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Research article

Seroprevalence of *Brucella* infection in sheep and goat in two Woreda, in Arsi Zone, Oromia, Ethiopia

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Abstract

The cross-sectional research study was conducted from November 2015 up to April 2016 in order to assess the prevalence of *Brucella* infection in shoats in two woredas in the Arsi zone, Oromia region, Ethiopia. The study was conducted in six Peasant Associations (3 PAs from each district). A total of 397 sheep and goats were sampled to be studied. Rose Bengal Plate Test (RBPT) was used as a Screening test for *Brucella* agglutinins, while Complement Fixation Test (CFT) was used to confirm the reactors by Rose Bengal Plate Test. The statistical analysis of the data was run by Fisher's exact test and the comparison of proportion was carried out too. Out of the 397 sera samples, 1.51% (n = 6) were seropositive for *Brucella* infection by RBPT; however, the CFT test indicated that only 0.5% (n = 2) sera were positive. Overall point estimation of 0.2% ovine and caprine *Brucella* infection was observed. High prevalence has been observed in aborted, parity one (1-5 kidding or lamping), female and in goats than non-aborted, parity two (> 6 lambing or kidding), male and sheep respectively. Especially high Seroprevalence in aborted goats indicates the presence of the illness in the two woredas'. The brucellosis presence in goats and sheep is significant as the infected animals can act as reservoirs of infection and may transmit the infection to human beings by several routes. So, Control measures should be designed and implemented by focusing on the prevention of further illness expansion in the study area by using an appropriate control practices approach.

Introduction

Sheep and goats are crucial domestic animals in lowland animal production systems. Shoat present in the African continent accounts for around 21% of the globe's sheep and goats. The whole count of sheep and goats in Africa represents 17% and 30% of this world's total shoat number respectively [1]. Ethiopia has the third-largest number of sheep and goats among African countries and ranks eighth in the world [2]. Sheep and goats give huge benefits to farmers: by acting as a source of animal origin food such as milk, and meat, fibbers, and saving money cash, due to their copping up to a wide verity of agro-ecologies [3]. Small ruminants' high production efficiency is the result of the high productivity rate of Sheep and goats. Under rearing livestock for lively hood (pastoralist) and mixed (agro-pastoralist) ways of the farming system, both species of sheep and goats are reared as a base of worth buying and as insurance against diseases [4].

Ethiopia is an agricultural-based country in which animal production act as a great role player for the country's resource and forms a major part of the agricultural lead production system. The animal production branch contributes forty-five percent of agricultural gross domestic product (GDP), fifteen up to eighteen percent of national GDP and five to seventeen total exports [5]. The country has a huge number of shoats, which is around 58.44 million heads of small ruminants [6]. Shoats is abundant in tropical agroecology which accounts for about sixty-five percent of the country, where twenty-five percent of ovine and nearly a hundred percent caprine density is there [7].

Except for the huge number of sheep and goats in the country, the competent large resources that are owned at the

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national level and income benefit gained from this branch do not balanced. Sheep and goat production system development is influenced by different constraints such as the absence of nutrition, less productivity, backward culture of taking care of animals, and infectious illness. The diseases are the main drawback and the major concern in the sheep and goats production system .yearly losses due to death of sheep and goats range from 12% – 14% and 11% - 13% respectively [8].

Brucellosis is the second most important zoonotic illness next to rabies, which results in great reproduction losses in adult animals and is known as a major drawback for livestock productivity and trade barriers in Ethiopia [1]. Brucellosis is a chronic zoonotic infection caused by *Brucella* species which are facultatively anaerobic, non-motile, gram-negative intracellular bacteria and are a known etiology of reproductive losses [2,9].

The causative agent of *Brucella* infection in shoat is mostly *B. melitensis* and in rare cases *Brucella Ovis and*/or *B.suis* [10]. The first zoonotic important species isolated in the genus *Brucella* was *B. melitensis*, which is the major causative agent of the disease in sheep and goats. Brucellosis is caused by *B. melitensis* found naturally in short and is very virulent for human beings, resulting in one of the most dangerous zoonotic phenomena globally. Sheep and goat brucellosis is endemic in most countries of the Mediterranean basin, middle and central Asia, Latin America, and parts of Africa [11]. Brucellosis is a zoonotic infection that exists globally and is prevalent within most nations of the African continent [12].

