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Brucellosis and Some Reproductive Problems of Indigenous Arsi Cattle in Selected Arsi Zone's of Oromia Regional State, Ethiopia

[1]*Teferi Degefa,* [2]*Asmamaw Duressa and* [3,4]*Reta Duguma*

[1]National Veterinary Institute P.O. Box 19, Debre Zeit, Ethiopia
[2]Microbiology Section, Asella Regional Veterinary Laboratory, Assela, Ethiopia
[3]Faculty of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia
[4]Prince Leopold Institute of Tropical Medicine, 2000 Antwerp, Belgium

Abstract: A cross-sectional study was carried out in Merti District of Arsi zone from October 2009 to March 2010 to determine the sero-prevalence of bovine brucellosis. A questionnaire survey on 97 households was also conducted to determine the prevalence of retained fetal membrane and abortion and their linkage to brucellosis sero-positivity. A total of 370 blood samples for sera were collected from indigenous Arsi cattle of both sexes and different ages. The Rose Bengal Plate Test (RBPT) was used on sera as a screening test for brucellosis. RBPT positive serum samples were further subjected to Complement Fixation Test (CFT) for confirmation of brucellosis positivity. The result showed that RBPT detected 2 (0.05%) of the 370 samples. The 2 RBPT positive sera were also confirmed to be positive (0.05%) by CFT. All the positive sera were from female cattle but male sera were not positive in both tests. Result of the questionnaire survey on 126 cows from 97 households indicated a prevalence of 11 (8.7%) abortion and 23 (18.3%) retained fatal membrane. Abortion and retained fatal membrane were significantly ($p< 0.05$) associated with brucellosis. This rapid study result showed that bovine brucellosis prevalence was low compared to prevalence of retained fetal membrane and abortion problems in the indigenous Arsi cattle in the study area. This reflected, in addition to brucellosis, some other agents were involved in retained fetal membrane and abortion. However, in-depth epidemiological study on brucellosis, retained fetal membrane and abortion is needed in the area to conclude and to implement control measures.

Key word: Abortion · Arsi Zone · Bovine brucellosis · CFT · RBPT · Retained fetal membrane · Prevalence · Oromia · Ethiopia

INTRODUCTION

Ethiopia has huge livestock resources in Africa, but it is the untapped resource. The reasons of under-utilization are multi-factorial. These include widespread infectious and parasitic diseases, poor management system and unimproved genetic makeup coupled with poor nutrition and malnutrition and absence of well developed market infrastructure [1].

One of the infectious diseases which are a major constraint for animal production is brucellosis. Brucellosis has a wide agent and host diversity. The genus *Brucella* contains six species: *Brucella abortus*, *B. suis*, *B. melitensis*, *B. ovis*, *B. canis* and *B. neotomae* [2, 3]. Animals primary affected include cattle (*B. abrotus*), sheep (B. *ovis and B. melitensis*), goats (*B. melitensis)* and camels (*B. abortus* and *metitensis*) [2, 4].

Brucellosis in cattle is characterized primary by abortion in late pregnancy, fetal membrane retention, endometritis and infertility in subsequent pregnancies. In bulls it usually causes orchitis, epididymitis, seminal vesiculitis, sterility and arthritis [4-6]. It is an important zoonosis, cause undulant fever in humans characterized by an acute septicemic phase followed by a chronic stage, which may extend over many years [7].

The pathogen can be killed by heating to 60°C for 10 minutes and is susceptible to an acidic pH, disinfectants and direct sunlight. It remains viable for long period of time at low temperature [3, 7].

Susceptibility of animals to brucellosis depends on their natural resistance, level of immunity and environmental stress [4]. Age, sex and breed and pregnancy status of the animal is also a risk factor for susceptibility. Younger animals tend to be more resistant to infection and frequently clear infections than sexually

Corresponding Author: Reta Duguma, Faculty of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia.

mature ones. Mature animals are much more susceptible to infection, regardless of sex. In female animals, pregnancy has positive contribution to the degree of susceptibility than their age. Bulls are relatively resistant than sexually mature heifers and less resistant than sexually immature heifers. In contrast to bulls, boars are more likely to be a source for introducing *Brucella* into a swine herd [8].

