	0.19
Presence of cats	No		1	
	Yes	0.77	0.39, 1.53	0.45
Presence of dog	No		1	
	Yes	1.38	0.57, 3.35	0.47
Communal grazing	No		1	
	Yes	2.79	0.67, 11.65	0.16
Supplementation	No		1	
	Yes	2.82	1.22, 6.50	0.02
Housing	adjoining house		1	
	Sheep house	1.23	0.47, 3.18	0.67
Presence of goat	No		1	
	Yes	0.47	0.14, 1.56	0.22
Presence of cattle	No		1	
	Yes	0.73	0.28, 1.89	0.51

P = 0.00). The incidence risk ratio for neonatal was significantly higher in flocks with size > 30 animals (IRR = 2.01,95% CI 1.43, 2.81;P = 0.00) and in the presence of cat in the household (IRR = 0.73, 95% CI: 0.55,0.97;P = 0.03). Similarly, neonatal losses was significantly higher in *C. burnetti* (IRR = 1.80 95% CI:1.15, 2.81 P = 0.01) and *T. gondii* positive flocks (IRR = 0.68, 95% CI:0.49,0.95; P = 0.03) (Table 6)

Table 4
Risk factors associated with flock level number of abortions using multivariable Poisson regression analyses.

Variables	Categories	Multivariable IRR	(95%CI)		P-value
District	Menz Gera	1			
	Horro	3.41	1.35	8.64	0.01
	Bonga	0.82	0.22	3.03	0.76
Flock size	< 15	1			
	≥ 15, < 30	0.35	0.14	0.83	0.02
	≥ 30	0.27	0.11	0.62	0.00

3.5. Seroprevalence of major infectious causes of abortion

The serological analyses revealed the presence of three abortive infections, namely *T. gondii, Chlamydia spp*, and *C. burnetti*, with none of the flocks testing positive for *Brucella* spp. or border disease virus. From 120 flocks tested, 107 (89.17%) were positive to chlamydiosis, 82(68.33%) to *C. burnetti*, 85 (70.83%) to toxoplasmosis and 102 (85.0%) showed mixed infections (Table 7).

At animal level seroprevalence was 58.20% (259/445) for *Chlamydia sp*, 37.98% (169/445) for Q -fever and 39.78% (177/445) for *T. gondii* and their prevalence were significantly different between study districts (P < 0.001). Of 445 sheep tested, 41.80% (95% CI:) were sero-positive to more than one species of abortive agents (Fig. 2).

3.6. Factors associated with seropositivity

The univariate analysis indicated that sero-positivity of three abortive agents (*Chlamydia* spp., *C. burnetiid, T. gon*dii) was associated (p = 0.00) with district (Table 8). Due to collinearity of district with breed, breed was omitted from all analyses. Sex, age, body condition, flock size and being a CBBP member did not show significant association (P > 0.05) with *Chlamydia* sero-positivity. However, for *C. burnetti*, age, body condition and flock size were associated (P < 0.05) with sero-positivity. Moreover, sex, age categories, flock size and being member of CBBP were noted as risk factors for *T. gondii* sero-positivity.