Brucella infection has been indicated that in beneath Sahara African countries, the epidemiology of *Brucella* infection in human beings and animals is not well understood and there is a scarcity of available data [13]. There is a scarcity of documented information on *Brucella* infection introduction and establishment in Ethiopia. However, many surveys conducted by serology have shown that brucellosis ruminants are prevalent and dispersed in different farm animals and some ranches found at the national level [14]. *Brucella* is indicated as the etiologic agent of fetal abortion, retired placenta, repeat breeding, infertility, and variability of the inter-calving period due to early embryonic death [15]. The disease is the second most common zoonotic illness with more than fifty thousand cases reported yearly [16]. Sheep and goat *Brucella* infection mostly occurred by *B. melitensis*, which contains 3 strains (srain1, strain 2, and strain 3). All the 3 strains induce infection in sheep and goats [17].

In livestock, *B. melitensis* is most likely transmitted by direct or indirect contact with the fetal membrane, fetus, and fetal fluids, and discharges from the vagina for at least ninety days, but shedding usually ends within 21 days in sheep. *Brucella* melitensis can also be found in milk and semen. Shedding in milk and semen can be prolonged or lifelong, particularly in young goats and sheep during their nursing seroprevalent mothers may excrete *Brucella Melitensis* in feces[18].

Ingestion of contaminated food products can transfer the disease from animals to humans by, direct contact with an infected shoat or droplet aerosol inhalation and is widely spread in developing countries [19]. For diagnosis of *Brucella melitensis* infection, the Rose Bengal plate test (RBPT) and complement fixation test (CFT) are the most widely used test. The RBPT is used as a screening test, while the CFT test is used as a confirmatory test. Both RBPT and CFT tests are based on t he reaction of antibodies produced as the response to *Brucella* infection and an antigen or entire cell of *Brucella* [20]. Livestock susceptibility to *Brucella* infection depends on their natural protection, age, sex, level of immunity, and stress induced by environmental factors [14]. Different researchers have studied the Endemicity of brucellosis of small ruminants in Ethiopia. Research conducted by [21] reported a prevalence of 31.5% and 3.3 % in the Afar region and the Somali region respectively. The result of [22] and [1] indicated that Mengistu (2007) reported a prevalence of 1.6% and 1.65% in Oromia and SNNPR respectively [23]. Recorded 4.89% *Brucella* infection in sheep in Eastern Amhara Regional State but [1], result showed that the endemicity of *Brucella* infection in shoat was found only around point seven percent in Kombolcha, Amhara Region. However, the prevalence status of shoat brucellosis in selected woredas of Arsi Zone has not been well documented. Based on the gap identified, this study's objective was, to assess the endemicity and its related risk factors of Brucellosis of shoat in the two selected woredas'.

Methods and materials

Study area

The selected woredas are located in the Arsi zone, Oromia region. Arsi zone is one of the administrative zones in the Oromia Region, that is known for its crop production especially, wheat production. It is located 6° 45' – 8°58' N latitudes and 38° 32' to 40° 50' E longitude southeast of the country. Topographically, the altitude of the Arsi zone ranges from 500-4130m above sea level. The 3 climatic divisions or zones, including an arid, tropical highland, and temperate are known to be found. An average annual temperature and rainfall of 10°c - 25°c and of 400 - 200mm are recorded respectively.

As in many other parts of the country, agriculture contributing 75.67% of the total GDP is the first ranking livelihood (economic) activity of the Arsi zone. Agro-pastoral farming system coverage is ninety percent of the total agricultural farming system, with the production of crop accounting for 45.33% and livestock for 30.34% of the GDP is typical of the area. Cattle stand as the dominant livestock asset followed by sheep and goats respectively in the zone. Arsi zone has a huge livestock population which include: cattle 2.52 million, sheep 1.66 million, goats 0.73 million, horse .24 million, mule .02 million, donkey.42 million camel 28.9 thousand,1.88 million poultry and .12 million beehives [6].

Target population

The study animals were shoat found in six selected kebeles of the study area. The study animals were managed almost under a pastoral and mixed agro-pastoral farming system. The number of sheep and goats population of the two districts

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was 1,180,423 (Agency of Livestock and Fishery of Tiyo and Jeju woreda, 2015). The selected PAs were included purposively based on their agroecology, accessibility to transportation, and density of the small ruminant population. All of the study animals were non –vaccinated against *Brucella* and all were above one year of age.

