



## **Review on Bovine Brucellosis in Ethiopia.**

**Tizazu Tamirat Ajabo**

Wolaita Zone, Boloso Sore Woreda Agricultural Development Office, Areka, Ethiopia.

### **Abstract**

Brucellosis is economically important zoonotic bacterial disease caused by genus *Brucella*. It contains different species such as *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotome*, *B. microti* that affect terrestrial animals and *B. ceti* and *B. pinnipedialis* affect marine mammals. The first three species are called classical *Brucella*. Three of them are differentiated into biovars. *Brucella* have no classic virulence genes encoding capsules, plasmids, pili or exotoxins contributing to the persistence in the host and multiplication within phagocytic cell. Brucellosis occurs worldwide, except a few countries that have been successfully eradicated. The aborted fetus, fetal membrane, and uterine discharges are considered as the major source of infection. Brucellosis is mainly transmitted to animals by ingestion of contaminated feed and water, by contact with infected aborted fetus, fetal membrane and genital discharges, and by artificial insemination from infected bulls. The bacteria are preferentially localized mainly in the reproductive tract of pregnant animals and consequently cause abortion (late abortion), retained fetal membrane and infertility, where as orchitis and epididymitis are seen in males. Among the serological tests, RBPT for screening and CFT for confirmatory are routinely used in Ethiopia. Brucellosis remains one of the most common zoonotic diseases worldwide with more than 50,000 human cases reported annually. It is mainly transmitted to humans through the consumption of unpasteurized dairy products and direct contact with infected animal parts. The disease also causes huge economic losses which arises from abortion culling of infected animal, hindering animal export trades of a country, treatment costs, time and costs allotted for research, and eradication programs. Formulating effective control strategies are needed that includes surveillance to identify infected animals, prevention of transmission to non infected animals and removal of the reservoir to eliminate the source of infection. In addition, vaccination of susceptible animals is also important in areas where high prevalence of brucellosis exists. In conclusions, Brucellosis has been widely reported from cattle as well as human cases in Ethiopia. This requires formulating effective control strategies are needed that includes surveillance to identify infected animals, prevention of transmission to non-infected animals and removal of the reservoir to eliminate the source of infection.

**Keywords:** Bovine, Brucellosis, Ethiopia, Occurrence, Public Health.

## Introduction

Brucellosis is one of the oldest zoonotic diseases which remain of economic and public health significance, today with major outcomes of reproductive losses in livestock and debilitating illnesses in humans [1]. The Bovine Brucellosis, usually caused by *Brucella abortus* and occasionally by *Brucella melitensis* and *Brucella suis*, is characterized by late term abortion, infertility as a result of retained placenta and secondary endometritis, and reduced milk production with the excretion of the organisms in uterine discharges and milk. The calves may die soon after birth. In fully susceptible herds, abortion rates may vary from 30- 80% [2].

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange Free State of South Africa in the year 1915, Chukwu [3]. However, the epidemiology of the disease in livestock and humans as well as appropriate preventive measures are still not well understood and such information is inadequate particularly in sub-Saharan Africa. The surveillance and control of brucellosis in this region is rarely implemented outside South Africa [4].

In Ethiopia, there is no documented information on how and when brucellosis was introduced and established. Even though, several serological surveys have showed bovine brucellosis is an endemic and widespread disease in urban, per-urban, highland and lowland, extensive and intensive farming, smallholder farms and ranches of the country [5]. So many studies carried on cattle brucellosis in central and northern Ethiopia did not provide an adequate epidemiological picture of the disease in different agro-ecological zones and livestock production systems of the country [6].

Brucellosis is a most serious disease that leads to considerable morbidity [6]. Also, it was characterized by abortion in females and epididymitis and orchitis in males [7]. So, brucellosis can cause significant loss of productivity through abortion, still birth, low herd

fertility and comparatively low milk production. In addition, it poses a barrier to export and import of animals constraining livestock trade and is an impediment to free animal movement and animal products, and can seriously impair socioeconomic development of livestock owners [8].

Sources of infection included aborted fetuses, fetal membranes, vaginal discharges and milk from infected cows [9]. Primary clinical manifestations of brucellosis among livestock are related to the reproductive tract. In highly susceptible pregnant cattle, abortion after the five month of pregnancy is cardinal feature of the disease [7]. In humans, the disease is characterized by fever, sweating, anorexia, malaise, weight loss, depression, headache, and joint pains that could be confused with malaria and influenza [10]. Brucellosis is transmitted to humans mainly by direct contact with infected livestock and the consumption of unpasteurized contaminated milk and dairy products [10]. The disease presents as an acute or persistent febrile illness with a diversity of clinical manifestations in humans [11].