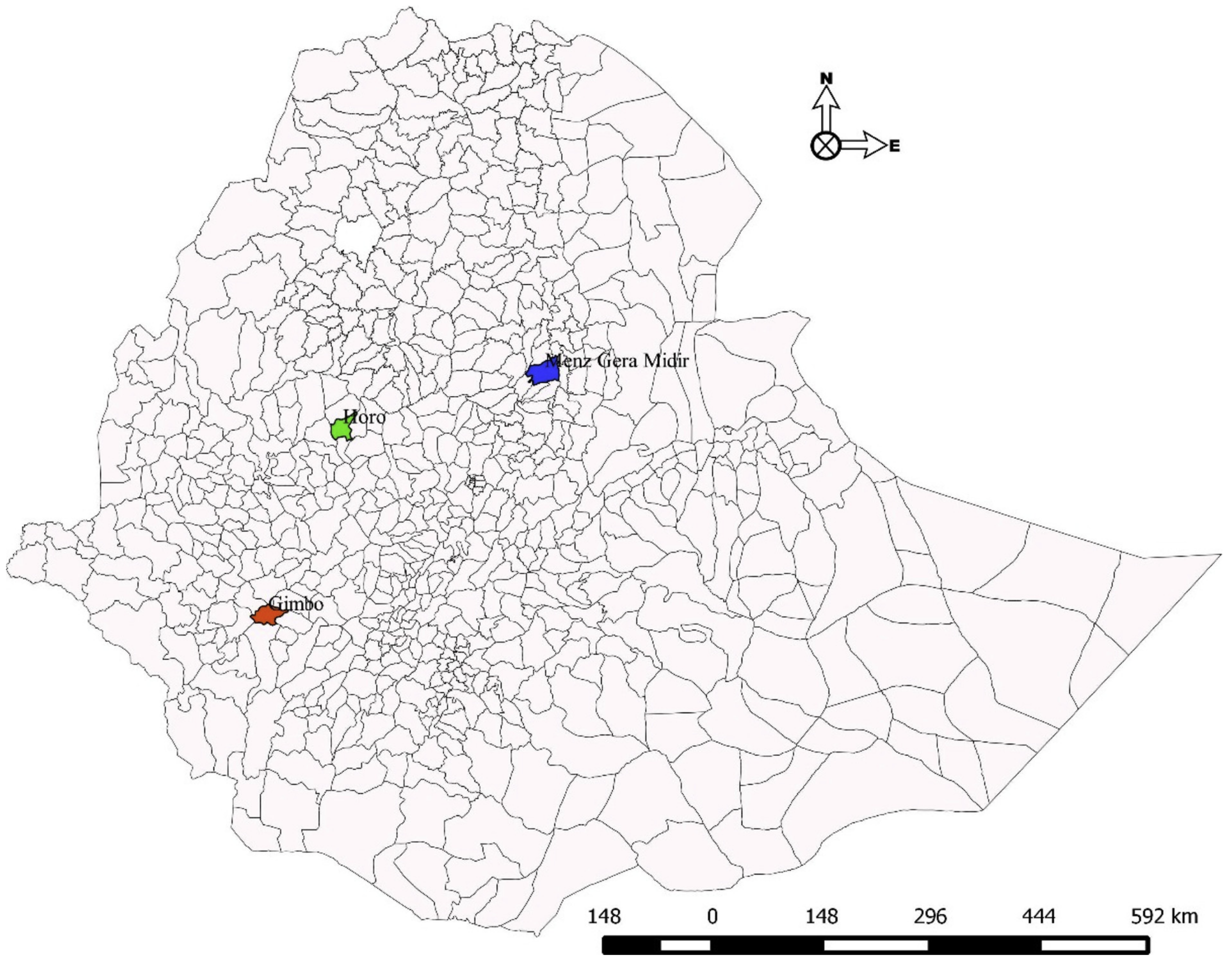

Fig. 1. Map of study areas.

Table 5
Risk factors associated with flock level number of neonatal loss using univariable Zero-Inflated Poisson regression analyses.

Variables	Categories	Univariate IRR	95%CI		P-Value
District	Menz Gera	1			
	Horro	1.83	1.33	2.50	0.00
	Bonga	1.04	0.71	1.53	0.85
Flock size	< 15	1			
	≥15, < 30	0.83	0.59	1.16	0.27
	≥ 30	1.78	1.32	2.41	0.00
CBBP	No	1			
	Yes	1.22	0.92	1.61	0.17
T. gondii positive	No				
	Yes	1.07	0.81	1.42	0.62
C. burnetti positive	No	1			
	Yes	1.65	1.14	2.39	0.01
Chlamydia spp. positive	No	1			
	Yes	1.22	0.82	1.80	0.33
Presence of cats	No	1			
	Yes	0.85	0.65	1.11	0.23
Presence of dog	No	1			
	Yes	1.23	0.93	1.62	0.14
Communal grazing	No	1			
	Yes	1.41	0.95	2.07	0.09
Feed supplementation	No	1			
	Yes	1.39	1.05	1.85	0.02
Housing	adjoining house	1			
	Sheep house	1.20	0.89	1.61	0.23
Presence of goat	No	1			
	Yes	0.96	0.68	1.35	0.81
Presence of cattle	No	1			
	Yes	1.05	0.72	1.53	0.81

Table 6
Risk factors associated with flock level number of neonatal loss using multivariable Zero-Inflated Poisson regression analyses.

