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Seroprevalence, Isolation, Molecular Detection and Associated Risk Factors of Brucellosis in Small Ruminants in Selected Pastoral Districts of Oromia and Somali Regional States, (Ethiopia)

Teferi Benti Moti [1*], Abebe Olani Bulto [1], Mekonnen Sorsa [2], Bayeta Sanbeta Wakijira [1], Abde Aliy Mohammed [1], Abdella Bulbula [1], Abdi Ahmed [1]

[1] Animal Health Institute, Sebeta, Ethiopia

[2] School of Veterinary Medicine, Ambo University, Ethiopia

Corresponding Author; **Teferi Benti Moti**

Abstract

Background: Brucellosis is a bacterial zoonotic disease that has important veterinary and public health consequences as well as economic impact in sub Saharan Africa, including Ethiopia. The purpose of this study was to detect Brucella DNA from isolates and blood clots, to estimate the prevalence of brucellosis and assess associated risk factors in sheep and goats**.**

Methods: The cross-sectional study was conducted in four selected districts of Oromia and Somali Pastoral areas, Southern, Ethiopia from April 2020 to December 2021. 14 vaginal swabs and 7 whole blood samples were purposefully collected from animals that had recently undergone an abortion. A total of 841 serum samples (267 from sheep and 574 from goats) were collected. Antibodies against Brucella infection was detected by multi-species indirect enzyme-linked immunosorbent assay, isolation of Brucella was performed by selective media, real time PCR for DNA detection, and Ct value. Using a standardized questionnaire, data from individual animals and flocks was collected to investigate the association between expounding and outcome variables. The data was evaluated using STATA version 14.0.

Results: The overall seroprevalence rate was 12.84% (108/841), 6.7% (18/267) in sheep and 15.6% (90/574) in goats. No Brucella organism was isolated from vaginal swabs and blood culture. An analysis of 108 seropositive blood clots for Brucella DNA revealed that 15/108 (13.9%) tested positive for the universal primers (IS711). For Brucella specious DNA detection, species-specific primers were employed, 13/15 (87%) positive for Brucella abortus and melitensis, while two genera were not respond. Furthermore, 95% of owners who were interviewed did not have knowledge of brucellosis. The univariable logistic analysis and a multivariable revealed statistically significant differences (p 0.05) among age groups (OR: 0.29 (95% CI: 1.8-9.94)), species (OR: 0.45, 95% CI: 0.26–0.77), and abortion history (OR: 0.05 (95% CI: 0.24-1.22)) While there was no significant difference between sexes (p-value > 0.05).

Conclusion: Brucellosis is endemic in the pastoralist area and could lead to abortion, infertility, and productivity losses. The diseases can spread from infected animals to humans and other animals. Therefore, one-health and integrated intervention strategies are needed to restrict the disease's spread, and pastoral communities should regularly raise awareness of brucellosis control methods.

Keyword: Brucella, Brucellosis, Small Ruminant, Risk Factor, Real Time PCR & Zoonotic

1. Introduction

Small ruminants are an essential part of the farming systems in the majority of developing nations, and they make up nearly half of all domesticated ruminants worldwide [1]. Between 1.35 billion and 1.94 billion small ruminants are thought to exist worldwide [2]. In developing nations,

particularly in areas where animals have historically been raised primarily for immediate revenue sources, milk, meat, wool, manure, and savings are an important part of livestock. Even in tropical and subtropical Africa, small ruminants serve a variety of social and cultural purposes that vary depending on the socioeconomic context, agro-ecology, culture, and geographic location [3]. For smallholder farmers, sheep and goats offer numerous benefits over large ruminants, including lower feed costs, faster returns, simpler maintenance, and suitable size at slaughter. Compared to large ruminants, they have a low mortality rate during drought, demonstrating their great degree of environmental adaptability. Furthermore, due to the significant danger of losing large ruminants, breeders choose sheep and goats [4].

Ethiopia is one of the African countries with the greatest number of small ruminants on the continent [5]. According to the Central Statistical Agency [6], there are an estimated 59.5 million cattle, 30.70 million sheep, 30.20 million goats, 8.44 million donkeys, 2.16 million horses, 1.21 million camels, and 0.41 million mules. Twenty-five percent of sheep and seventy-three percent of goats live in the lowlands of the country [7]. Ethiopian livestock production is classified under different agro-ecological zones, animal health care systems, and several management systems. The country's extensive husbandry system allows for the mixing of several animal species in common grazing areas and watering facilities [8].

Despite the fact that many small ruminant populations are crucial to farmers' livelihoods, the nation underutilizes the resources due to animal diseases. It is still unknown how the production and health systems for animals will be set up. Human zoonotic consequences result from brucellosis, which also damages wild animals and the livestock business [9]. Loss of progeny, decreased production of meat and milk, restricted economic processes, and obstacles to the international export of live animals and their products are all problems faced by small-scale livestock owners [10, 11].

Brucellosis primarily affects the reproductive tract of animals, characterized by inflammation of the genital organs and fetal membranes, abortion, sterility, and the formation of localized lesions within the system and joints [12]. In livestock, brucellosis is mainly caused *by B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, and *B. ovis*. Among these species, *B. melitensis* and *B. ovis* are the common causes of brucellosis in goats and sheep [13, 14]. Many researchers reported that farming and raising goats and sheep along with cattle was to be a risk factor for *Brucella* transmission [15, 16]. *Brucella* species are obligate intracellular parasites, requiring an animal host like ruminants, swine, rodents, canines, marine mammals, wild animals. Genus *Brucella* can survive and multiply within epithelial cells, placental trophoblasts, dendritic cells, and macrophages [17].

