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Seroprevalence and Public Health Perception of Small Ruminant Brucellosis in South Eastern Somali Region, Ethiopia.

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Abstract: A cross-sectional study was conducted to determine the sero-prevalence of small ruminant brucellosis and potential risk factors associated in South Eastern Somali Region, Ethiopia. A total of 226 (126 goat and 100 sheep) serum samples were collected using sample random and assessed using modified Rose Bengal Plate Test (mRBPT), Complement Fixation Test (CFT) and questionnaire survey were done to asses perception of the disease. Potential risk factors were analyzed using Fisher Exact and Chi-square tests. Accordingly, the presence study revealed the overall prevalence of brucellosis in small ruminants was identified to be 1.77% (95%CI: 0.4-3.8) with combined mRBPT and RBPT tests. Using companied RBPT and CFT results, a comparison of sero-prevalence of small ruminant brucellosis was carried out with in sex and the result showed a higher sero-prevalence of 2.2% (95%CI: 0.46-6.31) in Female and 1.1% (95% CI: 0.28-6.04) in Male respectively. However, the association of risk factors with seroprevalence of small showed that there are no statically significant difference (p>0.05) of small ruminant brucellosis among species, sex, districts and age groups considered. Although the result of the study was relatively low, the existence of the disease circulating in small ruminants and lack of awareness of the communities about the disease may result a potential hazard for zoonotic transmission of brucellosis in the pastoral communities. Implementing feasible control measures such as elimination of sero-positive reactors of sheep and goats and raising public awareness of the community towards brucellosis is an important aspect to reduce the transmission of the disease in small ruminants and risk of zoonotic transmission in man, respectively.

Keywords: Brucellosis •CFT •Fafen •mRBT •Sero-prevalence •Small Ruminants

INTRODUCTION

The domestic small ruminant population of the country is estimated to be 27.35 million sheep and 28.16 million goats. Brucellosis is known to be endemic and growing problems among small ruminant flocks of the country [7]. Small ruminant's meat and skin products represent an important export commodity, which significantly contributes to the national economy at optimum off take rates; Ethiopia can export 700,000 sheep and 2 million goats per year and at the same time supply 1,078,000 sheep and 1,128,000 goats for the domestic market which made small ruminants chief sources of cash income [2].

In spite of these large contributions to the national economy, the country fails to optimally utilize these resources as the it is suffering lower productivity in which diseases stand front line. One of the diseases that impede the productivity of small ruminant is infectious reproductive diseases and Brucellosis is among the major diseases affecting pastoral areas [13].

Brucellosis is an infectious bacterial disease which affects domestic animals (cattle, sheep, goats and camels), humans and wildlife; it is caused by various *Brucella* species such as *B. melitensis* in small ruminants, *B. abortus* in cattle, *B. suis* in swine and *B. canis* in dogs, while all the species mentioned are known to have zoonotic importance. *Brucella* species are slow-growing, Gram negative, small cocobacilli and intracellular bacteria that is capable to survive and multiply within epithelial cells, placental trophoblasts, reproductive tracts and mammary glands [10]. *Brucella melitensis* is considered to have the highest zoonotic potential followed by *B. Abortus* and *B. suis*. Brucellosis is prevalent in most countries and is one of the most important neglected zoonotic diseases which have serious public health significance. Globally, it is estimated that nearly 500,000 cases of brucellosis would occur in humans every year [19]. Brucellosis causes considerable major economic losses such as a barrier to trade of animals and animal products, an impediment to free animal movement [25]. It also causes losses due to abortion of fetus or breeding failure (culling) in the affected animal population and diminished milk production. The disease is often prevalent in traditional pastoral communities both in animals and humans but, due to lack of awareness the disease is not diagnosed and treated [8].

In Africa and central Asia, the incidence of brucellosis is generally considered higher in pastoral settings. However, because of the difficulty to access pastoral communities, the occurrence and

the control of brucellosis is poorly understood both in humans and their animals where the burden of the disease could be high [14].

In these rural pastoral communities, risk factors for brucellosis are living in close proximity of livestock, the habit of consuming raw milk, unsafe handling of aborted materials and other infected excretions of animals, rearing of diversified animal species together and herding of large number of animals. All of these are collectively practiced and the major route of human Infection in endemic areas is through ingestion of infected materials. The disease in man is referred to as undulant fever causing reduced work capacity through sickness of the affected people [5].