Variables	Categories	Multivariable IRR	95%CI		P-Value
District	MenzGera	1			
	Horro	2.17	1.53	3.08	0.00
	Bonga	1.74	1.10	2.75	0.02
Flock size	< 15	1			
	≥ 15, < 30	0.88	0.62	1.25	0.48
	≥ 30	2.01	1.43	2.81	0.00
Toxoplasmosis positivity	No	1			
	Yes	0.68	0.49	0.95	0.03
Coxiellosis positive	No	1			
	Yes	1.80	1.15	2.81	0.01

Table 7
Flock prevalence of chlamydiosis, Q -fever and Toxoplasmosis in sheep in study area.

Agents	Menz Gera (41)	Horro (39)	Bonga (40)	Total (120)	p-value
C. abortus	34(82.93)	33(84.62)	40(100)	107(89.17)	0.025
C.burnetii	3(90.24)	31(79.49)	14(35)	82(68.33)	0.000
T. gondii	21(51.22)	34(87.18)	30(75)	85 (70.83)	0.001
Mixed infection	35(85.37)	36(92.31)	31(77.50)	102 (85.00)	0.182

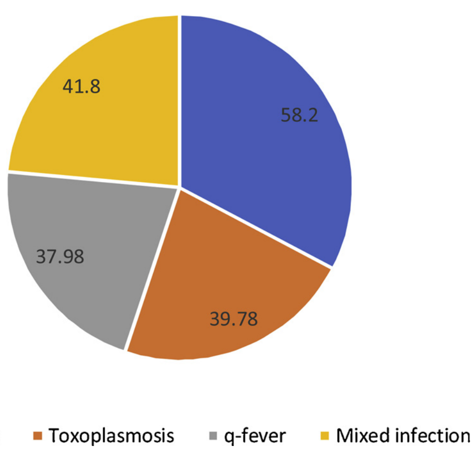

Fig. 2. Animal level sero-prevalence of chlamydiosis, Q -fever, Toxoplasmosis and mixed infection in sheep in study area.

For multivariatble analysis, stepwise mixed effect logistic regression technique was used and the relative effect of the independent variable on the outcome variable was determined. Variables with a p-value < 0.25 were in univariable analysis were included (Victora et al., 1997). The final multivariable mixed effect logistic regressions analyses indicated that for *Chlamydia* spp. only District remained significant, for *C. burnetti* district and flock size, and for T. gondii sex, district and CBBP-membership were identified as risk factors (Table 9).

4. Discussions

This study provided important insights into causes of reproductive failure in smallholder systems in Ethiopia by testing for several possible agents at the same time. It demonstrated the prevalence of reproductive failures (history of abortion, stillbirth, Neonatal losses and dystocia) in sheep flocks and the possible role infectious agents have in these failures. Neonatal loss was the main reproductive failure with 65% (78/120) flock level prevalence followed by abortion (20%) and stillbirth (10%). This is in agreement with reports of Gebremedhin et al. (2013a) and Fentie (2016) who indicated abortion, still birth and neonatal losses as major reproductive problems in small ruminants in Ethiopia. Flock level annual prevalence of abortion in this study was lower than reported by Gebremedhin et al. (2013a, 2013b) in Ethiopia and by Kardjadj et al. (2016) in Algeria who reported 57.5% and 75.33% flock prevalence of small ruminant's abortion respectively; the two figures may reflect variation between years. Our findings revealed that neonatal loss is widely prevalent in sheep flocks in all the studied districts. Prevalence ranged from 55 to 74.36%. The recorded flock prevalence of neonatal loss was higher than prevalence reported by Gebremedhin et al. (2013a). Since reproductive failure is multi-factorial in cause, variations between the results of this work and those reported by other investigators could be explained by differences in environmental factors, breeds and management systems. It should however be noted that in this study, recall bias may have occurred and to obtain more reliable data, longitudinal studies are needed. Furthermore, early abortion is difficult to detect, especially in sheep and may have been missed.