In pastoral and agro-pastoral farming, brucellosis is one of the neglected tropical zoonosis that is reemerging due to a lack of public awareness [18, 19]. Humans can be infected with Brucella spp., like B. suis, B. melitensis, B. abortus, and B. canis. In humans, B. melitensis produces deadly illnesses [20]. Human brucellosis can cause a variety of systemic symptoms, including fever of varying duration, whether acute or chronic, intermittent, undulant, or irregular; headache; chills; excessive sweating; widespread aching; weakness; and joint pain [21]. Every year, an estimated 500,000 human cases are reported [22, 23]. Direct contact with diseased animals or tissue secretions, ingestion of contaminated objects, or inhalation can all result in infection in humans [24]. Instead of molecular detection and isolation, antibody detection is used in most of the research that is done in our country. Thus, the aim of this study was to determine the seroprevalence rate of brucellosis in sheep and goats in four sites of the regional states of Somalia and Oromia. Real-time PCR was employed to detect Brucella DNA in isolates and blood clots.

2. Materials and Methods

2.1. Description of the study area

This study was conducted in four districts in Ethiopia's pastoralist regions of the Somali and Oromia Regional States (Figure 1). Filtu and Moyale districts are from the Somali Regional State's Liben Zone, whereas Dubuluk and Gomole districts are in the Borena Zone of Oromia. There are three types of livestock production systems in Ethiopia: mixed crop-livestock, agro-pastoral, and pastoral [25]. Pastoralists rely mostly on animals and their products for their subsistence on the sparsely populated pastoral rangelands that make up the pastoral production system. Due to a lack of rainfall, crop farming production is quite low in pastoralist areas. Cattle, sheep, camels, and goats make up the majority of animal husbandry systems. The pastoralists move between the two pastoral regions during the brief wet season.

The Borena Zone's lowlands include the districts of Dubuluk and Gomole; its capital, Yabello, is located 575 kilometers south of Ethiopia's capital (Figure 1). It is located south of the Oromia Region at $4^{0}2'56".58N$ and $38^{0}13'35".39E$. The Borena Zone shares a border with the neighboring Somali regional states on the east, the Southern Nations, Nationalities, and People Region (SNNPR) on the west, Gugi on the north, and Kenya on the south.

Filtu town, which is 650 kilometers south of Ethiopia's capital, serves as the capital of the Liben zone (Figure 1). Kenya borders the Liben zone on the south; Jubaland, the Somali federal state, borders it on the southwest, and the Oromia Regional State borders it on the northwest. Geographically, Filtu is located at latitudes 4°54'50" north and 40°40'29.29" east.

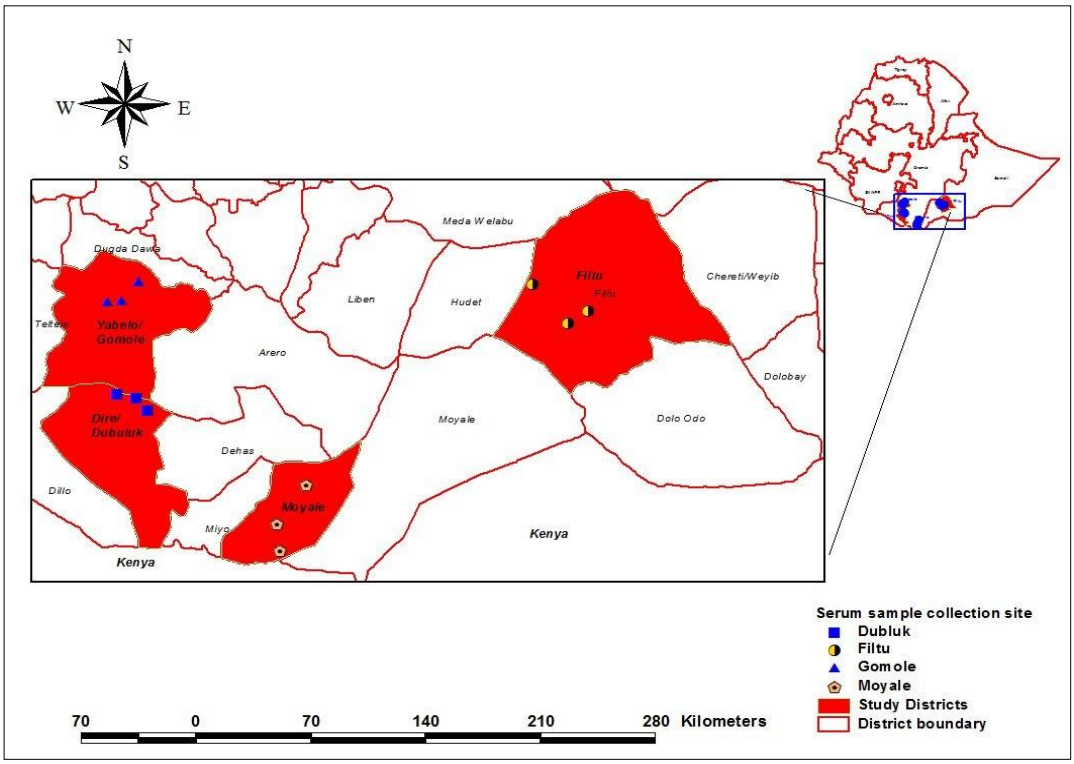

Fig 1: Map showing study area and sampling site

2.2. Study population and sources of data

The target goat and sheep populations were local and blackhead Somali long-eared goats raised in pastoralist areas under extensive management system. The Liben zone's animal population includes 711,000 cattle, 969,000 sheep, 1,874,000 goats, 954,000 camels, and 63,000 donkeys [26]. According to the Borena Zone Pastoral Development of the livestock population [27], there are 1,416,180 cattle, 1,262,782 goats, 776,870 sheep, 237,205 camels, 102,767 donkeys, 1,841 horses, and 4,433 mules. Participants in the study who had not received a brucellosis immunization and were older than six months were included. Each of the following was noted during sample collection: age, species, sex, flock size, and history of aborted animals. The sexual maturity of sheep and goats was used to classify them as young (less than a year old) or mature or adult (>1 years old) Megarsa *et al.* [28].

