

## Bovine *Brucellosis*: Epidemiology, Public Health Implication and Status of the *Brucella* Infection in Ethiopia

Dereje Tulu Robi\*

Department of Agriculture, Tepi Agricultural Research Center, Tepi, Ethiopia

\*Corresponding author: Dr. Dereje Tulu Robi, Department of Agriculture, Tepi Agricultural Research Center, Tepi, Ethiopia, E-mail: derejetulu5@gmail.com

Received date: November 26, 2021; Accepted date: December 13, 2021; Published date: December 20, 2021

Copyright: © 2021 Robi DT. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

*Brucellosis* is a serious economic and public well-being concern throughout the world. It is a global spreading and causes a serious problem to developing country similarly as Ethiopia. *Brucella abortus* is the foremost reason for this disease in cattle. Moreover, *Brucella melitensis*, and *Brucella suis* reason for *brucellosis* in cattle occasionally. It is recognized by causing abortion at third trimester, retained fetal membrane and sterility in female, and orchitis and bursitis in bull. *Brucella* organism is usually transmitted to different cattle through direct or indirect contact with diseased cattle or their discharge. Humans procure the disease mostly by drinking unpasteurized milk/product and contact with diseased cattle or their discharges. The incidence of disease is influenced by variety of factors associated with management system, host and environmental factors. Age, gender, species, herd size, herd type, hygienic status and agro ecology are among the most important factors of the disease. Work-related contact is observed in peoples who interaction with diseased cattle and their tissues. In Ethiopia, *brucellosis* is high in pastoral and mixed cattle production system where human live closely with cattle and so, are at higher probability of getting the *Brucella* organism. The most appropriate method of *Brucella* infection control is vaccination of young female cattle. *Brucella abortus* may be eradicated by isolation of diseased cattle, giving vaccine and test-slaughter methods. Thus, it is important to conduct applicable control methods, increase the public awareness on zoonotic transmission of *brucellosis* and conducted study on the epidemiology of *brucellosis* in higher risk group.

**Keywords:** *Brucellosis*; Cattle; Epidemiology; Public health

### Introduction

Ethiopia has a huge number of cattle in Africa, with a total cattle population of 59.5 million. Despite having large cattle population, the country is unable to optimally use this potential resource as result of different constraints affecting cattle production. Animal disease, management problem, poor genetic and nutrition deficiency are among the foremost constraints to cattle production in the country. Among the infection disease, *brucellosis* is widely prevalence disease which causes considerable economic losses and an important human disease in Ethiopia [1]. Introduction of higher yielding cattle breed is the major strategies to increased milk production of the country. However, *brucellosis* is the major dairy improvement problem in different part of the country. This disease is the leading cause of reproduction inefficiency and cattle abortion.

*Brucellosis* is causes huge financial losses and public wellbeing concern in the world including Ethiopia. *Brucella abortus*, *B. melitensis* and *B. suis* are the most important cause of bovine *brucellosis*. It is recognized by inflicting abortion at third trimester, retained fetal membrane and infertility in succeed pregnancies in bovine [2]. *Brucella* organism spread is through interaction with aborting cattle and aborted fetus or with contaminated fomites. Herd size, age and gender of cattle, management system, and contact with wild animals, environmental factors and herding different species in a herd are among risk factors reported.

In Ethiopia, several studies show that *brucellosis* is common disease in cattle. These investigations indicated that the highest seroprevalence of the disease in place where people live very closely

with livestock. Seroprevalence of *brucellosis* is higher in intensive farming than extensive cattle rearing systems. *Brucella* seroprevalence as high as ranging from 0.1%-14.1% has been reported in different parts of the country [3]. Recent reports from different areas of the country also indicated that *brucellosis* still widespread disease in the country, resulting in huge economic losses in cattle production. Nevertheless, there is limited evidence on epidemiology and public health implication of the *brucellosis* in cattle. Therefore, the aim of this paper was to review the epidemiology and public health implication of *brucellosis* in cattle.