The study found a higher prevalence of abortion in Horro compared to other districts. The higher prevalence in Horro district might be related with management and environmental factors. Horro sheep have more accesses for communal grazing pasture than Bonga and Menz. This might increase the exposure of flocks to abortion causing infectious agents which are easily spread among animals where communal grazing is common. As the infectious organisms are discharged into the environment from the uterus in huge numbers, together with the foetus and its membranes following abortion or lambing/kidding, spread of the agents within and between flocks can occur (Menzies, 2011; Crilly and Gascoigne, 2016). The reason for higher abortion in sheep housed in separate shelters than those kept adjacent to human house might be related to sanitation of the barn. Since adjoining house is attached to residence house of the family, the barn might be frequently cleaned. Poor hygiene allows dam to have increased contact with pathogenic agents (Dennis, 1980; Hovers et al., 2014). Moreover, pregnant animals kept in adjoining house were closely monitored by the household members than those kept in separate sheep house.

This study found high flock and animal level prevalence for three (*Chlamydia* spp., *C. burnetti* and *T. gondii*) important pathogens. Since there was no vaccination program for any of the tested pathogenic agents, there was high risk of flock exposure for these agents. However, no association was found between sero-positivity for tested infectious causes of abortion and flock prevalence of abortion. This result is in line with report from Gebremedhin et al. (2013a) who indicated that *T. gondii* sero-positivity was not associated with flock level abortion, still birth and neonatal losses. However, many studies (Givens and Marley, 2008; Hazlett et al., 2013) have demonstrated the role of *Chlamydia* spp., *C. burnetti* and *T. gondii* in abortion in sheep. Besides low sample size, a possible explanation for this finding is that overall apparent flock prevalence of those tested pathogens ranged between 68.33% and 89.17% indicating uniform distribution of pathogens prevalence among the three studied districts. Though serological testing of maternal serum samples may yield positive results, the abortion may not occur during study period since the development of immunity within the flock prevents subsequent abortions (Givens and Marley, 2008; Menzies, 2011, 2012; Borel et al., 2014). Reproductive pathogens are potentially a huge problem for reproductive efficiency in breeding programs and even though, this study did not find conclusive higher risks for animals enrolled in CBPP, the overall high prevalence of disease is of concern. This is particularly important in dissemination of improved rams to other communities.

Regarding flock size as risk factor for reproductive failure, results from earlier studies differ. Kardjadj et al. (2016) reported non-significant link between flock size and abortion. This result disagreed with report from central Ethiopia by Gebremedhin et al. (2013b) who indicated that abortion history was influenced by flock size. In our study, multivariable poisson regression of abortion indicated that animals of flocks sized between 15 and 30 animals and more than 30 animals had

Table 8
Risk factors associated with Chlamydiosis, Q -fever (*C. burnetti*) and *T. gondii* sero-positivity using univariable mixed effect logistic regression analysis using farm as random effect.

Risk factors		No. tested	C. abortus		T. gondii		C. burnetii		Mixed infection	
			No positive (%)	(P-value)	No positive (%)	(P-value)	No positive (%)	(P-value)	No positive (%)	(P-value)
District	Bonga	148	111(75)	0.000	61(41.22)	0.000	18(12.16)	0.000	56(37.84)	0.186
	Horro	151	79(52.32)		85(56.29)		72(47.68)		72(47.68)	
	MenzGera	146	69(47.26)		31(21.23)		79(54.11)		58(39.73)	
Sex	Female	318	191(60.06)	0.208	145(45.6)	0.000	128(40.25)		151(47.48)	0.000
	Male	127	68 (53.54)		32(25.2)		41(32.28)	0.118	35(27.56)	
Age	Lamb	68	46(67.65)		21(30.88)		8(11.76)		17(25)	
	Young	84	46(54.76)	0.331	21(25)	0.001	24(28.57)	0.000	26(30.95)	0.000
	Adult	90	49(54.44)		37(41.11)		38(42.22)		39(43.33)	
	Old	203	118(58.13)		98(48.28)		99(48.77)		104(51.23)	
BCS	Thin	319	179(56.11)	0.132	130(40.75)	0.799	134(42.01)	0.014	138(43.26)	0.609
	Medium	118	73(61.86)		44(37.29)		34(28.81)		45(38.14)	
	Fat	8	7(87.5)		3(37.50)		1(12.50)		3(37.5)	
Flock	< 15	196	116(59.18)	0.873	92(46.94)	0.018	62(31.63)	0.000	83(42.35)	
	≥ 15, < 30	166	94(56.63)		54(32.53)		57(34.34)		64(38.55)	0.436
	≥ 30	83	49(59.04)		31(37.35)		50(60.24)		39(46.99)	
CBBP	No	141	78(55.32)	0.401	39(27.66)		50(35.46)	0.456	49(34.75)	0.040
	Yes	304	181(59.54)		138(45.39)	0.000	119(39.14)		137(45.07)	
Overall		445	259(58.20)		177 (39.78)		169(37.98)		186(41.80)	