2.3. Study design and sampling method

A cross-sectional study was conducted in four pastoralist districts in the Somali and Oromia Regional states between April 2020 and December 2021. Brucella antibodies and DNA from isolates and blood clots were detected in the lab using the indirect enzyme-linked immunosorbent test (I-ELISA) and real-time PCR, respectively. Zones and districts were purposefully selected according to the number of sheep and goats, ease of access, and community interest in participating in further studies. Multi-stage sampling was used for the sample units, with flocks in each PA serving as secondary units, pastoralist associations serving as primary units, and sheep and goat sampling serving as tertiary units. The final selections were 212 animals from Dubuluk, 114 from Filtu, 240 from Gomole, and 275 from Moyale.

2.4. Questionnaire survey

Forty livestock owners from twelve pastoralist associations were interviewed in order to determine any possible risk factors on their holdings. Handling aborted fetuses, disposing of potentially infected objects, consuming milk and byproducts, and being aware of the zoonotic nature were among the human risk factors that were covered in the questionnaire.

2.5. Blood sample collection

Biosafety and personal protective equipment were used during the sample collecting process. Each sampling site's geographic coordinates were recorded. Each animal's jugular vein was used to draw blood using disposable needles and a sterile simple vacutainer tube. In light of this, 841 animals—267 sheep and 574 goats—were sampled. To separate serum from blood clots, each blood sample was labeled separately and allowed to coagulate at room temperature for the entire night in an extremely slanting position. Without coming into contact with the clotted blood, the separated serum was collected and labeled in sterile cryovials. Brucella DNA was extracted from the residual blood clots. Ultimately, all collected blood clots and sera samples were securely packaged and delivered in an icebox to the Animal Health Institute (AHI) in an icebox with ice packs and stored at -20 °C until further processing [29].

2.6. Sample size determination

The sample size was calculated using a well-known formula [30]. Small ruminant brucellosis has not been studied before in the Somali region's Liben zone, where it is estimated to be 50% prevalent and 8.1% [31] in the Borena zone of Oromia. Absolute accuracy was employed to reach the maximum sample size at a 95% confidence interval and a 5% precision.

Hence: $N=1.962*Pexp\ (1-Pexp)/d^2$

Where: n= required sample size, Pexp= expected prevalence, and d^2= desired absolute precision. As a result, 499 sample sizes from four districts, including 384 samples from the Liben zone and 115 from the Borena zone, were anticipated to be included in the study. The sample size was raised to 841 in order to improve accuracy and lower standard errors.

2.7. Serological test

The ID.vet Multiple Species Indirect ELISA was used to find antibodies against Brucella species in the serum. The test was verified in accordance with the manufacturer's instructions; the positive control OD mean value (ODPC) was larger than 0.35, and the ratio of the positive and negative control mean values (ODpc/ODnc) was greater than three. According to the sample's positivity percentage inhibition, the results were finally interpreted as follows: ≥ 110 negative, > 110 and < 120 questionable, and ≥ 120% positive.

2.8. **Bacterial culture**

Goats and sheep having a history of recent abortion during the previous 30 days were purposefully sampled in order to isolate and identify brucella. Seven whole blood samples with EDTA and fourteen vaginal discharge swabs in Stuart transport media were aseptically obtained. After being collected, the samples were kept at -20 °C until processing at the Animal Health Institute (AHI) via cold chain transportation. The use of bacteriological samples was carried out in a highly protected biosafety level III (BSL3) laboratory. The samples were streaked in duplicate on Brucella-selected basal media, incubated at 37 °C in aerobic circumstances, and anaerobically using a candle jar and for 20–30 days with frequent inspection [32].

2.9. DNA extraction and amplification

According to the manufacturer's instructions, the genomic DNA was extracted using the QIAMP DNA Mini Kit (QIAGEN GmbH Strasse 1.40724 Hilden, GERMANY). The DNA purification standard is raised by mixing blood clots with a vortex mixer for one minute to break them up and make them more uniform [33, 35]. A total of 108 blood clots (18 from sheep and 90 from goats) were retrieved for Brucella genomic DNA based on the results of serological tests.

Real-time PCR assay: Real-Time-PCR allows both detection and quantification of the PCR product in real-time while synthesizing [39] and is more sensitive, specific, reproducible, and rapid than conventional PCR [36- 38]. Real-time PCR was performed first for screening of Genus *Brucella* using universal primer for genus *Brucella* (IS711), (5'-GCT-TGA-AGC-TTG-CGG-ACA-GT-3') forward, (5'-GGC-CTA-CCG-CTG-GGA-AT-3') reverse primer and FAM-AAG-CCA-ACA-CCC-GGC-CAT-TAT-GGT-BHQ-1 probe with the internal positive control (IPC)10x Exo IPC Mix, and 50x Exo IPC DNA was used [40]. For *B. abortus* detection specific primers (5'-GCA-CAC-TCA-CCT-TCC-ACA-ACAA-3') forward primers (5'-CCC-CGT-TCT-GCA-CCA-GACT-3') reverse primer and the probe sequence. FAM-TGG-AAC-GAC-CTT-TGC-AGG-CGA-GAT-C-BHQ-1used.

For *B. melitensis* (5'-TCG-CAT-CGG-CAG-TTT-CAA-3') forward and 3'-CCA-GCT-TTT-GGC-CTT-TTCC-5' reverse primers were used. The probe for *Melitensis* FAM-CCT-CGG-CAT-GGC-CCG-CAA-BHQ-1 was employed [40].