Currently ten *Brucella* species are recognized including the better known six classical species comprised of *B. abortus* (cattle, biovars 1-6, and 9), *B. melitensis* (goats, sheep, biovars 1-3), *B. suis* (pigs, reindeer and hares, biovars 1-5), *Brucella ovis* (sheep), *Brucella canis* (dogs) and *Brucella neotomae* (desert wood rats). More recently, new members to the genus include *Brucella ceti* and *Brucella pinnipedialis* (dolphins/porpoises and seals respectively), *Brucella microti* (voles) and *Brucella inopinata* (reservoir undetermined) were identified [12]. Among the above species, *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, and *B. ovis* are transmitted from animal to human causing undulant fever in humans [13]. Brucellosis in animals called Bang's disease, contagious abortion, and infectious abortion. In case of human, it is known as Malta fever, Mediterranean fever, and undulant fever [14]. Of these species, *B. melitensis* has the greatest risk for human infection followed by *B. suis* and *B. abortus*, however other species have been shown to be virulent for humans [14]. Bovine brucellosis is

usually caused by *B. abortus*, less frequently by *B. melitensis*, and rarely by *B. suis*.

Brucellosis in animals and humans has been reported from different localities of the country, was particularly associated with cattle in different agro-ecology and production systems [15]. The prevalence studies in animals and human were largely confined to serological surveys and commonly targeted bovine brucellosis, occasionally sheep, goats, and rarely camels. So far, attempts to identify *Brucella* species in the country were unsuccessful; the distribution and proportion of their natural hosts was also not studied exhaustively [16]. This is largely attributed to the degree of laboratory development and lack of consumables for laboratory tests [17].

Diagnosis of the disease is based on the isolation and identification of *Brucella* from the animals aborted materials, udder secretions or from tissues removed at post-mortem or patient’s serum by detection of specific antibodies using appropriate serological methods. Presumptive diagnosis can be made by assessing specific cell-mediated or serological responses to *Brucella* antigens. All *Brucella* are related to lifelong chronic animal infection, since they are found within the cells of their milk glands and reproductive system.

This review aimed to through the light on the occurrence of bovine brucellosis in Ethiopia and the approaches for its control, prevention, and to

present an overview on the public health significance of bovine brucellosis in country.

### General characteristics of brucella

#### Etiology

The *Brucella* genus is composed of 12 recognized species after isolation and identification of novel species from the mandibular lymph nodes of the red fox [18]. There are six ‘classical’ species (Table 1): *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis* and *Brucella neotomae*, and the first three of these are subdivided into biovars based on cultural and serological properties [19]. They affect many animal species, but especially of those that produce food: sheep (especially milk Producing), goats, cattle and pigs and, on a more localized scale, camels, buffaloes, yaks and reindeer [20]. Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and rarely by *B. suis*. In general, *brucella* have a predilection for both female and male reproductive organs in sexually mature animals and each *Brucella* species tends to infect a particular animal species. The target organs and tissues of *Brucella* species are placenta, mammary glands, and epididymis in animal reservoir host [21]. Persistent (lifelong) infection is a characteristic of its facultative intracellular organism, with shedding in reproductive and mammary secretions [22].

Table 1. The table below summarizes *Brucella* strains, hosts and transmission mode [1]

Strain Symptoms	Principle Host	Other Hosts	Symptoms	Transmission	Human Disease
<i>Brucella abortus</i>	Cattle	Sheep, goats, pigs, horses, dogs, humans, wild ungulates	Abortion after 5 months	Ingestion, some venereal	undulant fever-control with antibiotics
<i>Brucella melitensis</i>	Sheep, goats, buffalo	cattle, pigs, dogs, humans, camels	Later term abortion, weak young, mastitis (goats)	Ingestion	Malta fever: can be fatal in human
<i>Brucella ovis</i>	Sheep		most often effects rams, rare abortions		

<i>Brucella suis</i>	Pig	cattle, horses dogs, humans reindeer, caribou	Abortion and infertility	ingestion and venereal	extremely deadly in humans
<i>Brucella canis</i>	Dogs	Humans	abortions at 40-60 days	Venereal	mild disease in humans

Sources: FAO, 2003

### Bovine *Brucella*

#### Characteristics of *Brucella* organism

*Brucella* species are facultative intracellular, gram negative, non-spore-forming and non-capsulated, partially acid-fast cocco-bacilli that lack capsules, endospores or native plasmids. They survive freezing and thawing but most disinfectants active against gram-negative bacteria kill *Brucella*. Pasteurization effectively kills *Brucella* in milk. The bacterium is of 0.5-0.7µ in diameter and 0.6-1.5µ in length. They are oxidase, catalase and urease positive. Although *Brucella* species are described as non-motile, they carry all the genes except the chemotactic system necessary to assemble a functional flagellum [23]. The genomes of the members of *Brucella* are very similar in size and gene make up Sriranganathan *et al.* [24]. Each species within the genus of *brucella* has an average genome size of approximately 3.29Mb and consists of two circular chromosomes, those are Chromosome I, is approximately on average 2.11 Mb and Chromosome II is approximately 1.18 Mb. The G + C content of all *Brucella* genome is 57.2% for Chromosome I and 57.3% for Chromosome II [25]. The *Brucella* have no classic virulence genes encoding capsules, plasmids, pili or exotoxins and compared to other bacterial pathogens relatively little is known about the factors contributing to the persistence in the host and multiplication within phagocytic cells. Also, many aspects of interaction between *Brucella* and its host remain unclear [26].