### Bovine *brucellosis*

Bovine *brucellosis* is mainly caused by *Brucella abortus* and a minimum of nine biotypes are recognized as well as variety of variants. *Brucella abortus* are small, gram-negative and facultative intracellular coccobacillus. *Brucella abortus* is principally causes of disease in cattle. However, sometime sheep, goats, swine, dogs, and horses could also be infected. Cattle will be also become disease by *B. suis* and *B. melitensis* once they grazing together with infected pigs, goats or sheep.

### Clinical signs

The principal clinical sign of *Brucella* infection is causes abortion at late term (5-7 months) in female cattle and inflammation of testis (orchitis) and lameness due to bursitis in male cattle. Immature may remain sub-clinically infected until maturity and pregnancy without showing any sign of the disease. *Brucella* infection is assumed in

herds when abortions and retention of placental occur at the last stage of pregnancies without concurrent disease. *Brucella* infection resulting in abortion, stillbirths, retention of placenta and weak calve. Retain fetal membrane and endometritis are outcome of abortion. Female cattle generally aborted just one, probably because of resistance. In general, abortion with retained fetal membrane and as result the endometritis may cause prolonged parturition interval and permanent sterility. The principal sign of *Brucella* infection particular for bulls are epididymitis, seminal vesiculitis, orchitis and testicular abscesses. Infertility occurs in male and female cattle may due to orchitis/epididymitis or metritis respectively. Hygromas on the leg joint of *Brucella* infected cattle is typical sign of the disease that is resulted due to chronic infection with *Brucella abortus*. Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections and deaths are common in the fetus or newborn.

### Pathogenesis

After exposure, *Brucella* organism enters intact membrane, survives and reproduces in cell of the system, just like the bone marrow, lymph nodes, liver, spleen, and also kidney. Reproduction of the organism here may last for several months, resolve itself, or be recurrent for at least two years in 5%-10% of animals. Recurrence occurs significantly at the time of parturition [4]. Throughout the bacteremia, organisms are carried intracellular in neutrophils and macrophages or free in plasma and localize in several organs, particularly the gravid uterus, udder, and supramammary lymph nodes. *Brucella* organism also localization in other lymph nodes, spleen, testes, and male accessory gender glands.

Occasionally *Brucella* organism localization happens in synovial structures inflicting a pussy tendovaginitis, arthritis, or bursitis [5]. The favored localization of the reproductive tract of the pregnant cattle is due to the presence of unknown factors in the gravid uterus. These are mentioned as allantoic fluid factors that stimulate the development of *Brucella* organisms. Erythritol (four-carbon alcohol sugar) is taken under consideration to be one in all these factors which are increased in placenta and fetal fluid from fifth month of gestation. The favored replication of *Brucella abortus* in extraplacental in trophoblast of the membrane causes rupture of the cells and ulceration of the membrane. The damage to placental tissue together with fetal infection and fetal stress will induce maternal hormonal changes. As result, abortion occur mostly within the last three months of pregnancy, the time period being reciprocally proportion to the stage of development of the fetus during infection.

### Literature Review

#### Epidemiology of *brucella* infection

*Brucella* organism is usually transmitted to other cattle through direct and indirect interaction with diseased cattle or their discharges. Transmission in cattle occurs mainly through the ingestion of contaminated feed and water by organisms that are presence in massive numbers in aborted fetuses, fetal membranes and uterine discharge. Moreover, cattle typically lick once birth, fetuses, and newborn calves, all of that can contain huge number of the organisms and constitutes the major source of infectious. Using pooled colostrum for feeding newborn calves may also transmit infection. Transmission sometimes play very little role in the epidemiology of *brucellosis* in cattle. On the other hand, artificial insemination will transmit the

disease and semen should be only collected from cattle illustrious to be free from infection. *Brucella* organism can resist drying and survive in dust and soil if there is an organic material in the environment. Survival rate of this organism is longer in low temperature especially in deep freezing.