Despite the huge population of small ruminant In Ethiopia, few studies have been conducted so far on small ruminant brucellosis particularly in pastoral areas of the country. According to previous studies, the prevalence of small ruminant brucellosis reported include 1.5% (in sheep) and 1.3% in goats (Tekelye and Kasali ,1990) in central Ethiopia .in Similarly, the report of Yibeltal (2005) showed a prevalence of 15% in sheep and 16.5% in goats in the Afar region and 1.6% in sheep and 1.7% in goats in the Somali region. The presence of this disease has also been reported in the Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) and pastoral areas of Borana (Teshale *et al.*, 2006). Regardless, there are very few reports focusing on Somali Regional State were done on small ruminant brucellosis and so far there is no report in Fafen zone of Somali Region on the status and risk factors of brucellosis infection in small ruminants of Jigjiga and Kerebeyah and districts. The objectives of this present study were to investigate the sero-prevalence, associated risk factors and perception of community for small ruminants Brucellosis in Fafan zone, Somali Region, Eastern Ethiopia.

MATERIALS AND METHODS

Description of Study areas: Jigjiga and Kebribeyah are two cities of Somali Regional State which are found in the eastern part of Ethiopia under Fafan zone which is 630 km and 103km away from Addis Ababa and Harar respectively. There are situated at an altitude ranging from 1,660 to 1,710 meter above sea level (m.a.s.l.) at geographic coordinates of approximately 9°20' North latitude and 45°56' East longitude. The climate of is semi-arid type which is characterized by high temperature and low rainfall. The mean annual temperature and mean annual rainfall is about 22°C and 543mm respectively. The production system practiced in the area includes agro-

pastoral management system. Kebribeyah is geographically similar with Jigjiga except that the it has slightly a higher temperature and low rainfall than Jigjiga [7].

Study population: The study population consisted of 226 (126 goat and 100 sheep) Somali goats and Black Ogaden sheep kept under agro-pastoral management system from two purposely selected districts of Fafen zone. The studied animals were all above 6 months of age, with no history of vaccination against brucellosis. Then risk factors such as age (<2 years (young) and \geq 2 years (adult)), sex (Male and Female), species (sheep and goat) and site with other relevant animal information were recorded.

Study Design and Sample Size Determination : A cross-sectional study design was carried out from December, 2020 to April 2021 to determine the sero-prevalence of brucellosis in small ruminants and as well as to assess potential risk factors associated with the host and animal owner's awareness of the disease in the selected study sites. The sample size was determined using the method recommended by [23]. Accordingly, the target sample size was calculated usin g the expected prevalence of 1.5% [5], 95% level of confidence interval and 5% desired absolute precision using this formula as follows.

$$n = \frac{1.96^2 P_{\exp} (1 - P_{\exp})}{d^2}$$

Where:

n= required sample size, p_{exp} = expected prevalence (1.5%) and d²= desired absolute precision (0.05); the minimum sample size calculated was 22.6 however; it was inflated to 226 sera samples for better precision. During sampling, both sexes and different age groups were considered so as to make the sampling representative with uniform distribution.

Sampling Method: The sampling method employed in this study for selecting districts was simple random. Then individual sampled animals were selected based on systematic sampling to represent equal proportion of small ruminant population in terms of species, sex and age on sample data sheets. Approximately 5-8ml of blood sample was collected directly from the jugular vein of each selected sheep and goat study animal using sterile plain vacutainer tubes, needle holder and needles. The blood from each animal was labeled and left tilted over night at

room temperature to allow for clotting. Next morning, sera were removed from the clots by siphoning them in to sterile cryovials. The sera samples were kept under -20° C at Jigjiga Regional Veterinary Diagnostic and Research Microbiology laboratory until positive sera were transported to National Veterinary Institute (NVI), Bishoftu for further Complement Fixation Test.

Questionnaire survey: A questionnaire was also performed simultaneously during sample collection to asses risk factors associated with community perception and their awareness about small ruminant brucellosis the questionnaire was administered to 60 sheep and goat owners residing in the aforementioned sites by personal interview. The questions were related to their awareness of small ruminant brucellosis among the respondents, their habit of consuming small ruminants' milk and their practices of handling aborted fetuses and retained fetal membranes.

