# Sero - Prevalence and Risk Factors of Cattle and Human Brucellosis at Seka Chokorsa and Shebe Sonbo Jimma Zone Districts, South West Ethiopia

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# Abstract

Background: Brucellosis is contagious bacterial disease of major socio-economic and public health importance globally and it is also one of the priority zoonotic diseases in Ethiopia. Across-sectional epidemiological study was carried out from April 2017 to April 2018 to estimate the sero prevalence of bovine and human brucellosis and to assess the associated risk factors of brucellosis in Seka Chokorsa and Shebe Sonbo districts of Jimma zone.

Results: The overall prevalence of cattle brucella infection at individual and herd level were 5.9% (95%CI: 4.1%-8.1%) and 26.6 (95%CI: 19.1%-35.3%) based on diagnosis using commercial kits of the competitive enzyme-linked Immunosorbent assay (CELISA) from Brucella abortus antibodies. Univariate logistic regression analysis at individual animal level showed that animals from large herd size and households which had a practice of introduction of new animals in their herds were 3.7 and 2.3 times more likely to be seropositive, respectively. The same scenario has been observed at herd level. Molecularly, five Brucella abortus was identified as the species affecting cattle in the study areas. From the total human serum samples tested only one serum was found to be positive for CFT and thus the prevalence is 0.42 %,( 95%CI: 0.01%-2.38%). From 238 respondents, 90% of them drink raw milk and milk products, and 70% of them also eat raw meat. Furthermore, slaughtering, assisting during delivery and poor management of aborted material is a common practice that favor transmission of the pathogen in the area.

Conclusions: In conclusion, the results of this study showed that brucellosis is an important and widely distributed disease in cattle in the study areas. The finding of the infection in human indicates the public health importance of brucellosis in the area. The risk behaviors and practices observed suggests the need to design all-inclusive health programmes, such one-health approach aimed at controlling brucellosis spread in the study area.


# Background

Brucellosis is contagious bacterial disease of major socio-economic and public health importance globally [1]. The disease affects humans, domestic life stock and wild life. The disease is caused by *brucellae* organisms which is gram-negetive coccibacilli. There are six “classical” species of this genus; *Brucella abortous* (Cattle), *Brucella melitenisis* (Goats and Sheep), *Brucella Suis* Pigs), *Brucella ovis* (Sheep), *Brucella canis* (Dogs), and *Brucella neotomae* B.neotomae) [2–4].

The disease causes a tremendous economic loss to the livestock industry via causesing low in milk yield (reduction 10% and 25% of the total milk yield), abortion (10–50%), infertility, stillbirth / birth of weak offspring and a major impediment for trade and export [5]. Thus brucellosis can have a considerable economic impact at the household and national level. Human brucellosis has a wide clinical spectrum, presenting various diagnostic difficulties because it mimics many other diseases like malaria, typhoid, and rheumatic fever, joint diseases and other conditions causing pyrexia [6, 7].

The prevalence of human brucellosis differs between areas and has been reported to vary with standards of personal and environmental hygiene, animal husbandry practices and local methods of food processing [8]. There is estimate show 500,000 human *Brucella* infections occurring per year in worldwide, with incidences ranging from less than one case per 100,000 population in UK, USA and Australia [1, 9].

The prevalence of brucellosis in livestock varies from country to country, for example the recent estimates for East Africa region suggests that there are approximately 21 million brucellosis cases in livestock annually [10]. In Ethiopia, brucellosis is considered to be endemic. The first report on livestock brucellosis in Ethiopia was made in 1970s [11, 12], since the disease has been noted as one of the important livestock disease in the country [13–16] a number of studies on bovine have been reported on brucellosis sero-prevalence ranging from 1.1 % to 22.6 % in intensive management system [17–20] and 0.05 % - 15.2 in extensive management system [21–24]. On top of this, brucellosis is now recognized as the second top zoonotic disease in Ethiopia [25].

In human all brucellosis infections are due to direct or indirect contact with infected animals or animal materials and the incidence is directly related to the prevalence of the disease in animals, socioeconomic level, eating habits, poor hygiene and practices that expose humans to infected animals or their products [26] .

It is acquired in people through breaks in the skin following direct contact with infected animals tissues or blood or their secretions. Infection may also result from consumption of contaminated unpasteurized milk and milk products [27] as well as undercooked contaminated meat [28, 29]. In Ethiopia, the rural people are mainly dependent on livestock and their relationship with them is very close. Moreover, people often consume raw animal products [30]. The mode of transmission of the bacteria varies with the epidemiological area animal reservoir and the occupational exposed groups [31, 32]. Sources of infection for the transmission of the livestock brucellosis are contaminated pastures, feedstuffs and water or licking infected placenta, foeti or the genitalia of infected female animals soon after abortion or delivery [31, 33]. Colostrum and milk from infected dams is also a potential source of infection for new born [31]. Venereal transmission in domestic ruminants is uncommon except in areas where artificial insemination is used. In order for a control programme of brucellosis to be efficient, it is important to know the prevalence of the disease with a good understanding of local knowledge, attitudes and practices relating to brucellosis to improve information delivery and initiate relevant control measures. It might also be advantageous for the outcome of a control programme to include disease education among community participants [34]. A knowledge, attitude and practice study of brucellosis conducted in Kenya among people with high level of contact with livestock implied that the disease awareness and knowledge of the transmission routes were poor [35] (. Knowledge, attitude and practice study conducted in Egypt showed a relative high general knowledge of brucellosis but still a high-risk behavior among livestock owners which the authors concluded might contribute to a high seroprevalence of brucellosis among large ruminants in the area [36]. A correct diagnosis of *Brucella spp.* infection is also important for the control of disease in animals and consequently in man.

More than 85 % of the Ethiopia population depends on agriculture for their livelihoods. The subsector contributes about 16.5 % of the national gross domestic product and 35.6% of the agricultural gross domestic product [37]. It also contributes 15 % of export earnings and 30 % of agricultural employment [38]. It has the largest livestock population in Africa, estimated at a total population of 183.04 million; out of these cattle are about 57.83 million, with the female cattle constituting about 55.38 percent and the male 44.62 percent. Regarding age groups, the majority of the cattle population (that is about 63.75 percent) is between 3–10 years age category, with about; 27.64 percent male and about 36.11 percent female. Moreover, about 16.30 percent are between age one and three years and those with age category 10 years and over, took small portion i.e. 2.13 percent of the total estimated number of cattle population, on the other hand, 98.59 percent of the total cattle in the country are local breeds, the remaining are hybrid and exotic breeds that accounted for about 1.22 percent and 0.19 percent, respectively.

The predominant livestock production system in Ethiopia is extensive, where indigenous local breeds are kept under low-input/low-output husbandry practices. The productivity of this sector is constrained by several factors and livestock mortality rates are high, with death estimates of 3.93 million in [39].Nevertheless livestock sector has been attributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. It is well-known that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply; provide the needed animal protein that contributes to the improvement of the nutritional status of the people. On the other hand livestock also plays an important role in providing export commodities, such as live animals, hides, and skins to earn foreign exchanges to the country [39]. Despite all these and many more, livestock diseases remain the greatest challenges as well as brucellosis which are prevalent both in livestock and humans in Sub-Saharan Africa [5]. There for the objective of this study is to assess the sero status and risk factors of brucellosis in cattle and human in Seka Chokorsa and Shebe Sonbo districts.

# Results

### Prevalence of Brucellosis at animal level

In this study, a total of 560 cattle serum samples were tested. The overall sero-prevalence of bovine brucellosis in the study areas were 8.9% (CI: 6.57-11.29) and 5.9 % (CI: 4.1 - 8.1) using RBPT and ELISA respectively. There was no statistical significant ($P > 0.05$) different between two districts (Table 2).

Table 2 : The overall prevalence of cattle brucellosis in the districts as it is tested by C-ELISA

| Districts | Sample size | No.positive samples | Prevalence(95%CI) | Chi-square, | p- value |
|---|---|---|---|---|---|
| Seka Chokorsa | 360 | 23 | 6.39 [3.86 - 8.92] | | |
| Shebe Sonbo | 200 | 10 | 5 [1.98 - 8.02] | 1.45, | 0.44 |
| **Overall** | **560** | **33** | **5.9 [4.1- 8.1 ]** | | |

CI = Confidence Interval

The distribution of brucellosis positive animals in the study villages within the two districts are presented in (Table3).