Table 9
Risk factors associated with Chlamydiosis, Q -fever and Toxoplasmosis using multivariable mixed effect logistic regression analysis.

Pathogenic agent	Risk factors	Predictors	Odd Ratio	95%CI		P-value
Chlamydia spp.		Bonga	1			
	District	Horro	0.3	0.15	0.6	0.00
		MenzGera	0.25	0.12	0.5	0.00
C. burnetii	District	Bonga				
		Horro	7.52	3.61	15.63	0.00
		MenzGera	9.33	4.33	20.12	0.00
	Flock size	< 15	1			
		≥15, < 30	0.82	0.44	1.51	0.53
		≥30	2.27	1.09	4.71	0.03
T. gondii	Sex	Female	1			
		Male	0.35	0.21	0.61	0.00
	District	Bonga	1			
		Horro	1.96	1.05	3.66	0.03
		MenzGera	0.4	0.2	0.78	0.01
	CBBP	No	1			
		Yes	2.51	1.39	4.54	0.00
Mixed infection	Sex	Female	1			
		Male	0.32	0.19	0.54	0.00
	CBBP	No	1			
		Yes	1.87	1.04	3.36	0.04

higher risks of abortion than animals belonging to small flocks. A possible explanation may be better control and follow up around the time of mating and during the pregnancy period when the sheep are kept in smaller groups. It may also be related to behavioral traits, as sheep in larger group show decreased synchrony in resting and feeding behavior. Moreover, larger flocks are more market oriented with a consequent higher flow of animals in and out with high probability of disease introduction by carrier animals.

Multivariable zero inflated poisson regression analyses revealed that incidence risk of neonatal loss varied with breed, flock size, *T. gondii* and *C. burnetii* positivity, grazing system and housing system. Thus, framers' awareness on application of appropriate management techniques for both the dam and neonate are needed. This includes training on importance of maintaining good hygiene at lambing, nursing sick lambs, fostering and maintaining ewes in good body condition during gestation.

The prevalence of *Chlamydia spp* in our study was 58.20% and this is lower than Bagdonas et al. (2007) from Lithuania who reported 67.18% seroprevalence. However, it is higher than reports from Greece (Bisias et al., 2009), Saudi Arabia (Abd et al., 2011), Morocco (Benkirane et al., 2015) and from Iraq (Fahad and Salman, 2017). Higher prevalence recorded in Bonga might be related with ecology and topography difference where sheep were kept. *C. abortus* is a major cause of abortion in sheep in almost every sheep-rearing region of the world which causes considerable economic losses in agricultural industries. In a newly infected flock, about one third of pregnant ewes may abort in the last stage of gestation or give birth to weak or dead newborns (Nietfeld, 2001; Rodolakis and Laroucau, 2015). Our results indicate that this is likely a problem for Ethiopian farmers that has been neglected in the past. In addition, *C. abortus* presents a zoonotic risk, inducing mild influenza-like illness or pneumonia in humans(Tontis and Zwahlen, 1991; Meijer et al., 2004).