Transmission occurs mainly by ingestion of contaminated feed and water by organisms, which are present in large numbers in aborted fetuses, fetal membranes and uterine discharge [8, 9]. However, infection through injured/intact skin, the mucosa at the respiratory system and conjunctiva frequently occurs [3]. Calves can be infected in uterus or suckling of infected dams. After entering animals body the bacteria localizes initially in the regional lymph nodes to stay there for 1 to 3 weeks [4, 8]. Then it is distributed to the lymphatic system, the parenchyma and other organs and tissue. In pregnant animals, the uterus and udder are preferred sites [3].

Brucellosis is distributed world wide, but varies with different species and geographic areas. Geographically *B. abortus* is the widest spread; *B. melitensis* and *B. suis* are irregularly distributed; *B. neotomae* was isolated from deserts wood rats (*Nestoma lepida*) in USA [8]. The distribution and prevalence of bovine brucellosis in east Africa was summarized as follows in that 4% in Djibouti, 8.5% in Eritrea, 15.8% in Tanzania, Somalia (11.9%), Kenya (19%), Rawanda (34.9%), Sudan (6.5 to 22.5%) and Uganda (1.8%) (as cited by Asfaw *et al*. [10]. In Ethiopia several investigators have established the endemcity of bovine brucellosis under ranch and intensive farming in different parts of the country. A study at the Abernosa ranch showed a prevalence of 19% in central Ethiopia [11] and 8.11% in and around Addis Ababa [10]. A prevalence of 38.7% (57/147) was reported at Bako Research Center semi-intensive farming at western Ethiopia [12]. Recently, limited studies conducted in extensive farming showed brucellosis is also endemic in extensive farming of Ethiopia. In this line, the prevalence was 1.66% of 1627 tested cattle in extensive farming in Sidamo in southern Ethiopia [13], 3.19% of 816 tested cattle under extensive farming in Tigray in Northern Ethiopia [14]. An overall prevalence of 4.9% of 1968 cattle and 7.7% of 1120 B*arka* breed cattle in semi-intensive production system and 1.2% of 848 A*rado* breed cattle from extensive system in Tigray in Northern Ethiopia [15]. These indicate the disease is widespread in exotic crosses and indigenous cattle both in intensive and extensive farming in the country.

The prevalence of abortion is varying in different production system and from place to place. It is 11.8% in Jersey cows of Wolaita Soddo Dairy farm [16], 6.8% in North Gonder zone [17] and 7.4% in Tigray [18]. However, a lower abortion prevalence 3.2% was reported at Kombolcha by a different author [19]. This difference in prevalence rate may be due to the variation in cattle breed and husbandry management system. Robert [20] indicated that incidence of abortion more than 2 to 5% should be viewed seriously and measures should be taken to control it.

The prevalence of retained fetal membrane also varies from place to place. It is 9.3% at Tigray [18], 2.4-9.1% prevalence in Debre Zeit [21]. However, 16.8% prevalence retained fetal membrane was reported [22]. The difference in the prevalence rate could be due to the variation in cattle husbandry and management system. Joosten *et al*. [23] indicated that retained fatal membrane causes considerable economic loss of farm, especially when incidence exceeds the average of 5-10%.

The aforementioned most studies on brucellosis, retained fetal membrane and abortion in Ethiopia so far focused on cross-bred cattle and (semi) intensive farming and ranching. However, the indigenous cattle and extensive traditional farming is less studied despite Ethiopia has a wide stretched extensive traditional farming system with diverse indigenous cattle breeds than cross-bred and intensive farming.

Therefore, the objectives of this study were (1) To determine the prevalence of bovine brucellosis, retained fetal membrane and abortion in indeginous Arsi cattle in selected Arsi zone, (2) To quantify the degree of association of retained fetal membrane and abortion with brucellosis positivity.