Serological Tests: RoseBengal PlateTest, All serum samples collected were first screened using the mRBPT at AAU, CVMA. This test is internationally acknowledged as the test of choice for the screening of Brucellosis in cattle as well as in small ruminants to detect Brucella agglutinins. RBPT is based on detection of specific antibodies and the antigen used was Rose Bengal antigen, which constitutes a suspension of B. abortus obtained from (Veterinary Laboratories agency, Addle stone, UK). Briefly, for the modified method, 25µl of rose Bengal antigen was dispensed on to card plate and then 75µl of sera samples were dropped alongside the rose Bengal antigen by using the tip of the automatic micropipette, the tips were used to mix the sera and antigen together and stirred for 4 minutes after 4 minutes of shaking a visible agglutination was considered as positive. Agglutinations were recorded as 0, +, ++ and +++, according to the degree of agglutination [17]. A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination, and +++ indicates coarse clumping. The presence of agglutination was considered positive reaction while the absence of agglutination was considered negative. Brucella positive and negative control sera were also tested along with the test sera to guide in the reading of the results. Then, the results were recorded and stored in Microsoft Excel 16[©].

Complement Fixation Test: Complement fixation test is the most widely used confirmatory test and recommended by OIE. All sera which tested positive by the mRBPT were further tested,

using the CFT, for confirmation. Sheep red blood cells (SRBCs) obtained from National

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Veterinary Institute (NVI), Ethiopia, were used to detect the presence of *Brucella* antibodies against *Brucella* antigen in the sera. Similarly, the control sera (Positive and Negative) and complement used in this test were also obtained from NVI, Bishoftu, Ethiopia. Preparation of the reagent and procedures were performed according to protocols recommended by World Organization for Animal Health [18] 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 were considered as positive and lack of fixation/complete hemolytic was considered as negative [15].

Ethical issues: During sample collection, Ethical consideration is an important factor to be considered so that permission was obtained from Somali Region Livestock and Pastoral Development Bureau and Small ruminant owners participating in the study were informed about the purpose of the study before any attempt to collect data. The owners were briefly discussed about the objectives of the study and the reasons why the research must to be done and after obtaining their oral consent the study was carried out accordingly. Blood sample collection was done with proper aseptic conditions and precautions.

Data processing and analysis: All the data obtained were entered in to MS excel 2016 spreadsheet and statistical analysis was performed using STATA® version 13.0. The sero-prevalence was calculated by dividing the proportion of sheep and goats whose sera was found to be positive for CFT by the total number of sample size, multiplied by 100. The association between each risk factor and the outcome variable was assessed using Fisher's exact test. Adjusted Odds ratio (AOR) and 95% Confidence interval (CI) were computed to see the degree of association of risk factors with *Brucella* seropositivity. For all analysis a *p*-value of less than 0.05 was taken as significant.

RESULTS

In the present study, among 226 serum samples from small ruminants (126 from goats and 100 from sheep) tested, 9 were found positive for *Brucella* infection by the mRBPT alone and 4

positive using combined mRBPT and CFT tests. Thus, the overall seroprevalence of Brucella infection in small ruminants in the two selected districts of Fafan zone in Somali Region was estimated to be 3.98% (95% CI: 0.15-6.3) using the mRBPT test and 1.77% (95% CI: 0.4-3.8) by the combined mRBPT and CFT tests. The study showed high sero- prevalence for brucellosis in goats than in sheep and the prevalence was 2.38% (95% CI: 0.49-6.80) in goats while it was showing only 1% (95% CI: 0.25-5.45) in sheep. But, relatively a higher prevalence was observed in Kebribeyah 2.59% (95% CI: 0.54-7.37) than Jigjiga with a prevalence of 0.91% (95% CI: 0.23-4.96). Based on sex, a higher prevalence was also found in females 2.21% (95% CI: 0.46-6.31) than males 1.11 %(95% CI: 0.28-6.04) (Table 1). The prevalence of small ruminant brucellosis was 2.31% (95% CI: 0.48-6.59) in the age groups of ≥ 2 years. The majority of seropositive sheep and goats were older than two years and only 1.04 % (95% CI: 0.26-5.67) was observed in younger animals (<2 years). However, in this study no statistically significance difference (p>0.05) was observed between age groups, sex, site and species categories considered. Analysis for association between risk factors and Brucella infection in small ruminants using was carried out using fisher's exact test and Comparison of the sero-prevalence of small ruminant brucellosis was carried out among different species, site of animal, sex and age groups. However the result of the analysis revealed that there was no statistical significant difference (p>0.05) in the seroprevalence of Brucella infection among any of the risk factors above considered in the study (Table 1).