Table 3: Prevalence of brucellosis in each of the 12 villages in the districts of Seka Chokorsa and Shebe Sonbo (C-ELISA confirmatory test).

| Village | No of animals examined | Prevalence (%) | (95%CI) | |
|---|---|---|---|---|
| Kishe | 38 | 2(5.3) | [0. 64- | 17.75] |
| Gaserokakero | 20 | 0 | - | - |
| Sebeka debiye | 34 | 2(5.88) | [0.72 - | 19.68] |
| Tagodogama | 31 | 0 | - | - |
| Sombodaru | 56 | 6(10.71) | [4.03 - | 21.88] |
| Atarogafere | 22 | 0 | - | - |
| Shashemene | 70 | 8(11.43) | [5.07 - | 21.28] |
| Atrosufa | 61 | 3(4.92) | [1.69 - | 13.71] |
| Guru ulake | 81 | 5(6.2) | [3.00 - | 14.00] |
| Gudobaka | 67 | 2(2.99) | [0.82 - | 10.25] |
| Gibeboso | 47 | 3(6.4) | [2.19 - | 17.16] |
| Meti | 33 | 2(6.06) | [1.68 - | 19.61] |
| **Over all** | **560** | **33(5.9)** | **[4.1-** | **8.1 ]** |

Pearson $\chi$ (chi-square) = 11.97 P-value = 0.36 CI= confidence Interval n =12.

The chi-square analysis showed that the sero-prevalence of brucellosis was not significantly different in the 12 villages (P =0.36).

**Risk factors study at individual animal level**

In this study the prevalence of brucella infection was of 6.9% in females and 3.8 % a nimals and the difference in sero status was not statistically significant between both sexes (Table 4). Similarly, the study fails to detect a significant variation in sero prevalence between the different age groups (Table 4).

Table 4 : Prevalence of Bovine brucellosis based on, sex, age, herd size history of abortion and introduction of new animal in the herd.

| es | Category | No Animals tested | Prevalence (%) | 95%CI prevalence | OR ,95% CI (univariate analysis) | P-Value |
|---|---|---|---|---|---|---|
| | Female | 375 | 26(6.9) | [4.36-9.50] | 1.81[0.7-4.26] | 0.13 |
| | Male(Ref) | 185 | 7(3.8) | [1.03-6.53] | | |
| | <5years | 178 | 6 (3.37) | [1.25-7.19] | 2.09[0.85-5.19] | 0.072 |
| | ≥5 years(Ref) | 382 | 27(7.07) | [4.50-9.64] | | |
| ze | Large (≥9) | 201 | 23(11.4) | [7.04-15.85] | 4.5[2.1-9.63] | 0.000 |
| | Small(<9) (Ref) | 359 | 10(2.8) | [2.32-3.29] | | |
| n in the old | Yes | 99 | 7(7.07) | [2.88-14.03] | 1.27[0.53-3.02] | 0.594 |
| | No(Ref) | 461 | 26(5.6) | [3.37-8.15] | | |
| ction animals | Yes | 235 | 25(10.6) | [7.0-15.30] | 4.7[2.1-11.6] | 0.000 |
| | No(Ref) | 325 | 8(2.46) | [1.10-4.46] | | |
| | | **560** | **33(5.9)** | **[4.1 - 8.1]** | | |

OR = Odds Ratio, CI = Confidence Interval

Animals from a large herd size were 4.5 times (OR 4.5, 95% CI = 2.1-9.63) more likely to be seropositive compared to smaller herd size, (Table 4). Serostatus of the cattle was not affected with previous history of abortion in the households (P > 0.05). However, introduction of new cattle into the herd influences the serostatus of the animals; herds introducing new animals were 4.7 times more likely to be seropositive (OR=4.7, 95% CI:2.1-11.6 than those that did not practiced introducing

new animals. The predictor variables that appear to be associated with brucellosis status of animals (with a P value < 0.25 in univariate analysis) were age, sex, herd size and introduction of new animals in the herd. These variables were examined further if they are related to each other too, regardless of their association to the outcome variable. Multiple logistic regression method was used for this analysis and results are presented in (Table5). In the final analysis individual animals sero-positivity influenced more by introduction of new animals and herd size of the individual animal cone from in this particular study.

Table 5: Multivariate analysis- associations of various risk factors to the brucellosis status at individual animal level in the study area

| Variable | Category | Prevalence (%) | 95%CI | | OR | 95%CI | | P-value |
|---|---|---|---|---|---|---|---|---|
| Introduction of new animal | Yes | 25(10.6) | [7.0 | 18.32] | 5.7 | [2.4 | 13.25] | 0.000 |
| | No(Ref) | 8(2.46) | [1.10 | 4.46] | | | | |
| Herd size | Large (≥9) | 23(11.4) | [7.04 | 15.85] | 5.4 | [2.4 | 11.99] | 0.000 |
| | Small(<9) (Ref) | 10(2.8) | [2.32 | 3.29] | | | | |

R = Odds Ratio, CI = Confidence Interval

## Herd level prevalence

In the current study we were able to visit 124 herds from Seka Chokorsa and Shebe Sombo districts, and took blood samples of cattle from each household Thus, based on finding at least one sero-reactor for C-ELISA test, the overall herd level prevalence of bovine brucellosis was 26.6% (95% CI; 19.1- 35.3).

Table 6: Herd level Prevalence of Bovine brucellosis

| Variables | Category | No herd Tested | Prevalence (%) | 95%CI | OR ,95% CI (univariate analysis) | P-Value |
|---|---|---|---|---|---|---|
| Introduction of new Animals | Yes | 72 | 24(33.3) | [22.6-45.4] | 2.3[1.1-5.6] | 0.042 |
| | No(Ref) | 52 | 9(17.3) | [8.2 -30.3] | | |
| history of abortion | Yes | 23 | 9(39.1) | [19.7-61.3] | 2.1[0.79 -5.3] | 0.13 |
| | No(Ref) | 101 | 24(23.7) | [15.8 -32.2] | | |
| Districts | Seka chokorsa | 79 | 22(27.8) | [17.9-37.7] | 0.58[0.5-1.35] | 0.6 |
| | Shebe sonbo(Ref) | 45 | 11(24.4) | [11.8-37.0] | | |
| Herd Size | Large(≥9) | 40 | 18(45.0) | [29.2-61.5] | 3.7[1.6-8.6] | 0.001 |
| | Small(<9) (Ref) | 84 | 15(17.8) | [10.3 -27.7] | | |
| **Overall** | | **124** | **33(26.6)** | **[19.1-35.3]** | | |

HH= house hold, CI= confidence interval

The predictor variables that appear to be associated with brucellosis status of herd (with a P value <0.25 in univariate analysis) were introduction of new animals, history of abortion, districts and herd size. These variables were examined further if they are related to each other too, regardless of their association to the outcome variable. Multiple logistic regression method was used for this analysis and results are presented in (Table7). In the final analysis individual animals sero-positivity influenced more herd size of house hold cone from in this particular study.

Table 7: Multivariate analysis- associations of various risk factors to the brucellosis

status at herd level in the study area.

| Variable | Category | prevalence | (95%CI) | OR | (95%CI) | P-value |
|---|---|---|---|---|---|---|
| Herd size | Large(≥9) | 45.0 | [22.6 61.5] | 4.1 | [1.6 10.5] | 0.002 |
| | Small(<9)(Ref) | 17.8 | [10.3 27.7] | | | |

OR: Odds Ratio CI: Confidence Interval.

### Nucleic acid detection (Genus-Species specific sequence based).

Nine Cattle blood clot samples from serologically positives (C- ELISA) were subjected for real time PCR to identify the species of brucella affecting the sampled animals, then the test were performed and interpreted results according to the manufacturer's instructions was done . As it is depicted in figure 3 of sample were able to amplify using the primers for brucella genus and species specific, and 5 of them were confirmed to be *Brucella abortus.*

**Figure 1:** PCR Amplification

*Brucella abortus* RFU assay. Fluorescence ratio (fluorescein/Cy 5) is plotted against number of PCR cycles to monitor amplification in real time mode. An increase in fluorescence indicated a positive reaction for *B.abortus*, (one control and five positive samples) while remaining samples were negative.

### Relationship between Cattle and Human

On the house hold level sero prevalence one household were both seropositive within cattle 2/560 (0.35%) and human 1/238(0.42%), this is warning of positive relationship between them.