MATERIALS AND METHODS

Study Area: The study was conducted in and around Merti District of Arsi zone. The site has attitude of 1780mm above sea level. The normal annual rain fall is within range of 750-1500mm and the daily maximum temperature mostly reaches 20-25°C. The minimum temperature normally falls within the range of 10-15°C (Tiyo District Agricultural Office of Asella 2006). Mixed farming of crop and animal production is practiced in the area. Usually animals are kept together in the barn at night time and are allowed to freely mix at grazing pasture land and watering sites during the day, especially during the dry season. In the area farming practice is biased towards crop cultivation. During cropping season the animals are kept in communal grazing land in order to protect them

Source: http:// www.younglives.org.uk/ countries/ethiopia and http:// www.ocha-eth.org/ Maps/ downloadables/ OROMIYA.pdf

Fig. 1: Study area in Arsi zone of Oromia regional state, Ethiopia

from damaging the widely cultivated crops on available land. This results in overgrazing of the communal land with subsequent malnutrition and starvation of the animals. Similarly, there is no supplementary feeding except crop residues at maximum dry season in the year. Thus, both cropping (rainy) season and dry season coincide with season of no pasture to graze and then animals suffer due to lack of feed in the area.

Study Design: A cross-sectional study was conducted from October 2009 to March 2010 to estimate the overall prevalence of abortion, retained fetal membrane and brucellosis by location and animal demography. The risk factors such as location, age, sex, parity, lactation and dry off were evaluated as to their association to abortion, retained fetal membrane and brucellosis.

Sampling Methodology: Simple random sampling method was followed to select the study animals. The numbers of animals to be sampled from each Kebele were determined by the proportion of the cattle population existing in each Kebele. From each Kebele three peasant associations selected. Actual peasant associations (Pas) were selected based on willingness of the owners. Animals within the Pas were selected using simple random sampling. The sample size for cattle was calculated on the basis of 5.6% prevalence of bovine brucellosis in Arsi region [24]. It was computed with the expected precision at 5 and at 95% confidence interval. The sample size was 210; however a total of 380 animals were sampled according to Thriestfield [25].

Questionnaires: A questionnaire survey on 97 households was conducted to determine the prevalence of retained fetal membrane and abortion and their linkage to brucellosis sero-positivity. The questionnaire was designed to collect information on factors that are believed to be a risk factor for *Brucella* infection. This include breed, sex, age of animal, purchase source, lactating, dry off, parity were recorded. In addition the clinical indicators including history of abortion and retained fatal membrane was interviewed and recorded.

Collection of Blood Samples: Blood samples were collected from jugular vein of each selected animal using plain vacutainer tube by needle. Identification of each animal was labeled on corresponding vacuatiner tubes and kept over night at room temperature to allow clotting. At the next morning sera were collected from the clot (unrestricted blood centrifuge) by siphoning in to the sterile cryotube (2ml), to which animals /identification was coincided and then RBPT was conducted. Serum samples were kept at -20°C at the Assela regional Laboratory until tested using CFT.

Serology Test: The Rose Bengal Plate Test (RBPT) was employed as a screening test on the serum samples for the presence of Brucella agglutinins.

Positive sera were then retested for confirmation by the complement fixation test (CFT) at Assela regional laboratory.

Rose Bengal Plate Test: The protocol of RBPT as recommended by OIE [2] was used as screening test to test the presence of *Brucella* antibody in the sampled sera. RBPT has a sensitivity of 98.3% and specificity of 68.8% [26, 27]. The test was performed according to manufacturer's literature. Before performing test, antigen and sera were brought to room temperature. One drop (0.03 ml) of serum was taken on a glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) of Rose Bengal antigen was added. The antigen and serum were mixed thoroughly with the spreader and then the slide was rotated for four minutes. The result was read immediately after four minutes.

The RBPT procedure reaction results were interpreted as 0, +, ++, +++ according to Nielsen and Dunkan [28] as follow:

N = no agglutinations
+ = barely perceptible agglutination (using magnifying glass)
++ = Fine agglutination – same clearing
+++ = Course clumping, definite clearing

Those samples with no agglutination were recorded as negative while those with +, ++ and +++ were recorded as positive. RBPT positive sera were then retested for confirmation by the CFT.

Complement Fixation Test: The principle of CFT is that *Brucella* antigen binds the *Brucella* antibody in test sera to form immune complex. This bound immune complex traps the complement. The complement is then unavailable to lyses the RBC (target cells) in the indicator system. In the absence of antibody (i.e. *Brucella* negative test sera), the complement remains free as it is unbound to the antigen and is available to lyses the indicator (Sheep RBC).

The interpretation of the test, therefore, full haemolysis indicates negative result, while sedimentation of Sheep RBC indicates positive reactions at different dilution concentration of the complement [2].