An extracted DNA was amplified with an Applied Bio systems 7500 PCR system. The master mix components are made for universal primers (IS711) for the genus *Brucella*. The reaction mixture (IS711) Real-time TaqMan® PCR was set up in a final volume of 25 µl and run for 45 cycles [36] with 3.9 µl of RNase-free water, 12.5 µl of TaqMan universal PCR master mix (2x), each primer forward, reverse, and TaqMan® probe at concentrations of 0.2 µM, 10x Exo IPC 2.5 µl, 50x Exo IPC DNA 0.5 µl, and 5 µl of DNA template. For *B. melitensis* and B. *abortus*, the master mix components were made separately in a test tube (0.20 µM primer F, 0.20 µM primer R, 0.20 µM probe, TaqMan universal PCR master mix (2x) 12.5 µL, RNAse-free water 6.9 µL, and DNA template 5 µL for each [40]). For double-strand DNA denaturation, a temperature of 95 °C for 10 minutes, amplification/extension at 95 °C for 15 seconds, annealing at 45 °C for 30 seconds, and 60 °C for 1 min for a final extension were used. This process adjusted to run for 45 cycles and accomplished in about 1.38 hours.

2.9. Data Analysis

All data collected during the study period were coded, entered into a Microsoft Excel spreadsheet, and analyzed using SPSS software version 20.0. The seropositive percentage was calculated based on the optical density (OD) of each sample measured at 450 nm using a Biotech ELx 800 ELISA reader. The prevalence rate of seropositive animals resolves by dividing the number of positive animals by the number of animals tested times 100. The univariate logistic analysis shows the significant difference between age groups, aborted animals, and species analyzed. The multivariable logistic statistically measures the significant association between risk factors and animal-level seropositivity. P-value < 0.05 taken as significant. The Applied Biosystems PCR platform and 7500 Fast SDS 21 CFR software were employed for the analysis of cycle threshold (Ct) values of positive reactions from *Brucella* isolates and blood clots in seropositive animals.

3. Results

3.1 Serological test:

Out of 841 sera samples that were tested for Brucella antibodies, sheep and goats had an overall prevalence rate of 12.84% (108/841) (Table 3). The seroprevalence rates in the Borena zone of the Oromia Regional State were 12.17% (55/452) and 13.62% (53/389) within the Liben zone of the Somali Regional State. Prevalence rates in sheep were 6.7% (18/267), and 15.7% (90/574) in goats were recorded.

Table 1: Seroprevalence of brucellosis in sheep and goat of Somali and Oromia regional states, (Ethiopia)

Region	Species	Examined	Positive	Prevalence rate %
Somali	Ovine	141	7	4.96
	Caprine	248	46	18.54
Oromia	Ovine	126	11	8.73
	Caprine	326	44	13.49
Total		841	108	12.84

In all four districts and 12 pastoral associations of study areas, brucellosis was detected in sheep and goats. The highest prevalence rate was recorded in the district of Dubuluk (28%), followed by Filtu (26.3%), Moyale (15.1%), and the lowest seroprevalence was recorded in the Gomole district (2.8%) by Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) in goats. Also, high seroprevalence in sheep was found at Dubuluk (12.33%), Filtu (10.53%), and Gomole (3.2%), and the lowest prevalence rate was recorded at Moyale (2.9%) (Table 4).

Table 2: Prevalence rate of brucellosis, in selected districts of Somali and Oromia regional states, Ethiopia.

Zone	Districts	Species	Examined	Positive	Prevalence rate %
Liben	Filtu	Ovine	38	4	10.53
		Caprine	76	20	26.3
	Moyale	Ovine	103	3	2.9
		Caprine	172	26	15.1
Borena	Dubuluk	Ovine	73	9	12.33
		Caprine	139	39	28.0
	Gomole	Ovine	63	2	3.1
		Caprine	177	5	2.8
Total			841	108	12.84

In terms of sex classifications, out of all the animals that were tested, 10.5% (9/86) of them were male and 13.1% (99/755) were female. There was no statistically significant difference between males and females (p-value > 0.05). Age-group differences in seropositivity were statistically significant (p-value <0.05), with young animals accounting for 3.67% (4/109) of the positive results and adults for the remainder, 14.2% (104/732) (Table 5). A higher seroprevalence rate was recorded in aborted animals, 25.3% (45/178), compared to animals that had no history of abortion, 9.5% (5/571). Statistically significant variation was observed among aborted and non-aborted animals (p-value < 0.05).

Table 3: Potential risk factors to sheep and goat brucellosis in four districts of Somali and Oromia Regional states, (Ethiopia).

Variables	Factors	Sample Examined	Positive for I-ELISA (%)	X^2	P-Value
Region	Somali	389	53(13.62)	0.40	0.529
	Oromia	452	55(12.17)		
District	Dubuluk	212	48 (22.64)	47.47	0.000
	Filtu	114	24 (21.04)		
	Gomole	240	7 (2.92)		
	Moyale	275	29 (10.55)		
Species	Caprine	574	90 (15.68)	13.00	0.000
	Ovine	267	18 (6.74)		
Age	Young	109	4 (3.67)	9.41	0.002
	Adult	732	104 (14.21)		
Sex	Female	755	99 (13.11)	0.48	0.487
	Male	86	9 (10.47)		
Abortion	Non-aborted	571	54 (9.51)	31.22	0.000
	Aborted	178	45 (25.28)		

Species, age, sex, and aborted versus non-aborted animals were all examined using animal-level multivariable logistic regression analysis. The findings of the logistic regression analysis, which was used to determine risk factors, showed that species, age, and aborted animals were statistically significantly ($P < 0.05$) correlated with sheep and goats infected with Brucella (Table 6). Multivariable logistic regression analysis revealed abortion (OR: 0.054 (95% CI: 0.24-1.22, $P < 0.05$)), species (OR: 0.45 (95% CI: 0.26-0.77, $P < 0.05$)), and age (OR: 0.29 (95% CI: 1.8-9.94, $P < 0.05$)). Sex and Brucella seropositivity in sheep and goats, however, do not appear to be correlated (OR: 1.29, 95% CI: 0.63-2.66, P-value > 0.05).