### Epidemiology of Brucellosis

#### Geographical distribution of brucellosis disease

The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated which include Australia, Canada, Cyprus, Denmark, Finland, Netherlands, New Zealand, Norway, Sweden and the United Kingdom which has been reported as eradicated it. This is defined as the absence of any reported cases for at least five years. However, the Mediterranean Countries of Europe, Africa, Near East countries, India, Central Asia, Mexico, Central and South America are still not brucellosis free. Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority, it is under reported and official numbers constitute only a fraction of true incidence of the disease [27].

Brucellosis is endemic in many developing countries and is caused by *Brucella* species that affect man, domestic and some wild animals, and marine mammals [28]. Ethiopia located in Eastern Africa, the country has diverse agro ecological zones, which have contributed to the evolution of different agricultural production systems. Animal husbandry forms an integral part of agricultural production in almost all ecological zones of the country [28]. In Ethiopia, brucellosis is endemic and the disease is highly susceptible more in cattle than in camels and small ruminants in pastoral and agro-pastoral areas. The highest prevalence is noticed in dairy cattle. It is more prevalent in developing countries and considered to be a serious health problem due to lack of effective public health measures, domestic animal health programs, and appropriate diagnostic facilities. Furthermore, the situation is also worsened by the resemblance of the disease with other diseases leading to misdiagnosis and under reporting [29].

The management systems as well as ecological conditions greatly influence the spread of *brucella* infection [30]. Ethiopia has several institutionally owned commercial dairy farms, mostly situated in and around Addis Ababa and in some regional towns. These farms have been the focus of most

of *Brucella* surveys, potentially producing a bias in reported findings. These prevalence reports below have been systematically reviewed as semi-intensive and extensive management systems of various regions in Ethiopia.

**Table 2. Sero-prevalence of bovine brucellosis in Ethiopia in different geographical areas under different production systems.**

<i>Study areas</i>	<b>N. animal tested (Prevalence)</b>	<b>Type of test</b>	<b>Authors</b>	<b>System</b>
<b>Jimma zone</b>	1,813 (0.61)	RBPT, SAT	[31]	Extensive & intensive
<b>Tigray</b>	1,951 (1.49)	RBPT, SAT	[32]	Extensive & intensive
<b>Bahr Dar</b>	1,944 (4.63)	RBPT, SAT	[33]	Extensive & intensive
<b>Cent. Oromia</b>	1,238(2.99)	RBPT, SAT	[34]	Extensive & intensive
<b>AA &amp;Suluta</b>	1,501 (1.3)	RBPT, SAT	[35]	Extensive & intensive
<b>Tigray</b>	1,968 (4.9)	RBPT, SAT	[30]	Semi-intensive & extensive
<b>East Shewa</b>	1,106 (11.5)	RBPT	[5]	Pastoral & agro-pastoral
<b>Sidama zone</b>	1,627 (1.66)	RBPT, SAT	[36]	Extensive
<b>Jijjiga</b>	435 (1.38)	RBPT, SAT	[37]	Agro-pastorals
<b>South &amp;East Eth</b>	1,623 (3.5)	RBPT, SAT	[38]	Extensive

Remark: AA (Addis Ababa), Eth (Ethiopia), N (number)

In general, at the country level brucellosis prevalence studies have been conducted in different localities of the country (Table 2). But there is little information on specific transmission dynamics within different agro-ecology in the country. Since prevalence studies in animals and human were largely confined to serological surveys and commonly targeted bovine

brucellosis, occasionally sheep and goats and rarely camels. Also attempts to identify *Brucella* species in the country were unsuccessful, the distribution and proportion of their natural hosts were also not studied exhaustively [16]. This is largely attributed to the degree of laboratory development and lack of consumables for laboratory tests [17].

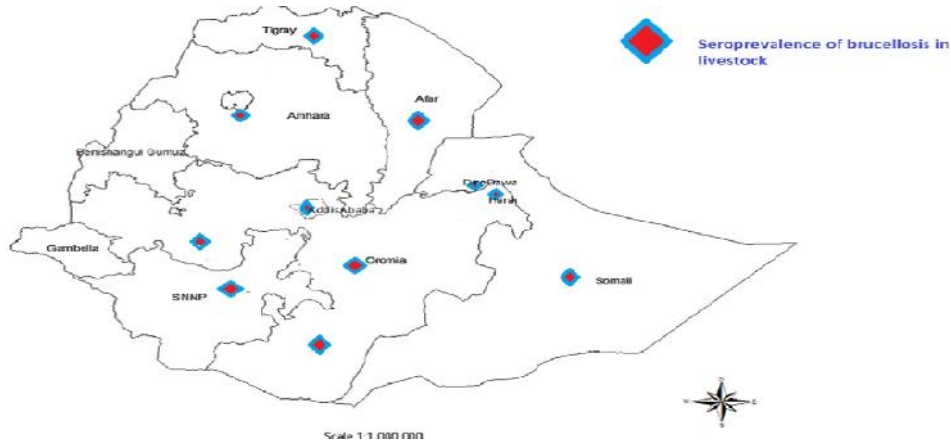


Fig1. Geographical location for studied report on the brucellosis in Ethiopia (Adopted from [38]).