Variable		Sera tested	RBPT-CFT+	95%CI	Fisher's exact	P-Vale
			(%)		value	
Site	Jigjiga	110	1(0.91)	0.23-4.96	0.62	-
	Kebribeyah	116	3(2.59)	0.54-7.37		0.36
Species	Goats	126	3(2.38)	0.49-6.80	0.63	0.49
Species	Sheep	100	1(1)	0.25-5.45	0.05	-

Table 1: Sero-prevalence of brucellosis based on the species, site, sex and age of the small ruminants using combined RBPT and CFT.

Sex	Female	136	3(2.21)	0.46-6.31	0.65	0.68
	Male	90	1(1.11)	0.28-6.04		-
Age	<2	96	1(1.04)	0.26-5.67	0.64	-
Age	≥2	130	3(2.31)	0.48-6.59	0.04	0.49

*Reference category; CI; Confidence Interval;

In multivariable logistic regression analysis, according to the effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis using combined RBPT and CFT fitted (Table: 2) indicated that statistically significance difference was not observed among districts, species, sex and age groups (p>0.05). However, relative differences in sero-prevalence were observed in the categories considered. Accordingly, goats were (OR= 2.41, 95% CI: 0.247-23.6) more likely to be at higher risk for getting *Brucella* infection than sheep although, it was not significantly associated with *Brucella* infection (p> 0.05).

The multivariable analysis also revealed that increased age of sheep and goats were more likely to be associated with an increasing risk of getting *Brucella* infection when evaluated collectively for other factors. Thus animals ≥ 2 (adult) were at higher risk of encountering *Brucella* infection (OR=2.25, 95% CI: 0.23 -21.9) than animals with <2 years (young). In spite, no significant difference was observed (p>0.05) (Table 2). Additionally, regression analysis, indicated that *Brucella* infection with in small ruminants were found insignificantly high in females (OR=1.52, CI: 0.21- 11.0) than in males. Furthermore, Kebribeyah district was (OR=2.89, CI: 0.295-28.2) more likely at high risk with *Brucella* occurrence than Jigjiga although, it was not significant statically (Table 2).

Table 2: Multivariable effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis using combined RBPT and CFT.

Risk Factors	Combined RBPT and CFT				
Variables	Categories	AOR	95% CI	P- Value	

	T • • •			
	Jigjiga*	-	-	-
Sites	Kebribeyah	2.89	0.295-28.2	0.36
Species	Goats	2.41	0.25-23.6	0.49
	Sheep*	-	-	-
Sex	Female	1.52	0.21-11.0	0.68
Bea		1.52	0.21 11.0	0.00
	Male*	-	-	-
Age	<2*	-	-	-
	≥2	2.25	0.23-21.9	0.49

*Reference category; AOR; Adjusted Odd Ratio; CI; Confidence Interval.

Questionnaire survey: The questionnaire survey was administered to 80 small ruminant owners in selected two peasant associations to assess their knowledge and awareness on small ruminant brucellosis using structure ques. Based on the analysis of the respondent the results were summarized in table 3. The questions were related to their awareness of small ruminant abortions, their consumption habits and methods of consumption of of small ruminants' milk, their practices of handling aborted foetuses and retained foetal membranes. Their responses were summarized as follows.

History of Occurrences and Stage of Abortion: Out of 80 household heads interviewed 69 (86.3 %) revealed the occurrence of abortion to be associated the disease of sheep and goats in their flock and 21(30.4%) reacted that abortion occurs more frequently in sheep, while 48(69.6%) responded it happens more frequently in goats (table 3). Out of 69 interviewed 32(46.4%) indicated that abortion occurred at late stage of pregnancy. Again, out of 69 interviewed 49(71%) responded abortion occurs in animals kidding/lambing for first time (table 3).