Table 4: Multiple logistic regression analysis of brucellosis sero-positivity with different risk factors

Risk factors	Total Examined	Sero positivity %	Crude odds ratio (95%CI)	Adjusted Odds ratio(95%CI)	P. value
Species					
Caprine	574	90 (15.68)	1	1	
Ovine	267	18 (6.74)	0.39(0.23-0.66)	0.45(0.26-0.77)	0.000
Age					
Young	109	4 (3.67)	0.23(0.08-0.64)	0.29 (1.18-9.94)	0.005
Adult	732	104(14.21)	1	1	
Sex					
Female	755	99 (13.11)	1	1	0.489
Male	86	9 (10.47)	1.29(.63-2.66)		
Abortion					
No	571	54 (9.51)	0.96 (0.46-2.02)	1.47(0.67-3.20)	0.921
Yes	178	45 (25.28)	3.12 (1.45-6.71)	0.54(0.24-1.22)	0.004
Total	841 108(12.84)				

3.2. Questioners survey results

Table 5: Questioners survey results

Factors	No. of interviewed	Response	Percentage (%)
Community awareness	40	38/40	95
Aborted animals	40	36/40	90
Handle with bare hand	40	39/40	97.5
Mixing animals/flock	40	39/40	97.5
Consumption of raw milk & milk product	40	38/40	95

3.3. Isolation of *Brucella*

Whole blood and cultured swab samples were not developed on selective media and were all negative. The animals' persistent infection may have prevented any viable bacteria from circulating, and the absence of live aborted animals at the time of sample collection may have contributed to the culture failure.

3.4. Real time PCR Results

The ELISA-positive samples were subjected to real-time PCR for confirmation of brucellosis and detection of *Brucella* species in positive samples of blood clots. The Brucella DNA was detected in blood clots using genus-specific primers (IS711). Real-time PCR results revealed that 15/108 (13.9%) of blood clots were positive. Of them, goat samples contained 86.7% (13/15), whereas sheep samples contained the remaining 13.3% (2/15). On the other hand, the results of real-time PCR using species-specific primers revealed that out of the total genus Brucella positive, B. abortus and B. melitensis were identified. (Figure 2).46.7% (7/15) of the goats and 6.7% (1/15) of the sheep were positive for B. melitensis, and 30.8% (4/15) were positive for B. abortus in goats. The co-prevalence of *B. abortus* and *B. melitensis* in positive samples was 13.35% (2/15*)*. While two genera of Brucella DNA that do not react with specific primers to both species are 13.3 (2/15) (Figure 3).

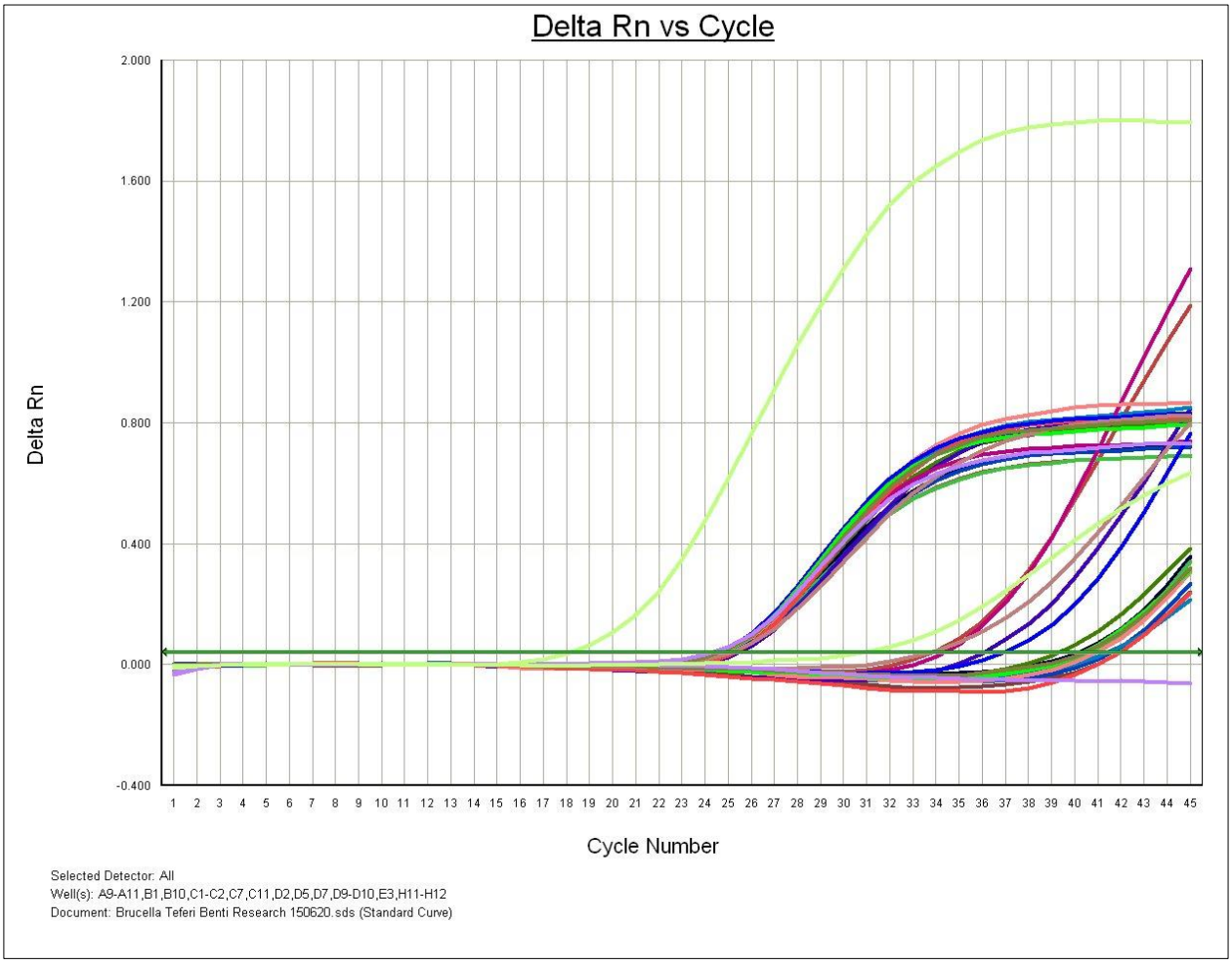

Fig 2: Real Time PCR amplification result of genus *Brucella* screening test using the IS711