**Status of Brucellosis in Ethiopia**

Ethiopia, located in Eastern Africa, is predominantly an agrarian country with over 85% of its population engaged in agricultural activity. Since the first report of brucellosis in the 1970s in Ethiopia, the disease has been noted as one of the important livestock diseases in the country [39,40]. A large number of studies on bovine have been reporting individual brucellosis seroprevalence ranging from 1.1% to 22.6% in intensive livestock management systems [40] and 0.05% -15.2% in extensive (Table 4) management systems [6,37]. Both husbandry systems as well as environmental conditions greatly influence the spread of *Brucella* infection [10]. Most brucellosis study report for highland agro-ecology was concentrated at urban and pre urban dairy farms. According to different authors herd level seroprevalence ranged between 2.9% and 45.9%.

Over half of the cattle are farmed under extensive lowland pastoralist and agro-pastoralist production system, *brucella* sero-prevalence within extensive cattle rearing systems (Table 4) is lower than that of intensive systems (Table 3). The highest sero-prevalence (50%) was documented using ELISA in Dedituyura Ranch [42], 2.91% in indigenous Borena breed cows in Borena zone in Southern Ethiopia [43]. In South Eastern Ethiopian pastoral zones of the Somali and Oromia regional state herds, sero-prevalence per species which were 1.4% were reported [17]. The same study in the area showed that anti-*Brucella* antibodies were prevalent in 10.6% [6]. In general, accordingly to region-based meta-analysis, forest plot revealed the highest prevalence in central Ethiopia followed by the southern part Figure 1). The lowest prevalence estimate was observed in the western part of the country [44]. The prevalence of disease in country ranged from 15% [41] to 12% [40].

Table 3. List of Prevalence of Bovine brucellosis in intensive and semi-intensive management systems in Ethiopia

Authors	prevalence	Management system	Diagnostic test
[36]	2.5	Semi-Intensive and Intensive	RBPT, CFT
[38]	1.9	Semi-Intensive and Intensive	RBPT, CFT
[45]	10	Semi-Intensive and Intensive	RBPT, CFT
[33]	3.4	Semi-Intensive and Intensive	RBPT, CFT
[30]	7.7	Semi-Intensive	RBPT, CFT
[39]	1.9	Semi-Intensive	RBPT, CFT
[34]	4.5	Semi-Intensive	RBPT, CFT
[40]	12.4	Semi-Intensive	RBPT, CFT
[41]	1.5	Intensive	RBPT, CFT
[16]	3.6	Semi-Intensive	RBPT, CFT

Table 4. List of the studies of *Brucella* sero-prevalence in the extensive management system in Ethiopia.

<i>Authors</i>	<i>Prevalence</i>	<i>Management system</i>	<i>Diagnostic test</i>
[36]	1.7	Extensive	RBPT, CFT
[32]	3.2	Extensive	RBPT, CFT
[37]	0.5	Extensive	RBPT, CFT
[5]	11.2	Extensive	RBPT
[17]	1.4	Extensive	RBPT, CFT
[30]	1.2	Extensive	RBPT, CFT
[39]	3.6	Extensive	RBPT, CFT
[34]	2.2	Extensive	RBPT, CFT
[40]	9.7	Extensive	RBPT, CFT
[6]	10.6	Extensive	RBPT, CFT
[31]	0.8	Extensive	RBPT, CFT
[16]	1.7	Extensive	RBPT, CFT

**Associated Risk Factor for Animal Brucellosis**

The risk factors can be categorized into those associated with characteristics of animal populations, management and the parasite biology [46].

**Risk factors associated *Brucella* spp (Agent)**

***Brucella* spp:** *B abortus* is an important risk for the maintenance of the agent in the animal population with special importance in areas where wildlife and cattle rearing occur together. Moreover, infections in wildlife can hinder eradication efforts in cattle. *B. abortus* is still a human pathogen and outbreaks associated from infected cattle and also from ingesting contaminated dairy products represent an important risk of infection [47]. *B. melitensis* is the main etiologic agent of brucellosis in small ruminants, although sheep can be also infected by *B. ovis*. Sporadic cases of brucellosis have been described in sheep and goats as *B. abortus* and *B. suis*. The dogs that guard the herds and flocks can also be infected [46].

**Risk factors associated with host (animals)**

Different *Brucella* species can affect the same livestock species and human. The principal strain that infects cattle is *B. abortus*, but also become transiently infected by *B. suis* and more commonly by *B. melitensis* when they share pasture or facilities with infected pigs, goats and sheep. *B. melitensis* and *B. suis* can be transmitted by cow’s milk and cause a serious public health threat [48]. The main etiologic agent of brucellosis in goats is *B. melitensis*. However, in certain countries like Brazil where there is no *B. melitensis*, goats can get infected with *B. abortus* [49].

**Age:**

It has been referred to as one of the intrinsic factors associated with brucellosis. Higher sero-prevalence of brucellosis has been observed in older animals. Brucellosis has traditionally been considered as a disease of adult animals since susceptibility increases after sexual maturity and pregnancy. However, variations in the age of sexual maturity among breeds could present differences between age and brucellosis positivity [6].