Table 3: Abortion Occurrences	s, Stage of Pregna	ancy and Parity Number.
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Occurrence	e (N=69)	Pregnancy Stage			Parity Number			
Sheep	Goat	Early	Mid	Late	1	2	3	≥4

21	48	25	12	32	49	16	3	1
30.4%	69.6%	36.2%	17.4%	46.4%	71%	23.2%	4.3%	1.5%

History of Retained placenta, Awareness, Method of Discarding and Handling: Out of 80 respondents 50(62.5%) respondents replied there are presence of retained fetal membranes of unrecognized causes in sheep and goat in their flock. Furthermore, the respondents were asked about the knowledge or awareness of small ruminant's brucellosis in their flock and 68 (85%) answered that they don't have knowledge about brucellosis. The respondents also asked about method of handling and discarding fatal membranes, aborted fetus and uterine fluid. These interviewees 76(95%) replied that they threw to open environment. About 79(98.75%) respondents replied that they handle the aborted fetus and fetal membranes without protection (table 4).

Table 4: Occurrence, knowledge, Handling, discarding methods of aborted fetus and fetalmembranes; Methods and Habit of Milk consumption

Perception about Animal Owner's	Frequency	Percentage
Knowledge About Brucellosis		
Yes	12	15%
No	68	85%
Occurrences of RFM		
Yes	50	62.50%
No	30	37.50%
AB		
Method of Discarding		
Thrown to Environment	76	95%
Burying	3	3.75%
Others	1	1.25%
Handling Methods		
With protection	1	1.25%
Without protection	79	98.75%
Small ruminant Milk Consumption Habit	70	87.5%
Yes	70	
No	10	
Habit of milk Consumption		

Boiled	6	8.57%
Raw	64	91.4%

Milk Consumption Habit and Method of Consumption: Out of 80 members of the interviewers 70 (87.5%) replied that they consume sheep and goat milk and only 10 (12.5%) answered don't consume sheep and goat milk. With regard method of consumption 6 (8.57%) replied that they consume boiled and 64 (91.4%) replied they consumed raw (table.5).

	Frequency	Percentage
Small ruminant Milk Consumption Habit		
YES	70	87.5%
NO	10	12.5%
Habit of milk Consumption		
Boiled	6	8.57%
Raw	64	91.4%

Table 5: Methods and habits of milk consumption

DISCUSSION

According on the result obtained from the present study, the overall sero-prevalence of small ruminant brucellosis in the two selected districts of Fafan zone, Ethiopian Somali Region was confirmed to be 1.77% (95% CI: 0.4-3.8) by combined RBPT-CFT tests. The result is in line with the previous studies conducted in Jijiga which reported a sero-prevalence of 1.2% in sheep and 1.9% in goats respectively, using CFT by [15] and the report of [22] which also indicated a sero-prevalence of 1.6% in sheep and 1.7% in goats in pastoral Somali Region. Similarly, the present result is inconsistence with another study conducted in yabello district which indicated the respective brucellosis sero-prevalence of 2.34% and 1.56% by RBPT and CFT respectively. This could be due to similarity of animal husbandry practices in communal grazing and watering areas and possibly similar climatic conditions [8].

On the contrary, the sero-prevalence of small ruminant brucellosis in this study was relatively lower than the study reported in Afar region with respective prevalence of 14.6% in sheep and 16.45% in goats [22]. This difference could be due to difference in agro-geographical location, sample size variation and animal herding practices. In afar Region mixing of the animals from various areas is common at communal grazing and watering areas. While in the current study

area of Somali region only animals belonging to a given clan are allowed to be mixed and there is a strong clan-based segregation of animals and use of range lands that restricts the transmission of the disease among flock of small ruminants [1].

The individual Sero-prevalence in this study indicated relatively higher sero-prevalence in goats (2.38%) than in sheep (1%) (Table 1) and the level of sero-prevalence is in agreement with some of previous studies done in the country. This finding is in confirmatory with the report of [5] who showed slightly a higher prevalence in goats (2.0%) than sheep (1.6%).