Data Analysis: The point brucellosis prevalence rate was calculated by dividing the number of RBPT and CFT positive animals by the total number of animals tested. Similarly, prevalence of abortion and retained fetal membrane (RFM) was calculated by dividing cows with history of positivity to abortion and RFM cases to the total cows inspected [25]. The brucellosis prevalence and the association of brucellosis with abortion and RFM were analyzed and tested using chi- square test in SPSS statistical software. The chi-square test of *Brucella* sero-prevalence rate and its association to reproductive disorders as a risk factor was tested at 95% confidence interval with 5% alpha.

RESULTS

- The studied Arsi cattle population by location, parity and sex.

A total of 370 serum samples were collected and tested for brucellosis whereby 206 serum samples were female and 164 were males.

- Prevalence of abortion and retained placenta by location.

A total of 126 cows managed in extensive management system of the 3 peasant associations were analyzed from questionnaire data. The abortion prevalence rate in Ashe Ejersa PA was found to be 4 (13.3%) which was higher compared to the other two PAs, Gado Galama PA was found to be 4 (12.5%) and Yiftu 3(4.7%). The variation in the prevalence rate of abortion was not statistically significant. Relatively higher retained fetal membrane prevalence rate was found to be Yiftu 14 (21.9%) and Gado Galama 7 (21.9%) than Ashe Ejersa which was 2 (6.7%) as indicated in Table 2. The difference in the prevalence rate of retained fetal membrane was statistically significant between the PAs ($P<0.05$).

Prevalence of Abortion by Parity: The overall prevalence of abortion in the study area in all parity level of 126 cows was 11(8.7%). Parity level 3 and 5 had higher abortion rate of 4.0%, 3.2%, respectively. But the parity level 1 and 6 showed lower prevalence of abortion (0%) as indicated in Table 3.

Prevalence of Retained Fetal Membrane by Parity: The prevalence of retained fetal membrane across parity levels was evaluated in a total 126 cows. 23 (18.3%) of the cows had history of retained fetal membrane. As the parity level increased the retained fetal membrane was also increased or the parity five and six had higher prevalence rate than the other the parity levels (Table 4).

Table 1: Frequency of the studied Arsi cattle by parity, sex and location

	Parity levels among female animals							Sex	
Locations	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5	Parity 6	Heifer	Female	Male
Ashe Ejersa	5 (31.2)	5(23.8)	11(27.5)	6(19.4)	3(20.0)	-	37(46.2)	67(32.5)	57 (35.0)
Gado Galma	3(18.8)	5(23.8)	9(22.5)	8(25.8)	5(33.3)	2(66.7)	18(22.5)	50(24.3)	39(24.0)
Yiftu	8(50.0)	11(52.4)	20(50.0)	17(54.8)	7(46.7)	1(33.3)	25(31.3)	89(43.2)	67(41.1)
Total	16	21	40	31	15	3	80	206	164

Table 2: Prevalence of abortion and retained placenta in Arsi cows of parity ≥ 1 at different locations

Locations	Total cows Examined	Abortion positive	Abortion Prevalence (%)	Retained placenta positive	Retained placenta prevalence (%)
Ashe Ejersa	30	4	13.3	2	6.7
Gado Galama	32	4	12.5	7	21.9
Yiftu	64	3	4.7	14	21.9
Total	126	11	8.7	23	18.3

Table 3: Prevalence of abortion in Arsi cattle across parity levels in the study area

	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5	Parity 6	Total
Examined	16	21	40	31	15	3	126
Abortion positive	0	1	5	1	4	0	11
Share of each parity in total abortion prevalence (%)	0	0.01	4.0	0.01	3.2	0	8.7

Table 4: Prevalence of retained fetal membrane in Arsi cattle across parity in the study area

	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5	Parity 6	Total
Examined	16	21	40	31	15	3	126
Retained placenta	0	2	10	5	5	1	23
Prevalence (%)	0	1.6	8.0	4.0	4.0	0.01	18.3

Table 5: Prevalence of brucellois in female and male Arsi cattle in the study area

	Examined	RBPT Positive	RBPT Prevalence (%)	CFT Positive	CFT Prevalence (%)
Female	206	2	1.0	2	1.0
Male	164	0	0	0	0
Total	370	2	0.5	2	0.5