### **Sex:**

Female ruminants presented a higher odd of brucellosis infection, the same has been observed in female dogs compared to male dogs. It could be associated with the intrinsic biology of the microorganisms and its tropism to the fetal tissue. Since brucellosis infection in males presented clinical signs such as epididymitis and orchitis, the prevalence in males could be lower than females because they may be culled faster. On the other hand, the absence of clinical signs such as abortion or metritis in non-pregnant infected females or the absence of observation/identification/ of abortions in extensive herds may also explain the higher prevalence in females [16].

### **Herd size:**

Herd size is another risk factor that affects occurrence of brucellosis. In Amhara region, Mussie *et al.* [50] observed significant differences of *Brucella* among three herd size categories in the semi-intensive production system whereas the difference was not statistically significant in the extensive production system. Their findings revealed comparatively higher sero-positivity in the larger herd categories than those herds with less than 5 cattle. A separate study in Addis Ababa area by Asfaw *et al.* [51] also found significant association between *Brucella* infection and herd size. Kassahun *et al.* [52] also reported that in both extensive and intensive systems, infection rates increased with herd size, but these differences failed to achieve statistical significance. On the other hand, Tolosa *et al.* [31] reported highly significant variation ( $p < 0.001$ ) between herds having 1 to 5 cattle and those with  $>5$  cattle.

### **Breed:**

The prevalence of brucellosis in farm animals seems to be lower in small ruminants than large ruminants and lower in sheep than in goats. In most of the circumstances, the main route of spread is the placenta, fetal fluids and vaginal discharges expelled after delivery or abortion. At

that time, large numbers of *Brucella* are released [53]. The vaginal excretion of *Brucella* spp. in goats is greater and more prolonged than sheep, lasting for 2-3 months. In sheep, it is generally lower and normally ceases within 3 weeks after birth or abortion. The excretion of *Brucella* in milk is generally intermittent and usually only appears 6 to 12 days after the abortion. In goats, the excretion is more abundant and more prolonged, so there is an increased risk of infection via the consumption of milk from this species [16].

### **Others risk factors**

#### **Managemental risk factors:**

The spread of the *brucella* pathogens disease are transmitted from one herd to the other and from one area to another is almost always due to the movement of an infected animal from infected herd in to a non-infected susceptible herd. Once infected, the time required to become free of brucellosis was increased by large herd size, active abortion and by loss housing, Radostits *et al.* [7].

#### **Intensive systems:**

Higher individual bovine brucellosis sero-prevalence has been recorded in intensively managed cattle herds as compared to those in the extensive management system. According to same authors, the reasons for the high prevalence of bovine brucellosis in same study areas were explained by low hygienic practices, no use of maternity pen and/or separation of cows during parturition, low cleaning and disinfection activities, low culling of infected animals. An overall sero-prevalence of 1.1% -22.6% was also recorded from many Parts of Ethiopia [47].

#### **Extensive systems:**

In Ethiopia, 95% of cattle are farmed under extensive systems. Accordingly the available of *Brucella* prevalence within extensive cattle rearing systems is lower than that of intensive systems. Tolosa *et al.* [31] reported overall individual animal prevalence and herd prevalence



of 0.77 and 2.9%, respectively in Jimma Zone. Recent reports from North West, Tigray region Haileselassie *et al.* [30] and Southern Sidama Zone Asmare *et al.* [36], recorded, an overall prevalence of 1.2 and 1.66% following screening 848 and 1627 cattle from extensive system, respectively. The overall prevalence of 0.05% - 15.2% in extensive management systems was recorded [6].

### Agro-ecological factors:

Few comparative studies have been under taken to show the status of bovine brucellosis in different agro-climatic areas of this country. For example, Mussie *et al.* [50] reported higher sero-prevalence in the midland areas (with individual rates of 5.61% compared with 22.4% at herd level), than highland areas (with individual rates of 1.97% and 6.33% at herd level respectively) within the Amhara Regional State. A possible explanation could be a consequence if higher stocking density in the midland area compared with the highland regions.

### Production system:

In infected cattle populations, brucellosis is might lead to a lower calving rate due to temporary infertility and/or abortion, resulting in decreased milk production, increased replacement costs, as well as lowered sale value of infected cows. General economic losses, however, go far beyond the financial losses suffered by cattle producers alone. Not only cattle but also other species might be affected by brucellosis, including humans Scholz *et al.* [13].

### Source of infection and mode of transmission in animals

In animals, the concentration of the bacteria is highest in pregnant uterus. The aborted fetus, placental membranes or fluids, and other uterine discharges were considered as major source of infection. Infected animals also shade organisms in milk which serve as source of infection for the new born. Contaminated feed can spread the infection from infected pasture over long distance

during purchasing and selling activities. The disease is transmitted to susceptible animals by ingestion of contaminated feed and water, contact with aborted fetuses, fetal membrane and uterine discharges; infection by inhalation is also possible. The use of infected bull for artificial insemination also poses an important risk and spreads the infection to many herds [54].