### Human sero-prevalence

Out of 238 samples screened using RBPT, five samples were found positive for Brucella. The reactive samples were further subjected to Complement Fixation Test (CFT) and one sample was confirmed by CFT respectively. An overall sero-prevalence of brucellosis in Seka Chokorsa and Shebe Sonbo was found to be 2.1 % and 0.42 % as tested by RBPT and CFT, respectively (Table8).

Table 8: Sero-prevalence of Brucellosis in humans in Jimma zone

| Test | n | Positive reactor | Prevalence(95% CI) |
|---|---|---|---|
| RBPT | 238 | 5 | 2.1% [0.92 4.83] |
| CFT | 5 | 1 | 0.42% [0.01 2.31] |

CI =confidence Interval

### Assessment of risky practices and attitude for brucella transmission

This study assessed the extent of risky practices and attitude for brucellosis in smallholder farmers and household level that might pose a risk for cattle and humans contracting brucellosis. Accordingly, out of the 238 participants interviewed the majority of them do not have knowledge about the mode of transmission of brucellosis when one look the raw milk and raw meat consumption pattern of the interviewed farmer families (Figure 4, Table10 and Table11).

### Demographic characteristics

The majority (58.8%) of the 238 included in the interview were males. 70.1 per cent of the participants did received no formal education and less than one percent was reached college level education (Table9).

Table 9: Socio- demographic characteristics of participants (n=238).

| Variables | Frequency | % |
|---|---|---|
| **Sex** | | |
| Male | 140 | 58.8 |
| Female | 98 | 41.2 |
| **Age** | | |
| 8-16 | 43 | 18.1 |
| 17-24 | 33 | 13.9 |
| 25- 35 | 52 | 21.8 |
| ≥ 36 | 110 | 46.2 |
| **Educational status** | | |
| Non formal education | 167 | 70.2 |
| Primary | 53 | 22.3 |
| Secondary | 16 | 6.7 |
| College/University | 2 | 0.8 |

## Assessment of consumption of raw animal products

The majority (90%) of the farmers' families reported to consume raw milk and its products, as it is depicted in figure 6. On top of this 70.5% of the participants reported that they consume raw meat. 202 (84.87%) of them were involved in milking practice.

### *Consumption of raw milk among different sex, age and education status*

Males were three times more likely to drink raw milk when compared with females respondents (Table10), however the level of education and age of interviewees were not significantly associated with raw milk drinking behavior participants.

Table 10: Univariate logistic regression results of self-reported practices among interviewed farmers and their families in relation with consumption of raw milk.

| Variables | Yes (%) | No (%) | OR | 95% CI | P- Value |
|---|---|---|---|---|---|
| **sex** | | | | | |
| Male ( n=140) | 99(70.7) | 41 (29.3) | 3.1 | [1.8 5.3] | 0.001 |
| Female ( n=98) | 42 (42.8) | 56 (57.2) | | | |
| **Age** | | | | | |
| Young ( n=48) | 34(70.8) | 14 (29.2) | 0.61 | [0.29 1.20] | 0.17 |
| Adult ( n= 190) | 107(56.3) | 83(43.7) | | | |
| **Level of education** | | | | | |
| Non-formal ( n= 167 ) | 98(58.7) | 69 (41.3) | 1.1 | [0.62- 2.02] | 0.70 |
| Formal ( n =71 ) | 43 (60.5) | 28 (39.5) | | | |

OR: Odds Ratio CI: Confidence Interval.

The prevalence of consumption of raw milk products like butter (kibe), skim milk (arera), cheese (ayib) and whey (aguat) is depicted in figure 4. Hundred percent (n=238) of the participant reported consumption of butter and skim milk, and 98% of the participants mentioned consumption of cheese and whey on regular basis.

Figure 2: prevalence of raw milk and its products.

### Prevalence of consumption of raw meat

The majority (70.6%) of the farmers' and their families affirmed eating raw meat. Univariate logistic analyses showed that Male participants were 2.4 time more likely consumed raw meat when compared with female participants (95%CI; 1.26-4.31,p- 0.008) and those who did not receive formal education (OR – 0.44, p- 0.011 were more likely to consume raw meat than their counter parts (Table11). No significant association was observed in eating of raw meat among different age groups.

Table 11: Univariate logistic regression results of self- reported practices among interviewed farmers and their families in relation with consumption row meat.

| Variables | Yes (%) | No (%) | OR | 95% CI | P- Value |
|---|---|---|---|---|---|
| **sex** | | | | | |
| Male (n=140) | 106(75.7) | 34(24.3) | 2.4 | [1.26 4.31] | 0.008 |
| Female (n=98) | 62(63.3) | 36(36.7) | | | |
| **Level of education** | | | | | |
| Formal (n=71) | 42 (59.2) | 29 ( 40.8) | | | |
| None-formal (n=167) | 126 (75.4) | 41 (24.6) | 0.44 | [0.263 0.82] | 0.011 |
| **Age** | | | | | |
| Young (n=48) | 38 (79.2) | 10 (20.8) | 0.55 | [0.25 1.12] | 0.15 |
| Adult(n=190) | 130 (68.4) | 60 (31.6) | | | |

OR: Odds Ratio CI: Confidence Interval.

### *Contact with animals and their products*

Risky activities and practices such as, birth assistance, Slaughtering, contact with aborted fetus at house hold level and milking cows that might pose a risk for humans contracting brucellosis were assessed. Accordingly 140 (58.8%) of the participants stated that they usually assist cows during birth at their household. Significant number 103 (43.3%) of the respondents reported not using any kind of protective material or chemical during handling or assisting birth furthermore 52 % of the participants partake in slaughtering of cattle in one way or another in their farms.

### *Slaughtering practices*

The interviewees were asked whether they have been involved in any animal slaughtering practices in the last 12 months. Accordingly 126 (52.9%) of mentioned were involved in slaughtering activity. Further analysis of the data

showed that among the demographic factors males were 29.2 time more likely to be participated during slaughtering animals in the community (Table12).

Table 12: Univariate logistic regression results of self-reported practices among

**interviewed farmers and their families in relation with slaughtering animals**

| Variables | Yes (%) | No (%) | OR | 95% CI | P- Value |
|---|---|---|---|---|---|
| **sex** | | | | | |
| Male(n=140) | 112 (80) | 28 (20) | 29.24 | [13.7 62.00] | 0.04 |
| Female(n=98) | 14 (14.3) | 84 (85.7) | | | |
| **Level of education** | | | | | |
| Formal (n=71) | 43 (60.5) | 28 (39.5) | 2.01 | [0.92 4.37] | 0.21 |
| Non-formal(n=167) | 97 ( 58.1) | 70 (41.9) | | | |
| **Age** | | | | | |
| Young(n=48) | 25 (52.1) | 23 (47.9) | 2.04 | [0.89 4.62] | 0.89 |
| Adult(n=190) | 101 (53.2) | 89 (46.8) | | | |

OR: Odds Ratio CI: Confidence Interval.

### *Assisting of animal during delivery*

One hundred forty (58.8%) of the participants mentioned assisting in deliver in the last 12 months.

Table 13: Univariate logistic regression results of self-reported practices among respondents in relation to assisting birth of animals

| Variables | Yes (%) | No (%) | OR | 95% CI | P- Value |
|---|---|---|---|---|---|
| **Sex** | | | | | |
| Male(n=130) | 90 (69,2) | 40 (30.8) | 1.81 | [1.06 3.10] | 0.028 |
| Female (n=108) | 50 (46.3) | 58 (53.7) | | | |
| **Level of education** | | | | | |
| Formal (n=71) | 43 (60.5) | 28 (39.5) | 1.1 | [0.62 1.96] | 0.72 |
| Non-formal(n=167) | 97 ( 58.1) | 70 (41.9) | | | |
| **Age** | | | | | |
| Young(n=48) | 23 (47.9) | 25 (52.1) | 1.55 | [0.802.97] | 0.188 |
| Adult (n=190) | 115(60.5) | 75 (39.5) | | | |

OR: Odds Ratio CI: Confidence Interval.

*Milking practices*

The vast majority (84.8%) of the respondents were involved in milking cow in the last 12 months. Females were frequently involved in milking than males. (Both the age and educational level of the participants did not showed any significant association with milking practice in the study community (Table14).