Table 6: Correlation of abortion and retained placenta in Arsi cows of parity ≥ 1 at different locations

		Location (PAs)	Parity	Retained M/M	Abortion
Location (PAs)	Pearson Correlation	1	.041	.145	-.136
	Sig. (2-tailed)		.645	.105	.129
	N	126	126	126	126
Parity	Pearson Correlation	.041	1	.210(*)	.145
	Sig. (2-tailed)	.645		.018	.105
	N	126	126	126	126
Retained M/M	Pearson Correlation	.145	.210(*)	1	.436(**)
	Sig. (2-tailed)	.105	.018		.000
	N	126	126	126	126
Abortion	Pearson Correlation	-.136	.145	.436(**)	1
	Sig. (2-tailed)	.129	.105	.000	
	N	126	126	126	126

* The correlation was significant at the 0.05 level (2-tailed). **The correlation was significant at the 0.01 level.

Table 7: Correlation of RBPT and CFT results in Arsi cows at different locations

		Kebele	RBPT	CFT
Kebele	Pearson Correlation	1	.035	.035
	Sig. (2-tailed)		.503	.503
	N	370	370	370
RBPT	Pearson Correlation	.035	1	1.000(**)
	Sig. (2-tailed)	.503		.000
	N	370	370	370
CFT	Pearson Correlation	.035	1.000(**)	1
	Sig. (2-tailed)	.503	.000	
	N	370	370	370

** Correlation is significant at the 0.01 level.

Sero-Prevalence of Brucellois by Sex: A total of 370 serum samples were tested for screening of brucellosis. 206 serum samples were from females in which 2 (0.5%) were positive for RBPT test. These 2 RBPT positive sera were further evaluated by CFT test and both cows (0.5%) were positive for CFT test too. The male serum samples were tested both for RBPT and CFT with negative reactors 0(0%). The overall prevalence of bovine brucellosis in the study area was found to be (0.05%) as indicated in Table 5.

Correlation Between Location and Parity Versus Abortion and Retained Fetal Membrane: Parity and retained fetal membrane ($p < 0.018$) was weakly positively correlated. Similarly, retained fetal membrane and abortion ($p < 000$) in Arsi cows was correlated. As parity increased retained fetal membrane occurrence also increased as well as abortion increased retained fetal membrane increased.

Correlation Analysis Between Location Versus Test Positivity by RBPT and CFT: The RBPT was positively correlated with the complement fixation test ($P<000$) as indicated in Table 7.

In this studies the highly sensitive (RBPT) and the highly specific (CFT) test had strongly correlated (100%) to detect brucellosis in indigenous Arsi cattle at equal rate of prevalence levels (0.05%) by the two tests.

DISCUSSION

In the present study, an overall prevalence rate of bovine brucellosis in the study area of Arsi was 0.05%. This low prevalence was in agreement with Fekedu [29] in which he reported a prevalence rate of 0.2% in the highland agro-ecological zone of Easter Amhara regional state, Taddesse [17] 0.1% in North Gondor. However, previous bovine brucellosis study in different part of the country indicated a high prevalence rate, 7.82% in the Arsi [24] and 11.6% in Sidamo region [30].

The current study in highland mixed crop-livestock extensive farming revealed lower prevalence than the reports of Dinka and Chala [31] who reported higher prevalence in pastoral and agro-pastoral production system in Ethiopia. The little amount of brucellosis positive reactor cattle in the extensive cattle management system of the three PAs could be too little contact between different herds. The spread of brucellosis under traditional methods of cattle husbandry is low. Daily movement of the herds, intensive solar radiation and a little contact between different herds may be the reason [32].

The RBPT is generally considered to be as a sensitive test [9]. Dohoo *et al.* [27] reported 97.9% sensitive for RBPT. The false positive results in the RBPT could be due to cross reactions with other bacteria such as *Yersinia entero colitica*, *E. coli*, *Salmonella* species and *Pastuerella* species [9, 33]. The CFT is recognized as the most reliable diagnostic test to be used routinely for individual animals [2]. It rarely exhibits non-specific reactions and does not work as the disease becomes chronic [4].