### Pathogenesis

The ability of *Brucella* spp. to cause disease requires a few critical steps during infection. *Brucella* spp. can invade epithelial cells of the host, allowing infection through mucosal surfaces: M- cells in the intestine have been identified as a portal of entry for *Brucella* spp. Once *Brucella* spp. has invaded, usually through the digestive or respiratory tract, they are capable of surviving intra cellular within phagocytic or non-phagocytic host cells. Then replicate within the phagocyte, release to circulation and colonization of the bacteria in multiple tissues, like lymphoid tissues, mammary gland and reproductive tract [55].

Invading *Brucella* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo-endothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape of *Brucella* from the lymph nodes in to the blood. During bacteremic phase, bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supra-mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. In bulls, the predilection sites for infection are also the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contains large number of *Brucella* but as the infection becomes chronic, the number of *Brucella* excreted decreases. However, it may also continue to be excreted for years or just become intermittent [54].

### Clinical signs

Brucellosis is a sub-acute or chronic disease which may affect many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male. According to WHO [10] *B. melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus*, and *B. suis* on those endemic regions. Although *B. abortus* is mainly associated with cattle, occasionally other species of animals such as sheep, swine, dogs and horses may be infected. In horses, *B. abortus* together with *Actinomyces bovis* may be present in poll evil and fistulous withers [56]. The mammary gland and regional lymph nodes can also be infected and bacteria can be excreted in milk [56]

### Diagnosis:

Diagnosis of brucellosis is the corner stone of any control and eradication program of the disease. Especially in humans due to its heterogeneous and poorly specific clinical symptoms, the diagnosis of brucellosis always requires laboratory confirmation. It is made possible by direct demonstration of the causal organism using staining, immunofluorescent antibody, culture, and directly demonstration of antibodies using serological techniques [21,57]. In cases of animal brucellosis diagnosis by cultural examination, the choice of samples usually depends on the clinical signs observed. The most valuable samples include vaginal secretions (swabs), aborted fetuses (stomach contents, spleen and lung), fetal membranes, and milk, semen and arthritis or hygroma fluids. From animal carcasses, the preferred tissues for culture are those of the reticulo-endothelial system (i.e. head, mammary and genital lymph nodes and spleen), the pregnant or early post-parturient uterus, and the udder. Growth normally appears after 3-4 days, but cultures should not be discarded as negative until 7-10 days have elapsed [58].

### Rose Bengal Plate Test (RBPT):

Often used as a rapid screening test; the sensitivity is very high (>99%) but the specificity is disappointingly as low as 68.8%. RBPT is a rapid, slide-type agglutination assay performed on serum. The general principle of this test is the agglutination of serum antibodies with Rose Bengal dye-stained *B. abortus* whole cells buffered at a pH of 3.65 to inhibit nonspecific agglutinins. Due to its simplicity and low cost, it is the most common test used for brucellosis screening purposes, especially in laboratories with limited resources. However, this is of value as a screening test in rural areas where it is not always possible to perform the other tests [59].

### Complement Fixation Test (CFT):

This test detects specific antibodies of the IgM and IgG1 type that fix complement. The CFT is highly specific but it is laborious and requires highly trained personnel as well as suitable laboratory facilities that makes less suitable for use in developing countries. Although it specifies is very important for the control and eradication of brucellosis, it may test false negative when antibodies of the IgG2 type hinder complement fixation. The CFT measures more antibodies of the IgG1 than antibodies of the IgM type, since it usually appears after antibodies of the IgM type, control and surveillance for brucellosis is best done by CFT [58].

### Public health and significant importance of *brucella*

Brucellosis (especially *B. melitensis*), remains one of the most common zoonotic diseases of worldwide with more than 50,000 human cases reported annually [60]. The significance of brucellosis as zoonotic has ever increased in recent times, due to the expansion of international commerce in animals and animal products, with increase urbanization, intensive farms and animal products, having nomadic animal husbandry [61]. Despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries due to various

sanitary, socioeconomic, and political factors [62]. As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection [63].

A study conducted in traditional pastoral communities by Ragassa *et al.* [64] using *B. abortus* antigen revealed that 34.1% patients with febrile illness from Borena, 29.4% patients from Hammer, and 3% patients from Metema areas were tested positive using *Brucella* IgM/IgG lateral flow assay. Studies conducted in high risk group such as farmers, veterinary professionals, meat inspectors and artificial insemination technicians in Amhara Regional State [50], Sidama Zone of Southern People Nations and Nationalities State [36]. In Addis Ababa, a seroprevalence of 5.30%, 3.78% and 4.8% by screening sera from 238, 38 and 336 individuals respectively were found [65]. The discrepancy between and others might be due to difference in milk consumption habits and sensitivity of test methods used [64].

Humans may become infected by ingestion of unpasteurized cheese or milk, by direct transmission through contact with infected animals or by handling specimens containing *Brucella* spp. in laboratory. It also transmitted to human by the consumption of raw dairy products and by direct contact with the skin or mucosa during parturition and abortion. Cattle are natural hosts for *Brucella abortus*, and sheep (*Ovis aries*) and goats (*Capra hircus*) for *B. melitensis* and *B. ovis*. Humans are susceptible to both *B. abortus* and *B. melitensis*, the latter being most frequently reported in humans [20].

Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be very diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis. Spontaneous abortions, mostly in the first and second trimesters of pregnancy, are seen in pregnant women infected with *Brucella* [66]. Symptoms and signs of brucellosis usually referred as fever of unknown

origin can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors [67]. Because of these rather non-specific signs, brucellosis is constantly mis-diagnosed as malaria, which is very prevalent in sub-Saharan Africa [68].

### Human Brucellosis

The true incidence of brucellosis in human and animals worldwide is obscure and the occurrence is expanding in low- and middle-income nations like Ethiopia. The bacterial pathogen is considered by US Centers for Disease Control and Prevention (CDC) as a category (B) pathogen that has potential for improvement as a bio-terrorism weapon with a capability of airborne transmission [69]. The incidence of human brucellosis is correlated with the level of incidence in domestic animals [1]. Human cases occur after ingesting raw milk and milk products and coming into close contact with infected animals. Human brucellosis can be a very debilitating disease, although the case fatality rate is generally low [1]. Brucellosis primarily affects livestock, but can be transmitted to humans (Figure 2) by ingestion, close contact, inhalation or accidental inoculation. 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 species of the causative agent and local methods of food processing [70]. In Ethiopia according to Regassa *et al.* [64] the major risks for brucellosis in the pastoral community are living in close proximity of livestock, milking and consuming raw milk and fresh dairy product.

As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection. For instance, (3.6%) were reported to be positive for *B. abortus* antibodies by RBPT and CFT [31]. A study conducted in traditional pastoral communities by Regassa *et al.* [64] using *B. abortus* antigen revealed that 34.1% patients with febrile illness from Borena, 29.4% patients

from Hammer and 3% patients from Metema areas were tested positive using *Brucella* IgM/IgG. The sero-prevalence studies conducted in high risk group such as farmers, veterinary professionals, meat inspectors and artificial

insemination technicians were reported 5.30% by Mussie *et al* [50], 3.78% and 4.8% by Kassahun *et al.*[52] and Asmare *et al.*[36] in different region of Ethiopia from individuals humans (Tale 50).

Table 5. Summary of humans tested for brucellosis in Ethiopia and its prevalence

<i>Study Area</i>	<i>Prevalence</i>	<i>Reference</i>
Hawassa	3.78	[52]
Addis Ababa	4.8	[36]
Borena	34.1	[64]
Amhara region	5.3	[5]
South Gonder	3.0	[64]
Yabello oromia	10.0	[16]
Hammer	29.4	[64]
Jimma zone	2.1	[71]

Source: Robinson, A., 2003

### Sources of infection and Mode of transmission in humans

The reservoirs of *Brucella* species comprise cattle, goats, sheep and some wildlife [72]. The disease is transmitted to man mainly by direct contact with infected animals or indirect contact and through consumption of raw or uncooked animal products [22]. Usually the main source of brucellosis for urban populations is ingestion of fresh milk or dairy products prepared from unheated milk. Cow, sheep, goat or camel milk contaminated with *Brucella melitensis* is particularly hazardous as it is drunk in fairly large

volume and may contain large numbers of organisms [20].

Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories. However, consumption of hard cheese, yogurt, and sour milk are less hazardous, since both propionic and lactic fermentation takes place. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver and spleen has been implicated in human infection [7].