Table 14: Univariate logistic regression results of self-reported practices among interviewed farmers and their families in relation with milking practice

| Variables | Yes (%) | No (%) | OR | 95% CI | P- Value |
|---|---|---|---|---|---|
| **sex** | | | | | |
| Male(n=140) | 120 (85.7) | 20 (14.3) | 0.03 | [0.014 0.064] | 0.001 |
| Female(n= 98) | 82(83.7) | 16(16.32) | | | |
| **Level of education** | | | | | |
| Formal (n=71) | 36 (50.7) | 35 (49.3) | 2.1 | [0.99 4.81] | 0.052 |
| Non-formal(n=167) | 101 (60.5) | 66 (39.5) | | | |
| **Age** | | | | | |
| Young (n=48) | 13(27.1) | 35 (72.9) | 2.2 | [0.83 5.6] | 0.112 |
| Adult (n=190) | 89 (46.8) | 101(53.2) | | | |

OR: Odds Ratio CI: Confidence Interval.

*Management practices in relation with aborted fetus*

With regard to handling of the aborted tissue, 52 (21.8%) of the participants sated that they will do nothing, 127 (53.36%) of them will bury the aborting animal and 59 (24.79%) of them said that will give to dogs (figure legend 4).

In relation with wearing any protective material during handling of abortion or during calving, the result showed that none of the participant used any protective materials. However, 45% the participant affirmed using soap after handling of calving, milking or slaughtering practices. We also confirmed that the absence of consumption of raw animal blood in the study area.

# Discussion

In the current research the overall sero- prevalence of brucellosis in cattle was found to be 5.9% that the disease is important in both districts of Jimma zone. Similar findings were reported by [52], [53], [18], [54]

and [55] with very close overall prevalence of 4.9 %, 4.6%, 4.3% and 3.5% by using CFT in Western Tigray, North Gondar Zone, in selected sites of Ethiopia, Adami Tulu, Southern and Eastern Ethiopia, respectively. Similar previous studies done in Africa by [56] in Uganda, [57] in Zimbabwe, [1] in Chad, reported a very comparable prevalence of 5.9 % 5.6 % and 6.6%, but slightly higher than 4.6 % in Niger by [58] respectively, when compared with the current study.

Comparatively our sero-prevalence report is higher than the previous overall prevalence reports in Ethiopia by the following authors:[59] 1.4% in Assela and Bisoftu towns, [24] (1.38%) in agro pastoral areas of Jijjiga zone of Somali Regional State, [17], reported (1.92%) in Sidama zone, southern Ethiopia, (1.97%), [60] in East Wollega Zone (1.97%) and [20] (1.5%) in Addis Ababa. However, the current prevalence report is relatively smaller than previous reports in some parts of Ethiopia: 7%, 11%, by [16, 52].

The possible reasons for these variations might be due to the difference in diagnostic test used, cattle management practice, sample size, sampling method, study design, test used, population dynamics, and biological factors. In pastoral production system, high herd mobility, increased number of animal stock and interaction of different domestic species and wild animals at grazing pasture and water points. The practice of mixing cattle, either through grazing or sharing of watering points is an important risk factor for Brucellosis [61, 62] These factors could potentially contribute chances of exposure and spread of the disease. The prevalence of brucellosis is influenced by a number of risk factors related to production systems, biology of the individual host and environmental factors [63]. Among the 12 villages where we collected samples 9 (75%) villages had at least one seropositive animal, among the nine positive villages the highest and lowest sero- prevalence recorded were at Shashemene (11.43%) and Meti (6.06%) villages. However, sero- prevalence of brucellosis was not significantly different between the 12 villages (P = 0.36). This finding is in agreement with the works done by [64–66]. The absence of significant variation in prevalence ofbrucellosis between Seka Chokorsa and Shebe Sonbo district village could be probably dueto the similarity in farming system, hygienic practice, and source of animal and herd size.

Risk factors assessment at individual animal level

Generally, the susceptibility of cattle to *Brucella abortus* infection is influenced by age,sex, breed and reproductive status of the individual animal [67]. Below, an attempt has been made to discuss the association of different risk factors with individual animal sero-positivity findings along these lines.

In this study the numerical sero-positivity was found to be higher in adult age groupanimals than young age group animals. Our finding consistent with previous reports inEthiopia [55, 68, and 69] .Brucellosis is more likely to infect sexually active animals and therefore older animals are more likely to test positive to brucellosis. This could be explained by sexual maturity and pregnancy due to the influence of sex hormones and placenta erythritol on the pathogenesis of brucellosis [70].

The current study showed that there was no significant difference in sero-positivity between male and female animals (P > 0.05). However the prevalence observed in female animals (6.9%) is greater than that of male animals (3.8%). This result is consistent with the finding of Yilkal *et al.,* (1998), who found higher proportion of female (8%) infected than male (0.11%), though the difference was not significant. [71]has

also reported higher proportion of female reactors than males in both extensive and intensiveproduction systems. Though the difference was again not significant. In some othercountry similarly, [72] had reported a sero-prevalence of 8.5% in females and 1.9% in males in Akwapim-Southern district of Ghana, yet the difference was not significant. The lack of significant difference in sero- positivity between male and females may be due to male and female cattle are usually kept in a single herd in this mixed type of farming system in Ethiopia, it may be reasoned that both sex are equally exposed to the risk of brucellosis.

The presence of seemingly higher sero-prevalence of Brucellosis in female animal could be due to infection within the female reproductive tract producing a potential reservoir for the organism to propagate. Our finding revealed that with large herd size or hold (>9 animals) were 4.5 times more likely to be c-ELISA positive for brucellosis when compared to those small herd size (< 9 animals) owned. One possible explanation for this condition could be large herd allows for increased contamination of pastures and would facilitate cross-infection within herd once abortion and deliveries occurred. The result is in agreement with several authors, who reported large herd size as one of the major risk factor for occurrence and higher prevalence of bovine brucellosis [21, 68, 71, 73–76] in Ethiopia and some other countries.

It is thought that purchase and introduction of new animals from a variety of sources without considering the disease history or prior screening of animals may be a major factor contributing to levels of infection at household level. So, an attempt was made to see the difference in sero-reaction between animals from households, who practice introduction of new animal or not. In this study we classified household practicing introduction of new animals in their herd or not and compare the prevalence of brucellosis in animals from those households, accordingly the result revealed that significantly higher sero- positivity (OR = 2.3) was observed in animals from household practicing introduction of new animals (33.3%) than animals from households which do not practice introduction of new animals in their herds (17.3%).

This could be explained by the high frequency of introducing new animals into the herds may increase chance of purchasing of infected animals and transmission of the disease [71, 77, and 78]. Abortion is the most obvious manifestations of brucellosis. We also assessed the individual animal sero-positivity with its previous history of abortion in the respective households. The result showed that history of abortion in a household where an individual animal sampled was shown to have no statistically significant associated with finding a positively testing individual animals in a herd. In extensive production system such as our study area abortion might be caused by other prevalent diseases such trypanosomosis, anthrax, black leg and pasteurellosis. Contrary to our finding in this regard, [68, and 71] and [79] reported the significant association between sero-positivity of individual animal and history of abortion.

In the current study, the multivariable model showed that herd size and introduction of new animals was found to be the predictors of individual animal sero- positivity for brucellosis in the cattle population of

the study area. Thus our finding is in agreement with many authors, who did a similar research in Ethiopia and other countries [55, 78, and 80].

During the study, 124 herds of cattle were tested and an overall of 33 (26.6%) herds were c-ELISA sero-positive. Similar findings has been reported by [55] (26.1 %) in southern and eastern Ethiopia and [52] (24.1 %) in western Tigray. In contrast the finding was lower, compared to the previous studies by using c-ELISA 61.4%, 90.0% in peri urban Juba town and rural Terekeka County [81] However the majority of bovine brucellosis herd prevalence reported previously so far in Ethiopia were between 11.2% and 15% [71, 82, 83] by using RBPT and CFT though, relatively higher 42.3% herd prevalence was also reported by [84] for the extensive system in Tigray.

The variation in the herd prevalence reported previously and ours may be the diagnostic test we used (c-ELISA) , which have a highly sensitive and specific characteristic when compared to the diagnostic tests (less specific) use by previous authors mentioned above, because the previously reported herd level sero-prevalence might be due to false- negative serum reaction.