A human is got *Brucella* infection mainly through ingestion of contaminated milk and unpasteurized milk or dairy product. Contact of mucosas and skin abrasions with fluids and tissues from aborted fetuses of diseased cattle are also important source of *Brucella* organism. *Brucella* infection may be a worldwide animal disease caused by *Brucella* organism in which domestic animals like cattle, goats, pigs, camel, buffalo and dogs serve as a reservoir hosts. Fresh milk and its product prepared from unpasteurized milk such as cheeses, yogurts, and ice creams contain a high concentration of the organisms and consumption of those is a significant cause for human *brucellosis*. *Brucella* organism can survive in proper environmental condition, damp soil and seawater and can be a source of infection [6]. The aborted materials such as placenta, fetal parts, and fetal membranes, amniotic fluid and vaginal discharges of diseased cattle may contain high amounts of the bacterium and act as source of the disease. Work related exposure to animals or their products is the most common risk factor for *brucellosis*. Abattoir employees, farm employees, veterinarians as well as laboratory employees are well-known risky group.

#### Risk factors for *brucellosis*

The occurrence of *Brucella* infection is influenced by variety of risk factors related to production systems, host and environmental factors. These include age, gender, breed, species, herd size, herd type, hygienic status and agro-ecology.

Age has been stated as the intrinsic factors related to *Brucella* infection. Higher seroprevalence of *Brucella* organism has been determined in adult cattle than in young. Mature and pregnant cattle are more prone to infected with the *Brucella* organism than immature cattle. Because of *Brucella* organism conferred a response to the productive tract due to the production of erythritol, sugar generated within the fetal tissues of cattle which stimulate the growth of *Brucella* organism [7]. However, higher seroprevalence of *Brucella* organism in adult has additionally been related to longer interaction with diseased cattle or environment. This potential risk could also be vital in those herds, while not culled the positive cattle.

The influence of gender on the prevalence of *Brucella* infection has been stated in cattle. Female cattle presented a higher odd of *brucellosis* compared to male cattle. Even if, this is difficult to explain, it may be related to the biology of the *Brucella* organism and its tropism to the fetal tissues. Since *brucellosis* in males conferred clinical signs like epididymitis and orchitis, the prevalence in males can be lower than females as a result of, they may be culled faster [8]. However, the absence clinical signs like abortion or metritis in non-pregnant diseased female may also described as higher prevalence in the female. Additionally, *brucellosis* is becomes chronic in non-pregnant cattle [9]. This has necessary for epidemiological consequences, when initial immune response cattle is also symptomless carriers, the antibodies disappear from circulation and are challenging to identify with old serologic methods.

There is argument among various researchers on the issue of breed prone to *Brucella* infection. However, higher seroprevalence of *Brucella* infection has been stated in cross than local breed cattle,

while other reports indicated no associated among breeds. Some of them reported higher seroprevalence of *Brucella* infection in local than cross breed.

Herd size is one of the major risk factor for *Brucella* infection, being higher in large herd size. This might be described by the larger odds of identifying a minimum of one seropositive cattle, the rise of spread of *brucellosis* by interaction among them, using of communal pasture areas or inadequate cleaning and disinfection techniques in big farms. The low prevalence of *Brucella* infection in small herd size might be related to the herd and/farm management [10]. As a result, small herd size sometimes graze at pastures close to, controlling interaction with other herds or using of communal methods. The small herd size could be simply management the partum period and frequently keep cattle removed from the herds throughout parturition. This is extremely vital in the case of abortion, in order to prevent pasture contamination. In small herd sizes, replacement is typically created by reposition and economic trade is not frequent. Hence, the lack an increased rate of cattle movement reduced the chance of the disease. However, cattle movement in large herds is common, each for replacement and/or trade, so increasing the risk of *Brucella* infection.

Farming many species within the same herd has been characterized as a risk factor for *Brucella* infection. Though there is no indication of higher proneness of *Brucella* infection in particular species. As a result, rise in prevalence of *brucellosis* where various species combine is difficult to describe but may be related to higher probability of being *Brucella* seropositive due to various sources of the disease [11]. It has been stated that *Brucella* infection is spread only seldom from small ruminant to cattle. Nevertheless, the risk for cattle on farms that additionally had sheep and goats propose that a number of the cattle *brucellosis* might have originated from sheep and goats because *Brucella melitensis* biovar three was isolated from cattle milk.