Even if the proportion of male animals tested were smaller as compared to female number, the positive reactor animals in the study area were all females. It had been reported that males are usually more resistant than female cattle [4]. This idea has been supported by different investigators in the country [10, 11]. Different factors are probably involved in the variation of sex susceptibility including physiological and behavioral differences between males and females. Because of the preferential growth of the *Brucella abortus* in the gravid uterus, it can enter the uterus as it disseminated from the principal site of carrier state (udder and superamammary lymph node). In latently infected cows depend on the number of pregnancy events and presence of infection; this will give the organism sufficient contact with lymph node system to stimulate a significant immunity response. On the other hand, some infected bulls in the testes are now to be non-reactors or only had low antibody titers [34]. This suggests that serological test may under-estimate *Brucella abortus* infection in males, because of the successful confinement of the bacteria in the testes and reticulo-endotelials system [35, 36]. Another factor which explains for the greater prevalence rate in the females is the behavior to lick or swift their infected newly born calves can lead to re-infection. Bulls are usually kept separate from cows. This management difference probably makes cows more susceptible to the disease [8].

The latent infection occurs in some animals which are serologically negative. In addition, serological diagnosis is considered to be unreliable when applied during the period of 2 to 3 weeks before and after abortion or calving suggesting that false negative results could occur [4].

The infection rate in the current extensive farming system involving indigenous animals was low. Similar findings to this report were recognized by different authors in local breeds that were kept at extensive management. A prevalence of 1.5% was noticed in Showa [37].

Using the questionnaire survey the prevalence of abortion in the study area was 8.7% and it was in agreement with other investigators such as prevalence of 11.8% in Jersey cows of Wolaita Soddo Dairy farm [16], 6.8% in North Gonder zone [17] and 7.4% in Tigrai [18]. However, lower abortion prevalence was reported by different author i.e. 3.2% in Kombolcha [19]. This difference in prevalence rate may be due to the variation in cattle husbandry management system. High abortion rate due to could exposure to physical exercise, stress long distance to search water point and pasture area and competition for available feed resource and conflict each other and infection. Robert [20] indicated that incidence of abortion more than 2 to 5% should be viewed seriously efforts should made to determine it cause.

Using the questionnaire survey the prevalence of retained placenta in study area was 18.3%. This was higher than the report of 92.4-9.1% prevalence in Debre-Zeit [21]. However, 16.8% prevalence retained fetal membrane was reported [22]. The difference in prevalence rate could be due to the variation in cattle husbandry and management system. Joosten *et al*. [23] indicated that retained fatal membrane causes considerable economic loss of farm, especially when incidence exceeds the average of 5-10% RFM in extensive management system was causes various factors. Various causes of RFM have been identified *i.e.* uterus paresis, abortion, stress, late or premature birth, dystocia, twinning, infections, seasonal and hormonal disorders. Additionally, some vitamin and mineral deficiencies induce or predispose animals to RFM [23, 38, 39].

CONCLUSION

The prevalence of brucellosis in the present study was 0.05% in indigenous Arsi cattle in Merti District of Arsi zone, South Eastern Ethiopia. It is much lower than the figures of previous reports from cross breeds at Assala and around Arsi zone. However, the current finding does not only suggest the presence of the disease in cattle population in the areas but also indicates the presence of foci of infection that could serves as source of the infection for the spread of the disease into the uninfected animals and herd. In addition, the current study revealed that the abortion and retained fetal membrane were higher in the area to pose important reproductive wastage and also they are important risk factors associated with the prevalence of the *Brucella* infection. The high prevalence of abortion and retained fatal membrane together with this low prevalence of brucellosis may suggest the presence of other causes of reproductive disease in addition to *Brucella*.

Knowledge of prevalence of brucelliosis and herd level risk factor is essential for introduction of cost effective and efficient control program. The present disease finding in indigenous Arsi cattle that were kept under extensive management system could give preliminary baseline information about brucellosis, abortion and retained fetal membrane.

Therefore, based on the current finding the following points are recommended.

- A detailed investigation should be conducted to characterize the isolates and determine the detailed epidemiology of brucellosis, retained fetal membrane and abortion in indigenous cattle under different production systems and.
- Isolation and characterization of the causes of abortion and retained fatal membrane of the cows should be studied in the study area and the country at large.

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