Assessment of risk factor in herd prevalence

In the univariate analysis, households having large herd size (OR = 3.7) and practiced introduction of new animals (OR = 2.3) were associated with sero-positive status of herds. This might be due to abortion being able to increase contamination of pasture and easy cross-infection within herd once abortion occurs. In addition survival of Brucella organisms may be prolonged, thus animals may become exposed to the infective agent for longer periods of time. Similarly the expiation for association of herd prevalence and practice of introducing new animals in herd may be explained by the fact that new animals are not screened prior to introduction, thus infected animals may spread the disease in the herds or flocks following parturition.

Similar findings were reported where introduction of replacement animals into the herd and flock was positively associated with c-ELISA sero- positive herds and flocks [31, 85]. Multivariate analysis of risk factors for herd level sero-prevalence showed that only herd size to be a good predictor of brucellosis status of the herd.

The result of the PCR assay was able to detect Brucella *abortus* from the blood clot samples. Thus it is possible to say that circulating brucella species is *Brucella abortus* by considering the absence of any vaccination practice for brucellosis in the study area, in additionthe c-ELISA test we used is known to be very specificity to differentiate field strains from vaccine strain S19 as reported by [86] .

The overall sero-prevalence of human brucellosis recorded in this study was (2.1 %) and (0.42%) by using RBPT, and CFT respectively. Similar RBPT/CFT test base sero- prevalence were reported in Ethiopia by various researchers, 1.2 % [87], 2.4% [71],0.8% [88], 3.6 % [89] and 2.15% [54] On the other hand, high prevalence was also reported by [90] 12.5% in Abernosa and [91] 34.9% and 29.4%, in Borana and Hamer, respectively. Elsewhere, brucellosis has also been reported in humans with sero-prevalence of 18 - 24% in Uganda [92], 3.8% in Chad [93], 0.04 - 35% in Saudi Arabia [94], India 25.5% [95], and 37.7% in Algeria [96].

Study on farmers and their family member behavior and farm management practices
In this study several known high-risk behaviors were common self- participant practicesamong the participants (farmers). Such behaviors were consumption of raw meat anddairy products and not wearing protective materials when dealing with cows having anabortion or with aborted materials. Furthermore participating in slaughtering activity wasalso common.All the above mentioned risky behaviors and practices can lead to contracting of brucellainfection, this is because the ingestion of foodstuff or fluid containing *Brucella* organism isa route of brucellosis transmission [97].

As such, there is a high risk for human infection with brucellosis among our studycommunity and other potential consumers who are exposed, given the habit of eating rawor improperly cooked meat, or milk and milk products which is common amongstlivestock keepers and meat handlers in Ethiopia and amongst Africans in general,[97–101]. Thus such habits could predispose consumers to various diseases and thus should not be ignored.

The majority (90%) of the farmers families reported consumes raw milk and its products,and that of meat was also over 70.5% among the interviewed communities of Sekachekorsa and Shebe sombo districts of Jimma zone. When we look the influence ofdemographic variables only being male was the significant factor associated with drinking raw milk (OR = 3.1, p = 0.000 and eating raw meat (OR = 2.4, P = 0.08), the reason could berelated to lack of awareness of the risks associated with drinking raw milk and eating rawmeat, consumer preference for raw milk or meat, and inaccessibility or limited to source ofcooking fuels and cultural practice may encourage consumers to drink raw milk or eat rawmeat[102, 103].

Four household activities were assessed including involvement in milking, slaughtering animals, assisting calving and removing aborted tissues. As indicated in this study the majority of the study community members are involved in milking regardless of their age, education level and sex. However the involvement of male individual in milking seems significantly high when compared with female (OR = 0.03, P< 0.001). The finding is as such new because under pastoral and most agro-pastoral setup, females do most of the work associated with harvesting of livestock products (such as. milking), cleaning of livestock houses, house repair using cattle dung and handling of the newly borne calves, which may predispose them to infection like brucellosis[104], this study showed that the involvement of males in slaughtering at household level is found to be 29 times more likely than females , again our finding is not surprising , because slaughtering animals is main activity of males when there is events such as weeding, cultural festivals and important holidays be it in Ethiopia and many African countries[103].

If we look in terms of likely hood of contracting brucellosis it has a negative impact, and the reason could be explained by, during slaughtering, they will have a more constant contact with viscera, uterus and fetal membranes of may be infected animals (the preferred sites of localization of the bacteria) and are generally more prone to contract brucellosis [105] It is also good to remember that women can also be while processing raw meat for different type of dishes prepared from meat. Of the 238(58.8%) total of the responded yes when they are asked if they have ever involved in assisting delivery of claves in the last 12

months. Further analysis of the result showed that male individual were more likely to assist delivery (OR = 1.8, P = 0.023).

Contrary to our findings in most agro-pastoral setup, females do most of the work associated with assisting of calf delivery and hand of the newly borne calves [91]. Generally in all practices discussed above and handling such activities under poor hygiene without protective materials could pose serious risks to handlers in addition to consumption of contaminated food [106, 107].

In addition different researchers suggested that the use of protective materials and disinfectants while handling such cases had never been practiced that much by livestock keepers in different part of Africa though potentially they could reduce risks to livestock keepers considerably [103]. Furthermore, different authors reports that frequent contacts with animal, unsafe handling of aborted fetuses and placenta, assisting birth, occupations related to animal contacts, raw milk and meat consumption and lack of awareness were found to be important risk factors for having Brucellosis in humans [91, 108–112].

# Conclusions

The sero-prevalence of brucellosis at individual and herd level reported in this studyshowed that the disease is important in this area. Herd size and introduction of newanimals in to the herd were identified as major risk factor for brucella infection. *Brucellaabortus* is identified, for the first time, as the species of brucella organism circulating incattle in the study areas. The study confirmed the presence of low level of *brucella*infection in human despite the existence of several risk practices that could expose them to brucella infection in the study area. Based on the result of assessment of risky practicesand attitude at individual and household level indicates the prevalent of poor knowledgeabout modes of transmission of brucellosis in human and animal.

Slaughtering of animal and milking activities are more likely practiced by male'sindividuals than female in the study area. The vast majority of the farmers poorly manage the abortion products (fetal membrane and aborted fetus) by either throwing to dogs orleaving them on the field. Consumption of raw dairy products is common practices in mosthouseholds, a practice which increase the chance of infection.

# Methods

## Study Area

The study was conducted in Seka Chokorsa and Shebe Sonbo districts of Jimma zone (Figure 1), southwestern Ethiopia (Jimma zone Livestock and Fishery Resource office, 2015). Seka Chokorsa district is situated 20 km southern part of Jimma town and located between $7^0$- 45 north latitudes and $36^0$ - 53' east longitudes and Situated a distance of 375 Km, South West of Addis Ababa. This

district gained annual rainfall varies between 1300mm-1700 mm. The mean annual maximum and minimum temperatures are 18 $^0$C and 22 $^0$C, respectively.

The second study area, Shebe Senbo district, which is located at about 55 km North of Jimma town and extends between 7º 17′-7º44′ north latitudes and 36º 17′-36º 52′. The altitude of the district is between 1500 and 2000 meters above sea level (63%) and annual rainfall varies between 1,400mm and 1900mm.The mean annual maximum and minimum temperatures are 20 $^0$C and 23$^0$C respectively.

## Study Population

Households who have at least two human and two cattle were included in the study. The cattle populations included in the study were all apparently healthy and indigenous breeds kept under extensive husbandry system from age of two year. Study population of human included in the study was both sexes with ≥ 8 years old.

## Study design

A cross-sectional study design was conducted. A pre- structured questionnaire survey was conducted to collect data on potential risk factors for cattle and human brucella infection transmission.

## Sampling Strategies

Study animals and human subjects were selected by a cluster sampling method. Samples were taken from Cattles and humans in the same household. The numbers of animals and humans to be sampled from each district were determined by the proportion of the cattle and human population existing in each district.

The two districts have 60 kebeles. Six kebeles were randomly selected from each district. The district Health Extension office record book of households was used as sampling frame for household selection, select households by online random number picker computer system and twenty five (25) households having at least two cattle  5years and 5 years of age and family member ≥ 8 years age having at least two volunteers were randomly selected, and after selected if the house hold which can’t full fill the criteria replaced by the neighbor house hold. Accordingly, a total of 124 herds, with herd size ranging from 2-9 cattle owners and family members ranging from 2-5 humans were sampled randomly. Earlier to sampling, oral consent was given by each farmer.

When permission for sampling or interview was declined, another house hold was selected from the listed houses on the basis of random sampling. A total of 560 cattle and 238 human blood samples were collected. Age of cattle was categorized as young (<5 year) and adult (≥ 5 years). The age estimation was done by the means of dentition as descript by [40].