Dairy cattle have a far bigger probability of not solely acquiring *Brucella* infection however additionally of spreading it more rapidly than beef cattle. This is not a genetic or physiological factor but because of husbandry. Cattle that live in focused smaller areas become close interaction once they are grazing and milked. Dairy cattle are exposed to additional stress on farms, causing a higher condition to *Brucella* infection.

Cattle purchase has been considered as a risk for *Brucella* infection and practice increases the risk of introducing diseased cattle into a herd. Introduction of animals from market fair also cause a higher risk of *Brucella* infection. Most of infectious disease in disease free herds starts through buying diseased cattle of unknown status. The effect of the agro-ecology has been additionally noted as *Brucella* infection risk issue, having prevalence in dry areas [12]. Because a shorted of pasture areas in dry areas, cattle should obtain pasture over large areas indicating uncontrolled cattle to cattle interaction with the potential transmission. Additionally, transmission because of aerosol inhalation of contaminated dust from fetus discharges or abortion is likely.

Large hers size can be probable to be related to intensive management systems that are generally tougher to manage and permit for nearer interaction between cattle and their surroundings, that can increase the potential for exposure to *Brucella* organism. Additionally, the nerve-racking conditions cattle exposed to intensive production system might create them a lot of prone to the infection. However, extensive production system may also indicate a risk of *Brucella* infection [13]. It could be related with controlling abortions, observation of diseased cattle or interaction with cattle among others.

Since in extensive system implies rearing many of cattle in large areas and/or sharing common pastures, the contamination of pastures with placentas or abortions may be as source of *brucellosis* to different animals in the herds. Farming and environmental condition are risk factors that affect the spread of *brucellosis*, such as birth and breeding in semi-dark setting, confinement in closed areas and high cattle densities. Another risk of intensive systems might be related with airborne transmission inside.

Similarly, season has an influence on herd management and animal nutrition, principally in production systems involving transhumance or nomadic practices. Rain influences the growth and nutritional state of the pasture. These factors have an effect on the reproduction of cattle in extensive systems so the time of delivery or miscarriages. In intensive systems, isolation of post-parturient cattle in maternity facilities decreases transmission of *brucellosis* to other herd.

## Diagnosis

The isolation and identification of *Brucella* organism offers a definitive diagnosis of *brucellosis*. It is important for epidemiology and to monitor the progress of a vaccination programs in cattle. The technique of the diagnosis includes the following:

### Direct diagnosis

*Brucella* infection can be confirmed by demonstration of the bacteria in smears with microscopic staining. The smears were prepared from vaginal discharges, placenta, colostrum, fetal stomach fluid or of the aborting cattle lochia and the abomasum of the aborted fetus with Modified Ziehl-Neelsen stain (MZN). Impression smears may be taken from freshly cut and blotted tissue surfaces, e.g. cotyledons, by firmly pressing the slide surface against the tissue [14]. Allow to air dry and heat fix. Smears are also prepared from fetus stomach fluid, cotyledons or lochia and stained with the improved Ziehl-Nielsen stain or stamp stain. In MZN-stained smears the *Brucella* organism seems as red intracellular coccobacilli or rode shape whereas other bacteria stain blue.

All *Brucella* strains are relatively slow growing, and as the specimens from that isolations best attempted are often heavily contaminated, the use of a selective medium, e.g. Farrell's medium is advocated. Incubation usually continues for 72 hours, but a negative diagnosis can only be made after week long incubation [15]. Sample that can be used for *Brucella abortus* isolation include: Fetus stomach fluid, spleen, liver, placenta, lochia, milk (especially colostrum or milk within a week of calving), semen and lymph nodes (supramammary for chronic and latent infections and retropharyngeal for early infections are favored, but iliac, prescapular and parotid may be used). If serological reactions are thought to be caused by S19 vaccine strain then it is vital to gather prescapular lymph nodes in addition. All *Brucella abortus* isolates should be furthered to laboratories capable of biotyping. Farrels' medium and Albimi *Brucella* medium are selective enriched media for isolation of *Brucella* species.