With regard to *C. burneiti*, the result of this study is in line with 37.22% seroprevalence reported from Eastern Slovakia by Dorko et al. (2010). Nevertheless, it is higher than 15.3% seroprevalence from Morocco (Benkirane et al., 2015), 9% from Italy (Masala et al., 2004) and 19.5% seroprevalence form Iran (Asadi et al., 2013) and lower than 48.8% seroprevalence form Greece (Bisias et al., 2009). The fact that there is a higher infection rate for *C. burneiti* in Menz compared to Horro and Bonga might be due to difference in management practice, husbandry system and availability of other favorable condition for *C. burnetii* transmission. Higher seroprevalence recorded in flock size more than 30 animals might be linked with crowding and overpopulation especially during night and watering point facilitate the transmission of these agents with the flock through aerosol. *C. burnetii* is zoonotic infection that has particular hazard to pregnant women and the developing fetus which result in fetal death and also cause severe illness in the mother(Buxton and Henderson, 1999).

Seroprevalence of *T. gondii* infection in sheep flock in the present study is similar to the finding of 37.0% from in Central Ethiopia (Gebremedhin et al., 2013b) and comparable to endemic situations elsewhere, such as the 38.75% from Italy (Zedda et al., 2010). However it is higher than 20.8% that reported from in Morocco by Benkirane et al. (2015) and lower than 55.18% report from Southwestern Ethiopia (Tegegne et al., 2016).

The present study indicated that sheep from the Horro and Bonga areas were significantly higher seroprevalence than those from Menze. Variation among districts can be explained by the variation in temperature and moisture in areas where these animals were managed. It is well known that the epidemiology of toxoplasmosis is influenced by the environment (Tenter et al., 2000). Humidity increases, the chance of oocyst survival in the environment, thereby contributing to the higher seroprevalence. The seroprevalence of *T. gondii* antibody was higher in female (45.6%) than in male (25.2%), which is in line with findings of Tegegne et al. (2016) who also found higher prevalence in female than male. The difference might be attributed to the management system in that ewes are retained in the farm for longer periods for breeding purpose than males. Few rams are retained for mating while the majority are culled and sold for cash purpose (Alemayehu et al., 2015). The hormonal difference in relation to stress of lactation and pregnancy leading to immunosuppression may also increase susceptibility to toxoplasmosis in females (Dubey, 2009). Higher seroprevalence was observed in flock supplemented with additional feed than unsupplemented flocks. Greece study also found correlation between the use of concentrate and the prevalence of toxoplasmosis in sheep (Tzanidaki et al., 2012). Storage of feed at farm may increase the abundance of rodents on the farm and subsequently cat because farmers often keep cats because to get rid of rodents. This might increase the chance of feed stuff contamination by *T. gondii* oocysts from rodents' and cats' feces.

Sheep under CBBP are well managed compared to non-participants (Haile et al., 2013). However, unexpectedly, higher seroprevalence of *T. gondii* were recorded in sheep kept by CBBP members. The possible explanation for this can be that CBBP members share rams for breeding purpose (Mueller et al., 2015). This might facilitate transmission of *T. gondii* between the flock. Experimentally it has been proven that *T. gondii* tachyzoites able to infect sheep via semen (Moraes et al., 2010b,a). This calls for rams in CBBP to be critically examined before dissemination.

Correspondingly, 41.8% of tested animals were infected by two or more infectious abortion causing agents. This result indicated that multiple agents are involved in reproductive failure which need careful investigation to identify actual pathogenic agents responsible for reproductive failure and comprehensive control strategies to minimize their economic and public impact designed. Pastoralists and small holder farmers should be educated to avoid the risk of zoonotic pathogens to themselves and their families.

5. Conclusion

Our study has demonstrated the importance of reproductive failures in sheep in different districts of Ethiopia and the likely contribution of different infectious agents to these disorders. While CBBPs are an attractive option for genetic improvement of small ruminants, care should be placed on the importance of health and serological testing of the rams for major diseases which can be transmitted through semen among CBBP member flocks and which should constitute the basis of a breeding ram certification. Reproductive failures affect the productivity of the flocks and consequently the livelihood of sheep owners'. Therefore, our study may be considered an opening research of its kind and more in-depth epidemiologic field studies and targeted intervention are needed.

Declarations of interest

None.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.smallrumres.2019.05.019.

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