## Sample Size Determination

The sample size was determined using the formula described by [41] by considering an absolute precision of 5% with 95% confidence level.

[Due to technical limitations, the formula could not be displayed here. Please see the supplementary files section to access formulas.]

Where, n = sample size; Z = Z statistic for a level of confidence (in this study is at 95%, Z = .96), P = expected prevalence = 50% and q= 1 – P; L = Absolute precision = 0.05 Therefore, a required sample size was 384. However, this sample size was recalculated in order to get closer accurate to simple random sampling use correction factor (design effect), average no sample taken from one cluster is 6 and 0.09 row for *burucella abortus* based on the author[42] . D= p (n-1) +1D= 0.09 (6-1) +1 = 1.45 Design effect, 1.45 was used. Therefore, a total sample size used for cattle was 384×1.45= 557. The total sample size was proportionally allocated between two areas based on Cattle population number of the area. However 560 blood samples was taken during actual work, a total sample population about 200 blood samples from Shebe Sonbo district and whereas about 360 from Seka Chokorsa district blood sample was collected.

Sample from Human was taken from people >8 and above 18 years of age cluster sampling method was applied. Blood samples were taken from Cattle owners. The sample size was determined using the formula described by [41] by considering an expected prevalence of 6% from previously reported works [43-46] an absolute precision of 5% with 95% confidence level.

Therefore, a total sample size used for Human was 87. However, this sample size was recalculated in order to get closer accurate to simple random sampling use correction factor (design effect), average no sample taken from one cluster is 6 and 0.09 row for B*urucella abortus* based on the author [42] . Design effect, 1.45 was used. 87x 1.45 = 126, however 238 blood samples were taken during actual work.

## Questionnaire survey

A pre-tested structured questionnaire was first designed in English then translated into local language (Afan Oromo) then the interview was made in Afan Oromo. For cattle, relevant data such as breed of animals, age, and sex, history of abortion, herd size and introduction of new animals to the herd were collected before blood sample collection. For human subjects, we collected information related to household to demography, contact with animal and animal products, habits of eating raw animal products and knowledge about zoonotic importance of brucellosis (figure .2).

Figure 3: Questionnaire survey (source self-Scheck, 2018).

## Blood sample collection

About 10 ml of blood was collected from the jugular vein and median cephalic vein of selected cattle and humans, respectively; by using plain vacationer tubes. The collected samples were centrifuged after 10 min at the field to separate clot and serum. Approximately 1.8ml to 3.6 sera samples were collected into cry-vials and stored at -20°C until serological and molecular testing could be performed.

## Laboratory Analysis and Interpretation

All sera samples collected were initially screened by RBPT using RBPT antigen (Veterinary Laboratories Agency United Kingdom BN i75221) according to [48] and [49] procedures. Briefly, sera and antigen were taken from refrigerator and left at room temperature for half an hour before the test to maintain to room temperature and processed according manufacturer instruction and then all positive sera by RBPT were confirmed by Competitive Enzyme Linked Immunosorbent Assay (C-ELISA), using commercially available kit (SVANOVIR Brucella–Ab C-ELISA), Boehringer ingelheim svanova, article number 10-2701-02 and 10-2701-10 Uppsala, Sweden. In cattle this assay is capable of discriminating between Brucella infected animals and animals vaccinated with brucella strain 19 test kits. All reagents were prepared and tests were performed and interpreted according to the manufacturer's instructions. The optical densities (OD) were read at 450nm in a micro-plate photometer (Multi Skan Ex, Thermo Electron Corporation, Finland). Conjugate control A, B, Strong positive control (SPC), Week positive control (WPC) and Strong Negative control (SNC) were run as duplicates in the micro – plate wells C, D, E, F and G, H respectively. Sera were run as a single spots in the remaining micro plate wells. Reading of the result for each sample was obtained as the percentage of the ratio between the sample optical Density (OD) and positive control OD, according to the ( PI) values for controls as well as samples,

formula, negative positive samples were determined based on the serum and plasma sample thresholds-values and status as recommended by the manufacturers.

[See supp. files for formula.]

The results was calculated in terms of PI (Percentage inhibition) value and interpreted accordingly. The serum samples showing PI value Less than 30%, was considered negative and those showing PI value equal or greater than 30%, was taken as positive.

### Molecular methods

A number of Serological positive blood clot sample was further tested with Real Time PCR enabling simultaneous amplifications of many targets of interest in one reaction by using more than one pair of primers specific for a genus and species of Brucella spp.

### Real –time PCR

This assay has intended for the qualitative detection of Brucella spp. DNA extracted from the blood clot was extracted using QIA amp DNA Blood Mini extraction kit (California, USA) according to the instructions of the manufacturer, procedure. The PCR reaction mixtures were placed in an ABI 7500 Real Time PCR System instrument. Real-time PCR primer and probes included 6 primers and 3FAM labeled probes were used for amplification and detection of PCR products.

Table 1: Sequences of primers and probes used for amplification and detection

| Gene Target | Species | Oligo | |
|---|---|---|---|
| *IS711* | Brucella genus | Forward | GCTTGAAGCTTGCGGACAGT |
| | | Reverse | GGCCTACCGCTGCGAAT |
| | | probe | FAM-AGCCAACACCCGGCCATTATGGT-BHQ-1 |
| BruAb20168 | *B.abortus* | Forward | GCACACTCACCTTCCACAACAA |
| | | Reverse | CCCCGTTCTGCACCAGACT |
| | | probe | FAM-TGGAACGACCTTTGCAGGCGAGATC-BHQ-1 |
| BME110466 | *B.melitensis* | Forward | TCGCATCGGCAGTTTCAA |
| | | Reverse | CCAGCTTTTGGCCTTTTCC |
| | | probe | FAM-CCTCGGCATGGCCCGCAA-BHQ-1 |

PCR was carried out in Aliquot 20µl of each master mix into pre-designated wells of a Micro Amp 96 – well micro titer plate and Add 5ml of positive control DNA to correct position for each assay. Positive control DNA were far away from test specimen on the 96 well plate apply adhesive film and open plate tray and place test plate in to instrument. The amplification was performed in a DNA thermal cycler at a denaturation temperature of 95 c for 10 min; followed by 40 cycles at 95 $^0$c for 15s, annealing 58$^0$c for 15s and 72$^0$c for 5 min. A typical run of 40 cycles was accomplished in about 1.5 hours and finally interpret the PCR results procedure.

### Compliment Fixation Test (CFT)

All human sera that tested positive to RBPT were further tested using CFT for confirmation using standard *B.abortus* antigen S99 (Veterinary Laboratories Agency United Kingdom BN TA0066/16). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health [51. 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 dilution of 1:10 and above were classified as positive and lack of fixation/ complement hemolysis was considered as negative.

### Data Management and Statistical Analysis

Factors believed to be associated with epidemiology of brucellosis in cattle were recorded. Therefore the risk variables considered in this study were:-Herd size, (small/large), Age of individual cattle (young/Adult), Sex of individual cattle (female/male), Abortion history refers to any history of cattle abortion in the sampled household in the last one year (yes/no), Introduction of new animal refers to if there was any livestock bought by the household and mixed with the herd in the last one year (yes/no).

All data collected from the study areas were entered into MS Office Excel 2007, spread sheet program and imported to the Statistical Package Social Sciences (SPSS) version 20, then some exploratory data like descriptive statistics analysis and logistic regression analysis were run. Method was set at enter, any explanatory variable with P-values < 0.25, was taken as an input to multivariable logistic regression analysis. Furthermore multicollinearity was also assessed for any correlation between the explanatory variables, no multicollinearity if tolerances values are > 0.1, and variance inflation factor

(VIF) less than three. Multivariable logistic regression analysis was conducted to calculate the probability of bovine brucellosis happening as a function of several independent variables, with forward conditional stepwise method and to control for confounding effect in the model. Interaction effect was also checked between the explanatory variables for inclusion or exclusion of interaction terms in the final model, and finally the model fit was assessed by Hosmer and Leme show test in all the analysis, confidence interval was set at 95% and significances assessed at P–value < 0.05.Human overall Sero-prevalence brucellosis was determined using descriptive statistics and logistic regression was used to assess risk factors associated with, practices regarding the use of animal products, birth assistance, slaughtering practices, practice of milking cows and handling aborted fetus at house hold level that might pose a risk for human brucellosis. For all statistical inference, 0.05 level of significance and 95% confidence was used.