### Indirect diagnosis

In the absence of culture facilities, the diagnosis of *Brucella* infection usually based on serological test with various agglutination tests like Rose Bengal plate test (RBPT), serum agglutination test and antiglobulin. Detection of antibodies (and at a lesser degree the measure of the cell-mediated immunity) against relevant *Brucella* epitopes is more sensible method. Serology can be used for an

apparent diagnosis of *Brucella* infectious or to screen herd. Indirect or competitive Enzyme-Linked Immunosorbent Assays (ELISAs) are also used.

Rose Bengal Plate Test (RBPT) may be a terribly sensitive test is conducted to screen serum samples. It does not distinguish between field and S19 vaccine strain reactions, but it is quick, inexpensive and easy to implement. False negative reactions are rare but may sometime be because of excessive heating in storage or in transit. Rose Bengal plate test has a sensitivity of 96.10% and specificity of 99.30%.

The diagnosis of *brucellosis* in cattle may be adversely affected the presence of cross-reactions that made false-positive serological test results due to S19 vaccine or other gram negative bacteria that share similar epitopes such as *Brucella abortus* O-chain polysaccharides. Thus *Yersinia enterocolitica* 0:9, 0157:H7, *Salmonella* group N (0:30), species and *Vibrio cholera* can react in serological tests for *Brucella* infectious in cattle.

The positive reaction ought to be investigated using appropriate confirmatory tests and/ or epidemiologic investigation. Rose Bengal Plate Test (RBPT) appears to be adequate as a screening test for identifying diseased herds or to guarantee the absence of infection in *Brucella* infection free-herds.

Complement Fixation Test (CFT) is that the most generally used test for serological confirmation of *Brucella* infection in cattle and counseled by OIE. The CFT based on the detection of particular antibodies of the IgM and IgG1 that fix complement. The sensitive and specific of CFT is in the hand of experienced users and used as a confirmatory blood test. Usually, the CFT is used on RBPT positive sera, but similar the RBPT it is also influenced a large extent by the misuse of strain 19 vaccine, mainly when recent or repetitive vaccinations have been used in mature heifers and cows. It is almost impossible to suggest strict cut-off reading that show *brucellosis* significantly once S19 vaccine reaction play a role because of its misuse. The CFT is comparatively complex test. The reagents include *Brucella abortus* CFT antigen, complement, amboceptor (haemolysin), ovine erythrocytes and test serum with Veronal buffer as the diluents.

The c-ELISA is usually conducted by choosing monoclonal antibodies (mAb) with slightly higher affinity for the antigen than most of the vaccine or cross reacting antibody, however with lower affinity than antibody arising from infection. The specificity of c-ELISA is very high and capable to identify all antibody isotype (IgM, IgG1, IgG2 and IgA). The c-ELISA has high diagnostic specificity (100%) and sensitivity of 98.8%, and it was observed to be the most specific test. The highest specificity just in case of c-ELISA is because of using particular monoclonal antibodies as a conjugate which has the ability to content with other non-specific antibodies and fix to certain specific epitope on SLPS antigen.

The confirmatory test has to demonstrate high level of diagnostic specificity and maintain effective sensitivity to reduce the number of false positive reactors to the minimal levels. The c-ELISA in additionally ability of reducing most reaction because of a residual antibody formed in reaction to vaccination with S19. Worldwide, the 3 OIE-ELISA standard sera ought to be used by National Reference laboratories to see or adjust the technique in query. The essay ought to be standardized like the Optical Density (OD) of the strong positive OIE ELISA standard serum ought to be close to highest inhibition. The only limitation of the c-ELISA is that it is more complex and cost

to conduct than the screening tests. The weak positive OIE ELISA common sera ought to provide a reaction that is moderate. The negative serum and the buffer or mAb control ought to offer responses that are always less than the test population.

### Occurrence of *brucellosis*

*Brucellosis* has a worldwide distribution and foremost problem to developing country similar as Ethiopia. It is one of the economically significant disease in animals and human in those countries. This disease has a significant influence on cattle and human wellbeing, further as extensive socio-economic effects, particularly in country that financial gain based on livestock production and dairy products.