In this study, there was no statistically significant difference (p>0.05) in the prevalence of brucellosis within the Species of ruminants (Table 1). This may be due to smaller sample size of sheep than goats which could affect the result. However, the reports of small ruminant brucellosis by [1] carried out in Afar region and [22] in Somali pastoral areas respectively indicated that there is a significant difference (p<0.05) with in species in which goats have high sero-prevalence of small ruminant brucellosis than in sheep.

Likewise, [21] explained briefly the possible higher prevalence of brucellosis in goats than sheep may be due to the greater susceptibility of goats to *Brucella* infection than sheep and the fact that unlike goats. Sheep do not excrete the *Brucella* organisms for longer periods of time (fewer carriers) which in turn can reduce the potential spread of the disease among sheep flock [21].

When the sero-prevalence of small ruminant brucellosis was compared between males and females in this study, higher sero-positivity was observed in females than males with no statistically significance difference (p>0.05) (Table 1). Similarly, previous study of [16] in Somali Region also showed insignificance difference of the risk factors considered. This insignificance can result from unequal proportion of the two sexes during sampling. However, the study is in contrary with the report of [24] who reported significantly (p<0.05) higher prevalence of brucellosis in female sheep than in male sheep which may be due to Production system variation. Similarly, it is an established fact that male animals are less susceptible to *Brucella* infection due to the absence of erythritol [11].

The study also revealed slightly a higher sero-prevalence of small ruminant brucellosis in the adult age groups (≥ 2 years) than in the younger ones even though, the difference was not statistically (p>0.05) insignificant in this study. Likewise, the work of [5] and [15] indicated the absence of significant variation in the seroprevalence of brucellosis between different age groups

of goats. However, [1] reported significantly (p<0.05) higher sero-prevalence of brucellosis in small ruminants more than 2 years than the other age categories in Afar region.

Similarly, it has been reported that sexually mature and pregnant animals are more prone to *Brucella* infection than sexually immature animals of either sex [20, 21]. The difference may be due to the fact that sex hormones and erythritol which stimulate the growth and multiplication of *Brucella* organisms tend to increase in concentration with the age and sexual maturity [21]. With respect to districts, a higher sero-prevalence of the disease was observed in sheep and goats from Kebribeyah (2.59%) while, the lowest prevalence was recorded in Jigjiga (0.91%) However, there was no statistically (p>0.05) significant difference in the sero-prevalence of small ruminant brucellosis among the study areas. The lack of significance difference between the districts might be due to similarities in agro-ecological location or condition and management as well as production systems of districts in the study areas [16].

The proportion of people relying on livestock for some or their entire livelihood is very high in pastoral communities. However, analysis of the questionnaire survey in this study suggests the risk of the community about brucellosis residing in sheep and goats. Brucellosis in human is danger in rural areas like Somali Region because people live in close contact with their animals and have traditional habits of consuming raw milk, handling of aborted materials and manipulation of reproductive excretions with their bare hands being at high risk of acquiring the infection. In addition, the questionnaire conducted in this study showed poor community's knowledge about brucellosis in which 80 small ruminant Owners interviewed 68 had no awareness about brucellosis which would favor the transmission of zoonotic disease among the pastoral community.

CONCLUSION AND RECOMENDATIONS

In conclusion, the finding of the present study revealed the existence of Small ruminant brucellosis in the two selected districts of Fafan zone, Somali Region. However, the seroprevalence of small ruminant brucellosis in the study area was relatively low. The study also revealed the lack of awareness of the community about small ruminant brucellosis and the existence of potential risk factors for zoonotic transmission in pastoral communities. While, the livelihood of pastoralist's community mainly depend on their livestock for sustenance and this may favor the transmission of zoonotic brucellosis in the study area.

Therefore, based on the present study and findings, the following points are forwarded:

➢ Further comprehensive study of isolation and identification of the biotypes of *Brucella* responsible for infection in the region should be carried out.

Appropriate prevention and feasible control strategy should be put in place to minimize the risk posed by brucellosis preventing further spread of the disease in the region including elimination of sero-positive animals and proper disposal of aborted materials.

An effort should be focused on improving Public health educational awareness; Pasteurization or boiling of milk before consumption to reduce the danger of the disease towards the community.

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