# Declarations

### Abbreviations

**CFT**: Complement Fixation Test; **C-ELISA**: Competitive Enzyme Linked Immune Sorbent Assay; **IS**: Insertion Sequence; **FAM**: Fluorescent antibody Monoclonal; **OD**: optical Density; **RBPT**: Rose Bengal Plate Test; **RFU**: Revolving Florescence Unit.

### Ethical Consideration

The study was approved by the Research and Ethics committee of Jimma University and written letters of clearance were obtained from Jjimma University College of Agriculture and veterinary Medicine. All participants were informed about the aim of this research and written consent was obtained from all participants.

### Consent to publication

Not applicable.

### Availability of Data and materials:

The data sets developed and analyzed during the existing study are accessible from the first author or from the corresponding authors upon request.

### Competing Interest:

The authors declare that they have no competing interests.

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**Authors' Contributions**

Considered and designed the study: FA, HD, BD. conducted filed surveys: FA, Analyzed the data: FA, HD, carried out laboratory work of RBPT, CFT C-ELISA and Real Time PCR: FA, TB, RB, wrote the paper FA, HD. All authors read and approved the final manuscript.

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# Figures

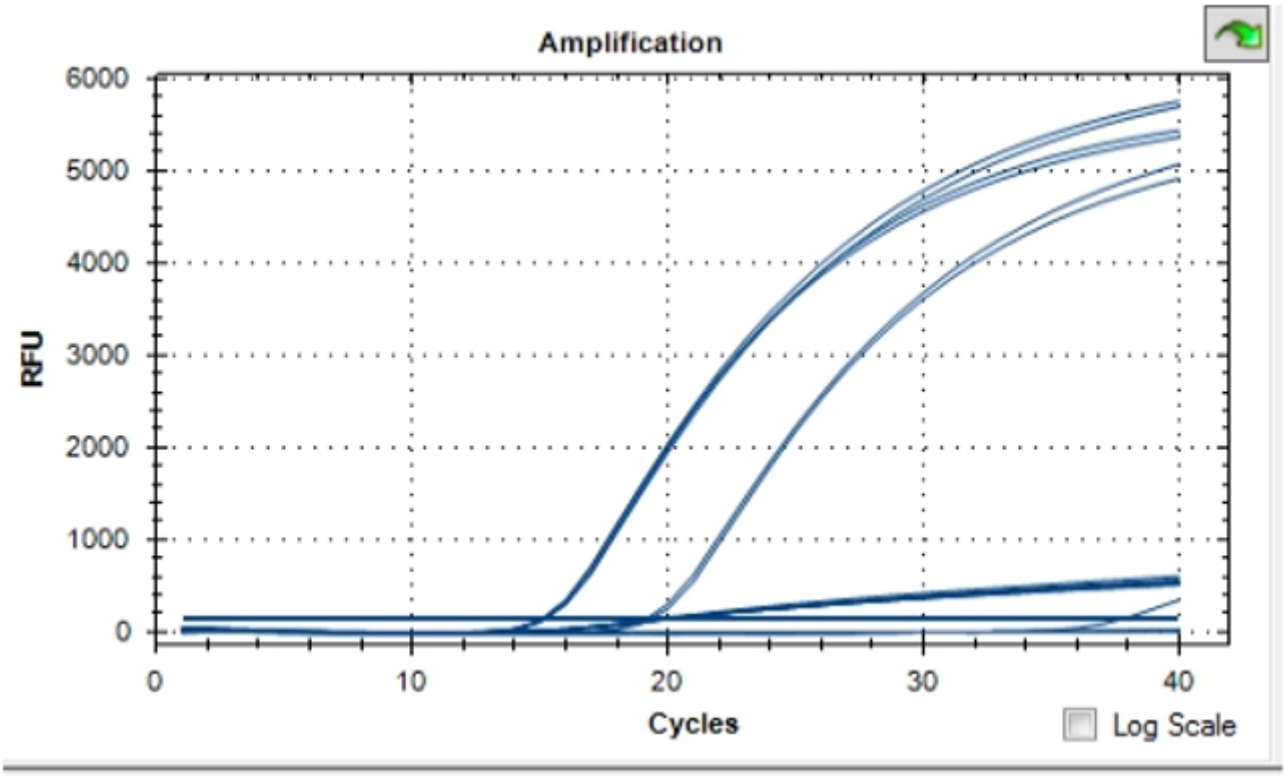


**Figure 1**

PCR Amplification

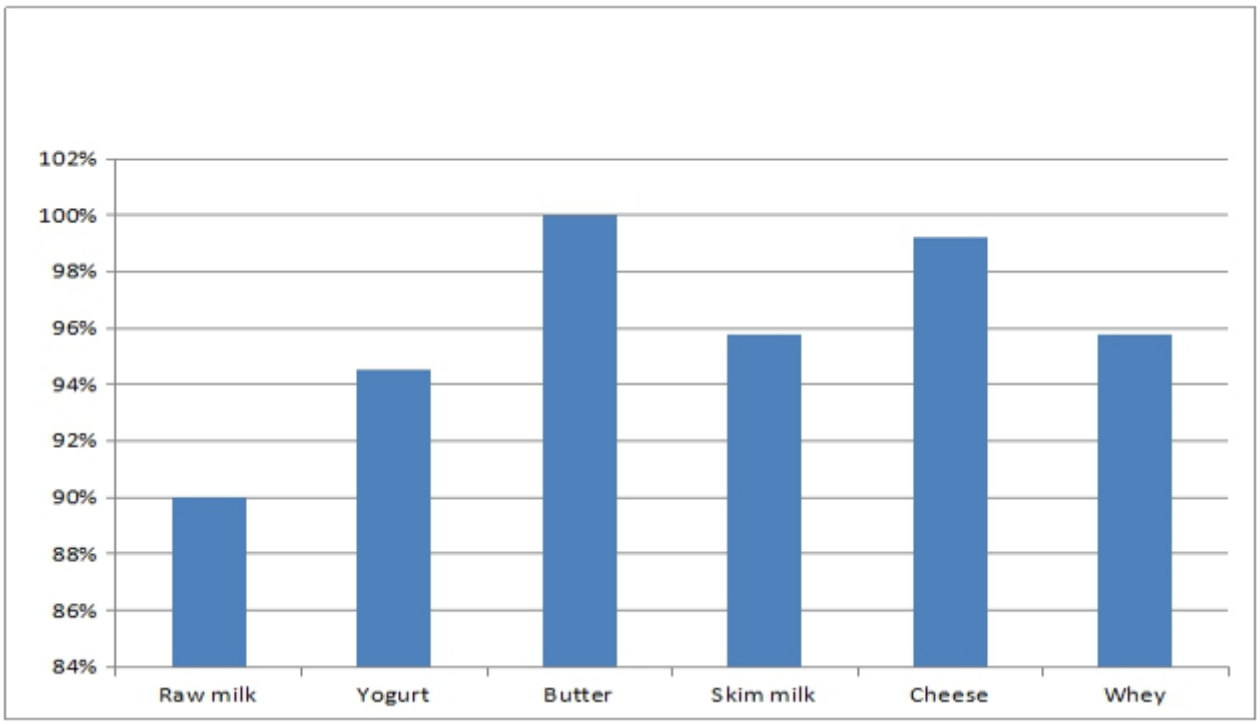


**Figure 2**

prevalence of raw milk and its products.