Design of study

The research study was conducted by using a cross-sectional study design starting from the tenth month of 2015 up to the fourth month of 2016 in order to assess the prevalence of *Brucella* infection of ovine and caprine in 2 woredas of Arsi zone, Oromia region, Ethiopia. The independent variable like district, agroecology, age of sheep and goat, their sex, parity status, and fetal abortion were evaluated as to their association to brucellosis.

Sampling method

Calculation of sample size: The formula given by (Thrusfield, 2005) was used to calculate the required Sample size with fifty percent expected prevalence, ninety-five percent confidence interval, and five percent of accepted error. Therefore, the calculated sample was 384 shots. However, 397 shoats were actually sampled by adding around 3 percent for increased precision.

Sampling strategy: Blood sample collection: five to eight milliliters of blood were taken from the jugular vein of each randomly caught animal using a needle and non-heparinized vacutainer tube. Labeling code of each shoat was done on vacutainer tubes which contain that animal blood sample and allowed to clot full night in a slant position at room temperature. The next day sera were then decanted to the non-contaminated cryovial tube of 5ml, to which the Labeling code of each shoat was done and Serum samples were kept at -20°C at the Asella regional veterinary Laboratory until they were transported in a cold chain to NVI for conducting RBPT and CFT.

Serological tests

A blood sample was collected in a plain vacutainer tube and allowed to clot overnight in a slant position at room temperature. The following day after the blood cell and serum were separated in the tube, a serum that was found on the upper part of the suspension was decanted to the sterile cryovial tube and kept at -20 °C at the Asella regional veterinary Laboratory until it was transported in a cold chain to National Veterinary Institute for conducting RBPT and CFT. A screening test of the serum was done by RBPT (Rose Bengal test) and for further tests, CFT was used for retesting the RBPT-positive serum sample. The test requires commercially available specific antigens, serum of the patients, the complement (rabies serum), and the sheep's red blood cells. To prepare sheep red blood cells, 75ml of sheep blood was drawn from the jugular vein of male sheep in 125 ml of Alsevers solution, after a small amount of crystalline penicillin was added to avoid bacterial contaminations stored at +4 °C overnight. Both prepared sera and available Antigens for the serological test were taken from the refrigerators and held at normal environmental temperature prior to testing being conducted. Rose Bengal Plate Test (RBPT) was used as a screening test for the presence of *Brucella* agglutinins. A test was conducted in the Laboratory of National veterinary Institute (NVI), at Debrazeit. The interpretations of RBPT output were done based on the agglutination degree. Those sera with null (0) degree of agglutination were recorded as a negative result while others were recorded as positive [20]. Sample that was positive by RBPT were retested further by the CFT test for confirmation. It is complex to perform modernized tests like indirect enzyme-linked immunosorbent assay (iELISA), requiring good laboratory facilities, and adequately trained staff to titrate accurate results and maintain the reagents. CFT Standard *Brucella abortus antigen* is used to identify the presence of anti-*Brucella* antibody in the sample serum. Sera which shows a strong reaction, that is above seventy-five percent complement fixation (+++) with a dilution of 1: 50; and at least fifty percent complement fixation (++) with a dilution of 1:10 were categorized as a positive result [20].

Analysis of data

The laboratory assay result Data was stored on a Microsoft Excel spreadsheet program and statistical analysis was done by using Intercooled STATA 7.0 version software. To assess the association, Both Fisher's exact test and Chi-square tests were used. In addition, univariable logistic regression analysis was used by calculating the odds ratio (OR) to see the degree of association between the independent variables and the disease in sheep and goats, and statistically significant results were considered at P values <0.05.

Results

All 397 sera (161 from sheep and 236 serum from goats) testing was conducted for the assessing endemicity of *Brucella* infection in shoat. Rose Bengal plate test showed Six (6) positive serum samples. Up on additional investigation on those RBPT positive sera by CFT, only 2(0.5%) sera were found positive (Table 1). All the Sero-prevalence estimates presented are with reference to CFT results.