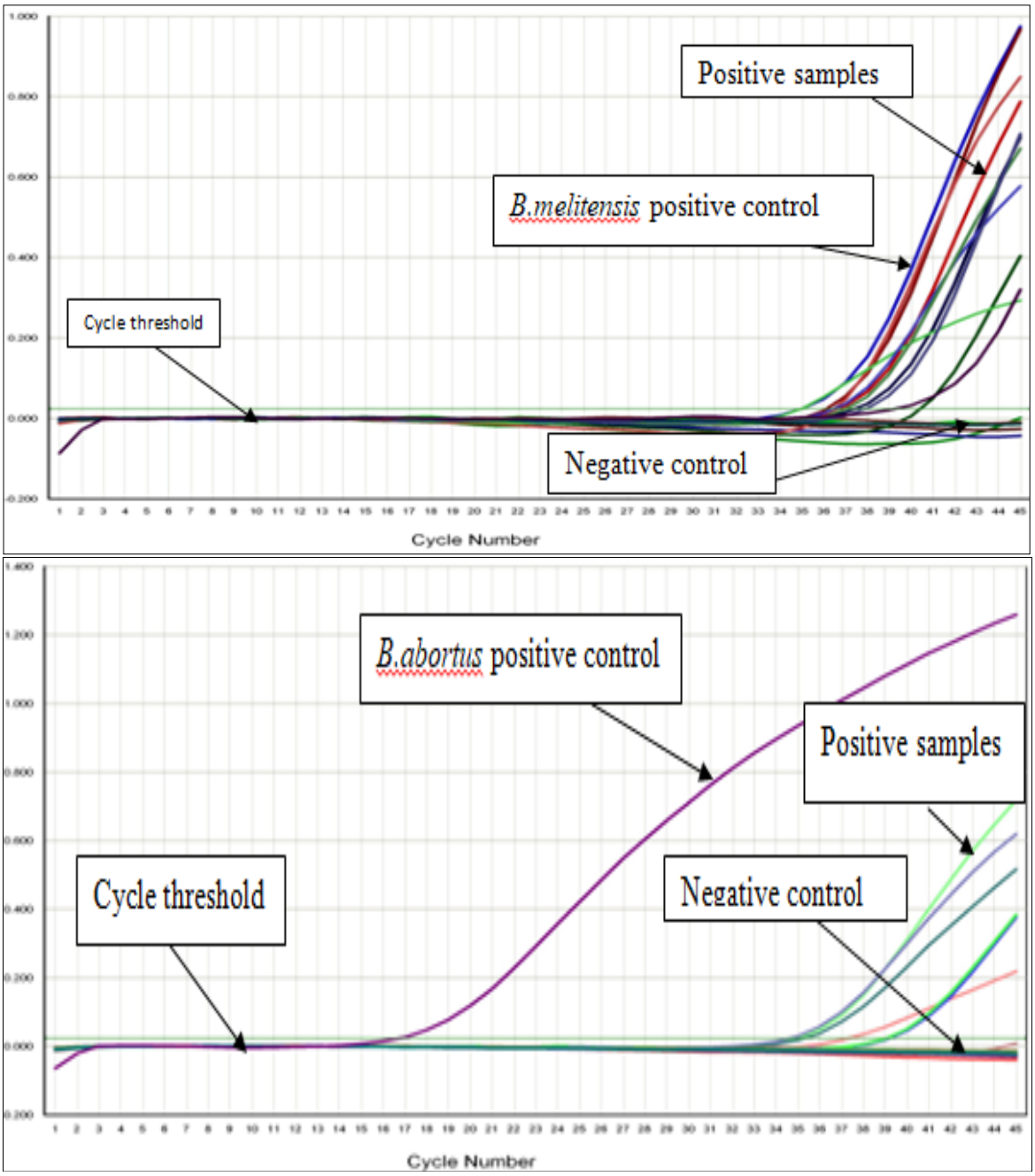

Fig 3: Real time PCR amplification result of primer specific. Left (*B. melitensis)* and Right *(B. abortus)*

4. Discussion

The Seroepidemiological studies in various parts of Ethiopia indicated that brucellosis is widespread among the small ruminant population [41, 42]. Similarly, this study shows a high prevalence rate in individual animals, and *B. abortus and B.* melitensis were detected from blood clots by real-time PCR. The current study showed that the overall seroprevalence rate was 12.84% in small ruminants within the Borena zone of Oromia and the Liben zone of Somali Regional states. In the same way, [43]; 13.6% reported in four districts of Afar [44]; 12.35% [45]; 13.7% in Tellalak District of Afar [46]; 12.35% in Chifra and Ewa Districts of Afar, in Ethiopia. In contrast, low prevalence rates were reported in Ethiopia by [22]; 1.7%; [47]; 1.37%; [48]; 1.9%; [42]; 3.6%; [49]; 3.5%, and (9.7% in Afar and Somali pastoral areas in small ruminants). However, the prevalence rate is less than the previous reports in other countries by [50]; 27.6% and [51]; 34.88% in sheep and goats. The variation in previous and current studies attributed to small ruminants that had close contact with seropositive herds of cattle may be a possible source of infection, management practices, and the level of frequent introduction of the latest animals. In pastoral areas, livestock owners often sell Brucellosis-positive animals, allowing infected animals to enter Brucella-free herds and spread, leading to increased prevalence rates due to lack of vaccination and control mechanisms. Especially, mixing of various herds and among species can even facilitate transmission and cross infection [52].