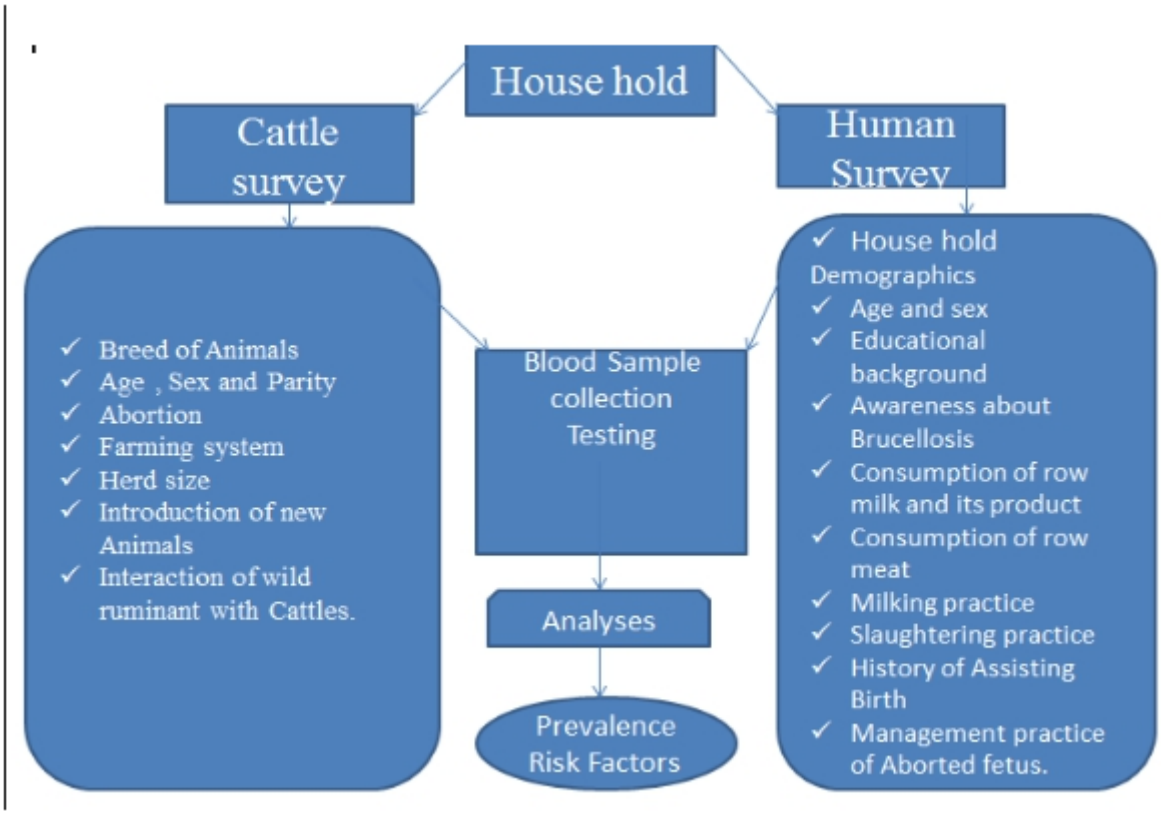


**Figure 3**

Questionnaire survey (source self-Scheck, 2018).

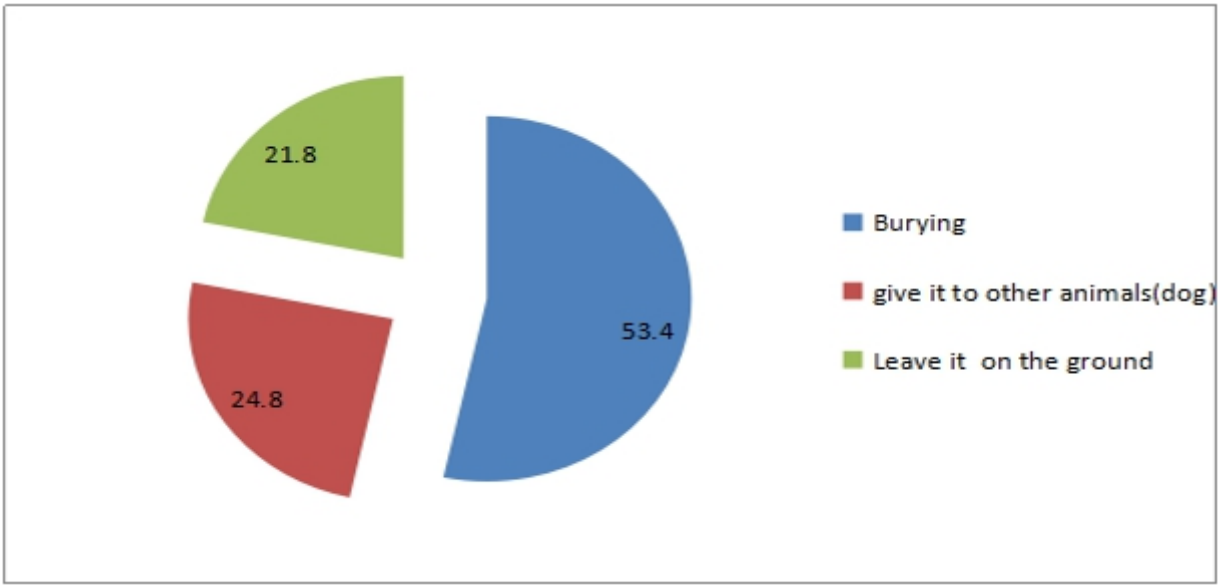


**Figure 4**

Management practice of aborted material by proportion

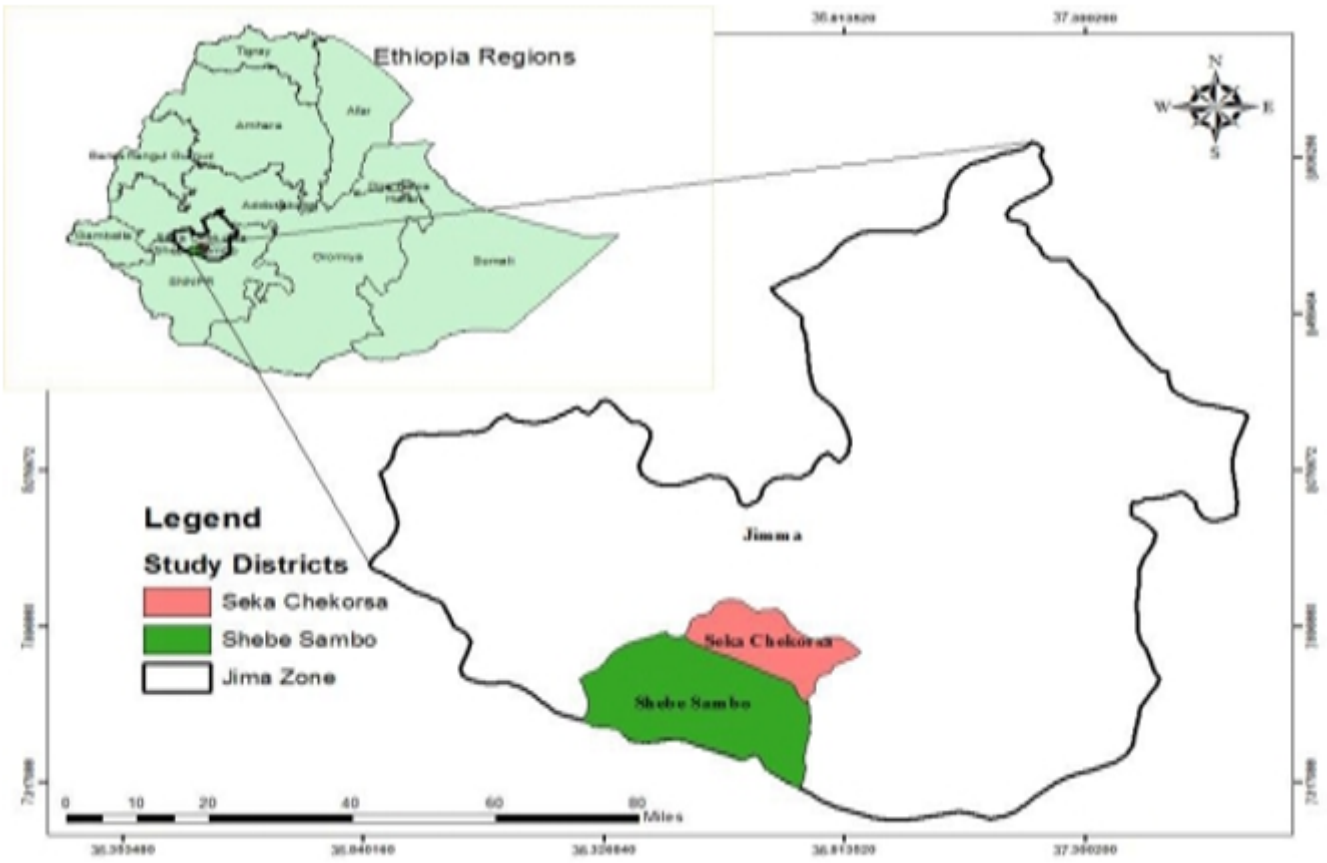


**Figure 5**

Map of the study Area (source self-Scheck, 2018).

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