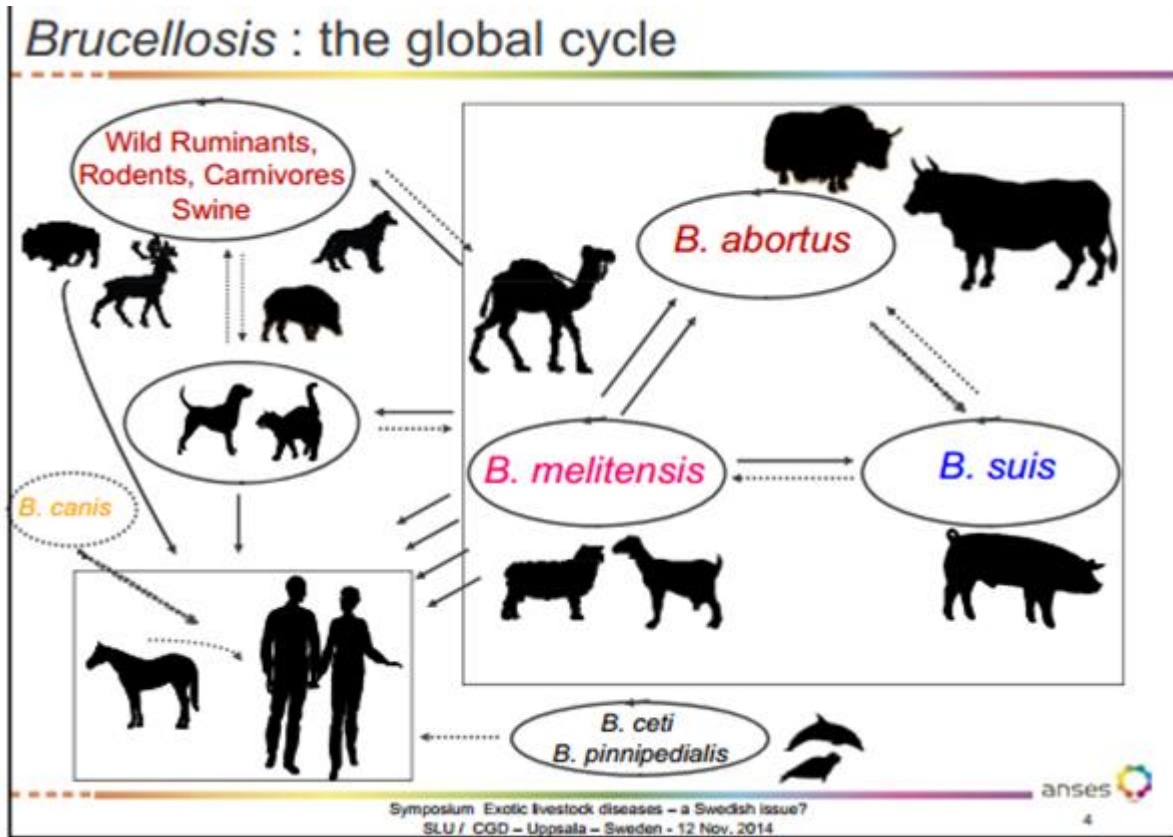


Fig 1. Transmission mode of brucellosis within its range, Garin-Bastuji, B., 2014.

### Treatment, prevention and control

Due to the intracellular localization of *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage [26], treatment of domestic animals with antibiotics is not usually successful. Even though, treatment failure and relapse rates are also high in humans, treatment depend on the drug combination of doxycycline with streptomycin which is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis [74]. A combination of doxycycline treatment (6 weeks duration) with parentally administered gentamicin (5 mg/kg) for 7 days is also considered an acceptable alternate regimen [75]. The initial aim of surveillance and control programs is the reduction of infection in the animal populations to reduce the effect of the disease on animal health and production, thus

minimizing its impact on human health.

An effective control of animal brucellosis requires the following elements:

- 1) Regular schedules of surveillance to identify infected animal that may causes herds infections,
- 2) Prevention of transmission or spreads of infection to non-infected animal herds
- 3) Eradication of the reservoir to eliminate the sources of infection in order to protect vulnerable animals or herds coupled with measures to prevent re-introduction of the disease [60]. In areas where a brucellosis free status has been established or where such a status is assumed from epidemiological data, the risk of importing the disease by means of animal movement must be protected. Movement of infected animals must be prohibited and import permissions should be given only to certified brucellosis-free farms or areas.

This is also true for national and international transport of animal products, in accordance with the general principles and procedures specified in the International Zoo-Sanitary Code of the OIE. This code also describes the testing procedures for animals and quarantine measures [46].

### **Suggested prevention and control strategies for livestock Brucellosis in Ethiopia**

As the source of human brucellosis is direct or indirect exposure to infected animals or their products. Prevention must focus on various strategies that will mitigate infection risk. To our knowledge, there has been no national program proposed for prevention and control of brucellosis in Ethiopia. Similarly at regional levels, no strategy is in place to control brucellosis. This is largely a result of lack of facilities and budget to run such a program. Moreover, many responsible bodies may not recognize the significance of brucellosis given the contradictory and sometimes low prevalence data. However, at this time, it is crucial to define geographical extent of the problem and then allocate resources and funds to initiate prevention and control strategies in this country [76].

### **Conclusion and Recommendations**

Brucellosis remains one of the most common livestock and zoonotic diseases worldwide except in those countries where bovine brucellosis has been eradicated. In developing countries brucellosis appears to be more endemic especially in sub-Saharan countries including Ethiopia and its prevalence is increasing due to sanitary, socio-economic and political factors. Existence of brucellosis in a population is detected by identification and isolation on culture, serological tests and PCR based molecular tests although each has limitations. Brucellosis is responsible for abortion, retained fetal membrane, endometritis, orchitis, epididymitis in animals and undulating fever in humans. The worldwide economic losses due to brucellosis are extensive not only in animal production but also in human health, but surveillance and control measures are not

instituted adequately. Even though the disease is prevalent in Ethiopia, few reports in human are available. This may be due to absence of appropriate diagnostic facilities. Based on the above concluding remarks, the following recommendations are forwarded:

- ✓ In order to reduce the economic losses and public health impact of the brucellosis disease, control and eradication of disease of animals should be prepared or designed at the national and regional level.
- ✓ To convince the decision makers, prevalence, distribution and public health impact of the disease should be further studied and well documented.
- ✓ Suitable laboratories for study of the disease have to be established at national and regional level.
- ✓ Public education on the transmission and source of infection of the disease as well as control and prevention method should be taught or awareness creation should be applied.
- ✓ For both human and animal brucellosis, extension services should include emphasis on addressing the impacts of risk factors for the occurrence of brucellosis.
- ✓ Avoid eating or drinking unpasteurized milk, cheese, or ice cream.
- ✓ The necessary precautions should be taken to reduce occupational risks.
- ✓ Aware people to use Pasteurized milk widely practiced to prevent human infections.
- ✓ Eradication of the reservoir to eliminate the sources of infection in order to protect vulnerable animals or herds coupled with measures to prevent re-introduction of the disease.

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