An overall prevalence of 2 (0.51%) with Seroprevalence of 1 (0.71%) in Jeju and 1 (0.78%) in Tiyo was recorded. Comparatively higher Seroprevalence was recorded in goats 2 (0.89%) compared to sheep, in females, 2 (0.85%) compared to males, in the age group between 1-5 years 2 (0.57%) compared to age group ≥ 6 years, in parity group 1 (0.91%) compared to the other group, in a group with abortion 1 (4%) compared to the group without abortion and finally in *Woina Dega* agroecology 1 (0.71%) and *Kolla* agroecology 1 (0.66) compared to Dega agroecology. But none of the risk factors associated proved to be statistically significant.

Table 1: Point estimation of *Brucella* infection in goats and sheep by woredas.

District	Number of tested animals	RBPT Positive (%)	CFT positive (%)
Jeju	142	2 (1.41%)	1(0.71%)
Tiyo	255	4 (1.57%)	1(0.78)
Total	397	6 (1.51%)	2(0.51%)

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Discussion

The point estimate of shoat *Brucella* infection in the two woredas in the Arsi zone was founded to be 1.5% by RBPT and 0.5% (0% in sheep and 0.85% in goats) by CFT. The difference in the result of RBPT and CFT is due to the difference in sensitivity and specificity of the two tests. The result of RBPT was higher because RBPT is more sensitive than the CFT: which is specific. The RBPT result of this study is greater than the result of [24] who reported a prevalence rate of 1.2% in ovine and 1.9% in caprine in Jijiga surround. But it is almost similar to [17,22], who reported a prevalence of 1.56% in the Finale district, in East Shewa [1]. Indicated that Mengistu (2007) reported a prevalence of 1.6% and 1.65% in Oromia and SNNPR respectively. The CFT Seroprevalence finding of this study is similar to [25], whose result indicates the prevalence proportions of 0.87% in caprine in Bahir Dar, Amhara Region, and the result reported by [1], with the rate of prevalence 0.7% of small ruminants at Kombolch, Amhara Regional State show the similar result with this finding

The higher prevalence rates were recorded by [22], with rate prevalence of 5.6 % and 13.2% in sheep and goats [26] with a rate prevalence of 5.8% in goats and 3.2% in sheep and [27], with a prevalence rate of 4.2% in goats respectively in Afar and Somali areas, pastoral regions of Afar and in South Omo zone are higher records compared to the result of this research [23]with 4.89% *Brucella* infection in sheep in Eastern Amhara. The variability in the rate of prevalence might be due to the variability in agroecology and animal management system. Arsi zone animal husbandry system is known for mixed agro-pastoral farming, in which somewhat few sheep and goats are kept in separated herds beside the high turnover of short stocks to central market; But, Afar and Somali regions pastoralists and south Omo kept a huge number of various livestock species [28] the result reported a higher prevalence of 4.6% in Arsi and East Shoa. The variability in sample size taken and serological tests conducted for confirmation i.e., iELISA may result in the difference in prevalence rate obtained.

Conclusion

The current study has shown the distribution of *Brucella* antibodies in 0.5% of the small ruminants tested in Jeju and Tiyo woredas of the Arsi zone, Oromia region, Ethiopia. Despite the fact that low Seroprevalence of *Brucella* infection was recorded in this study result, the easy ways of transmission, zoonosis nature, and the raw milk consumption behavior still pose a considerable risk to small ruminant husbandry. The independent variable assessed including species, sex, age, parity, dams' abortion history, and agroecology was not associated significantly with the prevalence of *Brucella* infection in the two woredas. The absence of association could have resulted from a high turnover of sheep and goats for sale to the central market which can affect the overall Seroprevalence of the disease. But still, the present study could give an indication of brucellosis can be a disease of importance since most factors underestimated can be important in wider studies. Generally, this finding provided the status of sheep and goats *Brucella* infection in Arsi Zone and indicated a considerable risk for transfer of the illness in shoat and community of the two woredas of Arsi zone.

Based on the above conclusion the following recommendation are addressed: The implementation of the test–and–slaughter policy remains unfeasible for the time being since other provisions are not met. However, the very low prevalence rate observed in the study area may suggest. In addition, society in general and high-risk groups such as farmers and animal attendants, in particular, should be trained and aware of the zoonotic importance of some *Brucella* species and their transmission mechanisms. Further investigations should be done on small ruminant brucellosis to elucidate the isolation and identification of specific biotypes and livestock and the public health significance of the disease in the two woredas.

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