Among the two regions, the Liben zone of the Somali regional state, 13.6% (53/389) of tested samples were positive for Brucella antibody, and a high prevalence rate was recorded. In contrast to this study, lower prevalence rates were reported by [47]: 1.37%, [28]: 1.2% in sheep and 1.9% in goats in Jijiga, [53]: in goats 3.02% and 1.9% in sheep at Dire Dawa. In the Oromia Region of the Borena Zone, 55/452 (12.17%) were found to be seropositive. There was no significant variation between the two pastoral regions. This may be the similarity in agro-ecological conditions and livestock management systems.

In the Borena Zone of Dubuluk and Gomole districts 55/452, the (12.2%) prevalence rate was recorded. These results were higher as compared with previous reports in the pastoral area of the Borena zone [54], which reported 3.2% [55], 8.8% [31], 8.1% [48], 3.3% [56], 11.3%, and 9.6%. This difference between the previous and current studies may be due to variations in the test method in sensitivity, specificity imparted, the population sampled, and the level of frequent introduction of new animals in the area. Free movement of animals during the shortage of pastures and water in large areas that imply animal contact with potential transmission may contribute to the relatively higher prevalence of small ruminant brucellosis.

There was statistically significant variation (p-value < 0.05) between goats (15.7%) and sheep (6.74%) in the seroprevalence of brucellosis. This report is in line with [46], in goats, 14.4% than in ovine 6.7% [43], reported 13.6% in goats and 7.1% in sheep. In contrast, [53] reported (2.6%) in goats (3.0%) and 1.9% in sheep, and [47] reported 1.37%, in sheep 1% and goat 1.57% with no significant variation among sheep and goats. In this study, species prevalence differences might be that ovine are more resistant than caprine, and they do not shed the bacteria for a long time, and flocks with high numbers of ovine would have low prevalence [57]. Excretion from the vagina in goats is more numerous and prolonged than in sheep and lasts for at least 2-3 months. In addition, goats are the principal host of *Brucella melitensis*, whereas sheep are not significantly infected even when kept in close contact with goats. These may reduce the potential spread of the disease within and between sheep flocks. Infection in caprine can vary from acute to persistent occurrence for years. In ovine, the course of infection depends upon the dose of bacteria, and they are resistant to reinfection [58].

Among age groups of animals, adult and young variation in seroprevalence of brucellosis (p < 0.05). In adults, high seroprevalence was recorded (14.2%) compared to in young animals (3.7%). The significant variation was in agreement with previous reports of [54, 59, 60, 61, 50]. Meta-analysis of brucellosis was reported higher in post-pubertal than in pre-pubertal animals [62]. In contrast to this study, no significant variation in the seroprevalence of brucellosis between different age groups was reported by [47, 63, 28]. In the current study, the possible reasons for the significant differences observed among age groups could be that older animals had greater exposure to infection, and younger animals are usually resistant to the diseases [61].

Colostrum from infected does is used to passively immunize [64]. Due to sex hormones, adult and pregnant animals are more likely to contract Brucella than sexually immature animals of either sex; erythritol in female allantoic fluid and meso-erythritol in male testicles and seminal vesicles promote growth [57]. Brucella organisms proliferate, and their concentration tends to rise with age and sexual maturity [57, 65]. Animal age has a beneficial impact on seropositivity to brucellosis and is a significant epidemiological determinant [66].

In this study, seropositivity to *Brucella* infection between sheep and goats was significantly associated with the history of abortion (p < 0.05) that was observed. The prevalence of brucellosis was found to be higher in goats and sheep with a history of abortion (25.28%) than in animals with no abortion (9.51%), and abortion is strongly associated with brucellosis. Similar results had been reported by [54, 67, 68]. The study indicated that the chances of abortion in goats and sheep increase due to brucellosis and a higher incidence of abortion in the advanced stage of pregnancy. This is due to the uterine environment being conducive to the multiplication of the bacteria that leads to placentitis and the destruction of villi [69].

According to the sex category, higher seropositivity was observed in females (13.1%) over males (10.4%). However, there was no statistically significant variation in the seroprevalence of *brucellosis* among female and male groups (p-value > 0.05). This is in agreement with previous reports by [43, 47, 50, 70, 71] indicating that there is no observable sex variation and significant difference in the prevalence of brucellosis in males and females of small ruminants. In contrast, [46, 48] reported that observable significant variation in the prevalence of brucellosis in males and females of small ruminants. In the current study, the absence of sex variation in seropositivity may be because most pastoralists preferred to keep a large number of females and a few males in the flock. The pastoralist used males for marketing and replacement. A small number of sampled males might have contributed to not observing the statistically significant difference between the sexes.

In general, the distribution of brucellosis among different districts, animal species, and pastoral associations (PAs) was variable. This could be associated with the variability of the herd sizes and samples tested per visited PA. Animals were traveling long distances in search of pasture and water in different ecologies and uncontrolled movement of domestic animals. This results in a massive concentration of animals in areas with relatively better pasture and watering points. This may contribute to the increased transmission of *Brucella* organisms among different flocks, resulting in the emergence of new infectious foci, creating variation in the distribution of *Brucella* infections among different pastoralist PAs and districts [54].