### Control and eradication of *brucella* infection

*Brucellosis* treatment in animal has typically ineffective attributable to the intracellular nature of organisms and the bacterium are facultative intracellular that persist and multiply in the cells. *Brucellosis* is typically introduced into a herd with an infected animal, but it can also enter in semen from diseased bulls and on fomites. In an endemic area, vaccinated calves or non-pregnant heifers are the best herd addition in uninfected herds. Newly introduced or pregnant cows should come from *Brucella* free areas or herds and have to be sero-negative. New animals should be quarantined for about a month and retested for *Brucella abortus* before they introduced to the group. *Brucellosis* can be eradicated by isolation of diseased animals, giving of vaccine, test and slaughter methods. Moreover, several forms of investigation and trace backs are important in eradication program. *Brucella* organism is killed by most typically available disinfectants together with formaldehyde, glutaraldehyde and xylene; however, organic substance and low temperatures decrease the efficacy of disinfectants. Two *Brucella abortus* vaccines, strain nineteen and RB51, is accustomed management this disease in endemic areas, or used as a part of eradication program. Regular vaccination is done in calve to minimize the production of persistent antibodies that can interfere with serological tests.

### Discussion

#### Public health significance of *brucellosis*

*Brucella abortus* is one of the most pathogenic and invasive species for humans. Work-related exposure is seen in people who interacted with diseased cattle or tissues. *Brucella* infection is among the principal easily acquired laboratory infections. Human also infected by ingesting unpasteurized milk or dairy products [16]. The Strain nineteen *Brucella abortus* vaccine is additionally pathogenic for humans and should be handled with carefully to prevent unintentional injection or contamination of mucous membranes or scraped the skin. Adverse events are also reported with the RB51 vaccine, although it appears to be safer than Strain 19.

The prevalence of *brucellosis* in human is associated to the amount of prevalence in domestic animals nearby them. *Brucellosis* may be a comparatively common disease among animals and human in developing countries and it constitutes a large and uncontrolled public health problem [17]. Several case of *brucellosis* in human occurs in each year. Human is infected one there is indirect contact with animals at abortion, parturition, or post parturition from splashing of infected droplets into the eye or drinking unpasteurized milk or milk product [18]. The *brucellosis* in human is described by a multitude of somatic



complaints, such as fever, sweat, anorexia, malaise, weight loss, depression, headache and joint pains. This disease is confused with disease like malaria and influenza. The highest seroprevalence of human *brucellosis* was reported in Afar region (12%) whereas low seroprevalence was reported in Fafan zone (0.4%) in Ethiopia using complement fixation test.

### Status of bovine *brucellosis* in Ethiopia

Various investigations have indicated that cattle *brucellosis* is common disease in Ethiopia [19]. These investigations revealed that *brucellosis* is more common in pastoral and mixed production system where people live closely with cattle and so, at higher probability to acquire the *brucellosis*. *Brucellosis* has been evaluated serologically in different areas of the country. Higher seroprevalence of *brucellosis* was reported in intensive farming than in extensive cattle rearing systems [20]. The highest *Brucella* seroprevalence (50%) was recorded in Borena zone. In addition, seroprevalence of 0.77% was reported in Jimma zone and prevalence of 0.2% and 1.7% was reported in extensive production system. Another investigation carried out in southern and eastern part of the country indicated that 3.5% of cattle were positive for *Brucella* antibody.

### Conclusion

*Brucellosis* is a worldwide distribution that causes the most important public wellbeing problem in *developing* country. It is the major dairy improvement problem, public health problem and economically important disease in Ethiopia. *Brucellosis* is higher in pastoral and mixed cattle rearing systems wherever human closely live with animals and so, are at higher probability to have *brucellosis*. Human is infected one there is contact with animals at abortion, parturition, or post parturition from splashing of infected droplets into the eye or drinking unpasteurized milk or milk product. Work-related exposure is observed in people who interaction with diseased cattle or their tissue. This disease is the most important cause of reproductive inefficient and cattle abortion. The occurrence of *brucellosis* is affected by factors related with production system, host and environmental factors. *Brucella abortus* can be eradicated by isolated diseased cattle, given vaccine for young female cattle and test-slaughter methods. As a result, it is critical for conducting applicable control methods and increasing the community awareness on zoonotic transmission of *brucellosis* are suggested. In addition, further study should be carried out on epidemiology of the *brucellosis* in higher risky groups in Ethiopia.