Bacteriological isolation and identification are time-consuming, expensive, and require well-equipped, safe laboratory facilities [32]. However, isolation of *Brucella* is not always successful due to the fastidious nature of the organism. It has often been reported that the sensitivity of culture medium depends on the stage of the disease, *Brucella* spp., number of circulating bacteria, culture techniques, culture medium, type of clinical sample, and history of antibiotic used [72, 74]. In this study, no isolates of *Brucella* were recovered from vaginal swabs and whole blood. According to [23], a vaginal swab taken immediately after abortion or parturition is an excellent source for the recovery of *Brucella*.This could be attributed to the delay in the isolation attempts, as the samples were preserved for a long time in the deep freezer. When the animals were infected chronically, no living bacteria were circulating any longer, and when a few organisms were present in the samples, they were contaminated [75]. The isolation of *Brucella* is definitive proof that the animal is infected, but not all infected animals give a positive culture, and the methods and facilities that must be employed are not always readily available [76]. The ability to direct isolation and culture of *Brucella* spp. can vary between acute and chronic manifestations. Although 50% - 80% of acute cases yield positive blood cultures, only 5% of chronic cases are culture-positive [77]. To increase the sensitivity, multiple blood sampling should be conducted in the acute phase of brucellosis [78]. The frequency of bacteremia episodes is another factor that should be considered in terms of the time, frequency, and volume of blood collected for culturing [79].

In this study, real-time PCR was employed for the detection of the genus *Brucella* and two *Brucella* species in sheep and

goats of blood clots. For the genus Brucella, universal primers (IS711) and species-specific primers for *B. abortus* and *B. melitensis* were used. In this study, *Abortus* was detected in sheep with a blood clot. This result comparatively agreed with [70, 80, 81] in Egypt, [82] from Tanzania, [83] from Iran, [84] from Nigeria, and [85] from the USA.

Brucella abortus and *melitensis* were detected using species-specific primers by real-time PCR assay. Small ruminants that were infected by *B. abortus* are said to be spillover infections from infected cattle when kept close, particularly during common grazing and/or watering points [86]. Infection of *B. abortus* can occur because of natural exposure to infected materials and infected animals or indirectly through contact with soil and pasture contaminated with abortion secrets [90]. In this study, *B. abortus* was detected as the causative agent of brucellosis in sheep and goats. This result was supported by different authors at the national and international levels [81, 87, 89] who reported B. *abortus* in sheep and goats. In one sheep and six goats of blood clots, *B. melitensis* was detected. A comparative study was reported on the milk of goats and sheep from Iran, buffaloes, and cattle [92-94].

Mixed infection of *B. abortus* and *B. melitensis* DNA was detected in two goats. This result indicated that one host could be infected with two different species of *Brucella* at the same time [95, 81]. The potential host range of *Brucella* may also depend on breeding conditions [96]. Co-habitation and close contact with different animal species increase the risk of a pathogen crossing the species barrier [90]. In reality, inter- and intra-specific transmissions of different *Brucella* species can occur in larger herds and livestock production systems where cattle, goats, and sheep are put together [97, 98]. However, it is important to point out that several *B. abortus* isolates have been reported from milk and abortion products of sheep and goats [99]. Accidental *B. abortus* infections in small ruminants may even play an understanding role in the persistence of brucellosis in cattle [100]. From blood clots of small ruminants in Ethiopia, no reports could be found. To the best of my knowledge, this is the first report on the detection of *B. abortus* and *B. melitensis* from blood clots.

The questionnaire results indicated that 95% drank raw milk and dairy by-products, 97.5% disposed of aborted fetuses in environments and handled them by bare hand, 97.5% kept their animals mixed, 95% of participants had no awareness of brucellosis, and 90% had an abortion in their flock. This result is in line with the previously reported by [101, 31, 46]. In pastoral communities, handling of aborted materials, manipulation of reproductive excretions with bare hands, and herding of a large number of animals mixed with other animals are widely practiced. These may be considered a risk factor [76, 102-104]. The occurrence of the disease in humans is largely dependent on the animal reservoir, and high rates of brucellosis infection in sheep and goats usually cause the greatest incidence of infection in humans. Factors that expose humans to *Brucella* infection are ingesting the raw animal product, contact with aborted materials, working with specimen-infected animal products, and occupational contact with infected animals; handling *Brucella* isolates in laboratories; and inhalation of the aerosolized droplet are the major public health hazards [105-107].

6. Conclusion and Recommendations

In this study, the overall seroprevalence rate was 12.84%, with sheep at 6.74% and goats at 15.68% recorded. In sheep and goats, two species of *Brucella* (*B. melitensis* and *Brucella abortus*) and mixed infection were confirmed as the causative agents of brucellosis from blood clots using real-time PCR. Brucellosis causes devastating losses to the livestock industry, small-scale holders, hindrance live animal export, and international markets. The potential risk factors are age, species, and aborted animals found to be significant risk factors for brucellosis in sheep and goats. The traditional mixed livestock farming system in pastoral areas, supplemented with recurrent livestock mobility, continues to enhance the endemicity of the disease in the area. It poses a public health and economic threat since the livelihood of the pastoralist's community mainly depends on these species of animals, providing milk, meat, and cash income to cover family expenses for food and other essential consumable goods. Therefore, the results of this study warrant the need for appropriate control strategies to reduce the economic and zoonotic impacts of brucellosis. Thus, based on the study results, the following recommendations were forwarded:

- To prevent human infection and reduce the risk of transmission of the diseases, community awareness creation, proper disposal of an aborted fetus, pasteurization of milk and by-products.
- One Health approach and Safe husbandry practices
- The shorter shipment, storage time of samples, and quick delivery to the diagnostic laboratory increase the probability of isolating the *Brucella* spp.
- Surveillance of Brucellosis should be carried out to indicate the status of the disease and sketch its distribution.
- Strengthening the diagnostic capacity of veterinary and public health laboratories should be carried out.

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6.2 Compliance with ethical standards

Ethical clearance on the use of goats and sheep for this study was obtained from the ethical board clearance committee of Animal Health Institute Sebeta, Ethiopia, which offered ethical clearance (Minutes number and date of review: ARSERNC 009/20, December 30, 2020).

6.3 Data availability

No data was used for the research described in the article.

6.4 Declaration of Competing Interest

The authors have no conflicts of interest regarding this work

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