### References

1. Adone R, Pasquali P (2013) Epidemiological surveillance of brucellosis. *Rev Sci Tech* 32: 199-205.
2. Adugna KE, Agga GE, Zewde G (2013) Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. *Rev Sci Tech* 32: 765-773.
3. Al Dahouk S, Nockler K, Scholz HC (2006) Immunoproteomic characterization of *Brucella abortus* 1119-3 preparations used for the serodiagnosis of *Brucella* infections. *J Immunol Methods* 309: 34-47.
4. Alemu F, Admasu P, Feyera T, Niguse A (2014) Seroprevalence of Bovine Brucellosis in Eastern Showa, Ethiopia. *Acad J Anim Dis* 3: 27-32.
5. Asfaw M, Ameni G, Kassa T, Tuli G, Arenas A, et al. (2016) Seropositivity and risk factors for *Brucella* in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. *Afr J Microbiol Res* 10: 203-213.
6. Asgedom H, Damena D, Duguma R (2016) Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia. *Springer plus* 5: 1-8.
7. Ashagrie T, Deneke Y, Tolosa T (2011) Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. *Afr J Microbiol Res* 5: 1682-1685.
8. Asmare K, Asfaw Y, Gelaye E, Ayelet G (2010) Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. *Afr J Agr Res* 5: 257-263.
9. Bashahun GD, George WN, Benti DG (2015) Seroprevalence and risk factors for brucellosis in cattle in selected districts of Jimma zone, Ethiopia. *Trop Anim Health Product* 47: 1-7.
10. Bekele M, Mohammed H, Tefera M, Tolosa T (2011) Small ruminant brucellosis and community perception in Jijiga district, Somali Regional State, eastern Ethiopia. *Trop Ani Heal and Prod* 43: 893-898.
11. Berhe G, Belihu K, Asfaw Y (2007) Seroepidemiological investigation of Bovine Brucellosis in the extensive cattle production system of Tigray region of Ethiopia. *Int J Appl Res Vet Med* 5: 65-71.
12. Beruktayet W, Mersha C (2016) Review of cattle Brucellosis in Ethiopia. *Acad J Anim Dis* 5: 28-39.
13. Bikas C, Jelastopulu E, Leotsinidis M, Kondakis X (2003) Epidemiology of human brucellosis in a rural area of northwestern Peloponnese in Greece. *Eur J Epidemiol* 18: 267-274.
14. Borba MR, Stevenson MA, Goncalves VS, Neto JF, Ferreira F, et al (2013) Prevalence and riskmapping of bovine brucellosis in Maranhao State, Brazil. *Prevent Vet Med* 110: 169-176.
15. Bwala DG, McCrindle C, Fasina OF, Ljagbone L (2015) Abattoir characteristics and sero-prevalence of bovine brucellosis in cattle slaughtered at Bodija Municipal Abattoir, Ibadan, Nigeria. *J Vet Med Anim Health* 7: 164-168.
16. Degefa T, Duressa A, Duguma R (2011) Brucellosis and some reproductive problems of indigenous Arsi cattle in selected Arsi zones of Oromia Regional State, Ethiopia. *Global Vet* 7: 45-53.
17. Delrue RM, Lestrade P, Tibor A, Letesson JJ, Bolle XD (2004) *Brucella* pathogenesis, genes identified from random scale screens. *FEMS Microbiol Letters* 231: 1-12.
18. Deselegn TB, Gangwar SK (2011) Seroprevalence study of bovine brucellosis in Assela government dairy farm of Oromia Regional State, Ethiopia. *Int J Sci Nat* 2: 692-697.
19. Dinka H, Chala R (2009) Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. *American-Eurasian Journal of Agricultural and Environmental Sciences* 6: 508-512.
20. Etman RH, Barsoum SA, Ibrahim IG, El Ashmawy WR, Abou-Gazia KA (2014) Evaluation of efficacy of some serological tests used for diagnosis of brucellosis in cattle in Egypt using latent class analysis. *Sokoto J Vet Sci* 12: 1-7.