Bovine Brucellosis and its Public Health Significance in Ethiopia

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Abstract: Brucellosis is a contagious and economically important bacterial disease of animals worldwide and it is considered as one of the neglected zoonoses in the world. Despite being endemic in many developing countries, brucellosis remains under diagnosed and under reported. This disease is an important disease among animal and people in Africa. Bovine brucellosis is an infectious and contagious disease and is predominantly a disease of sexually mature animals which usually caused by B. abortus; occasionally by B.melitensis and B.suis.The disease poses a barrier to trade of animals, economic loss due to delayed heat, loss of calves and reduced milk production. Brucellosis is considered as one of the most widespread but neglected zoonoses in the world. B.melitensis, B.suis, B.abortusand B.canis are zoonotic pathogenic species of Brucella. Human clinical disease is characterized by severe flu-like illness, serious complications of joints (arthritis) or internal organs (heart failure). Even though a large number of studies on bovine brucellosis have been reported in different part of the country, the is no documented review on the disease. The finding of positive serological reactors did not only suggest the presence of the disease in the cattle population, but also indicated the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and humans.In conclusion the implementation of test and slaughter policy with compensation payment to the farmers as the prevalence of the disease is low in the study area.Awareness creation among farmers, butchery men, abattoir workers and animal health workers about the nature and effect of the disease through formal and informal educational channels is required.

Keywords: Abortion, Bovine Brucellosis, Human Brucellosis, Risk factors, Seroprevalence.

1. Introduction

Brucellosis is a contagious and economically important bacterial disease of animals worldwide and it is considered as one of the neglected zoonoses in the world. Despite being endemic in many developing countries (Donev *et al.*, 2010), brucellosis remains under diagnosed and under reported. This disease is an important disease among animal and people in Africa (Smits *et al.*, 2007).The genus *Brucella* consists of commonly known six classically recognized species based on antigenic/biochemical characteristics and primary host species: *B.abortus* (cattle), *B.melitensis* (sheep and goats), *B.suis* (swine, cattle, rodents, wild ungulates), *B.ovis* (sheep), *B.canis* from dogs and *B.neotomae* of rodents (Tiller *et al.*, 2010).

Bovine brucellosis is an infectious and contagious disease and is predominantly a disease of sexually mature animals which usually caused by *B.abortus*; occasionally by *B.melitensis* and *B.suis*. It is of major economic importance in most countries of the world. It affects approximately 5% of the livestock population worldwide and continues to increase in distribution. The disease poses a barrier to trade of animals and animal products, represents a public health hazard, and is an impediment to free animal movement. Economic loss due to delayed heat, loss of calves, reduced milk production, culling and economic losses from international trade bans in tropics and subtropics (WHO, 2001).

In cattle the mode of transmission is usually from animal to animal by contact following an abortion and retained placenta. Pasture or animal barn may be contaminated and the organisms are most frequently acquired by ingestion but also inhalation and conjunctival inoculation are other possibilities. The use of pooled colostrum for feeding newborn calves may also transmit infection. Sexual transmission of the disease is very low in bovine. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection (WHO, 2001).

Brucellosis is considered as one of the most widespread but neglected zoonoses in the world. It was the second most important zoonotic disease in the world after rabies (WHO, 2006). It is also most important zoonotic disease in most developing countries, which have no national brucellosis control and eradication program(Donev *et al.*,

2010). Brucellosis in human known as "undulant fever", "Mediterranean fever" or "Malta fever" and the infection is almost invariably transmitted by contact with infected animals or their products either directly or indirectly. *B.melitensis*, *B.suis*, *B.abortus*and *B.canis* are zoonotic pathogenic species of *Brucella* (WHO, 2006, OIE, 2009).

Human clinical disease is characterized by severe flu-like illness, with a high fever that comes and goes (hence the name "undulating fever"), which may progress to a more chronic form with serious complications in joints (arthritis) or internal organs (heart failure). In this chronic, recurring form, humans can be so debilitated that they are no longer able to work and they become a health care burden on their families (Pappas *et al.*, 2006).

In Ethiopia there is no documented information on how and when bovine brucellosis was introduced and established. However, in the last two decades several serological surveys have showed that it is endemic and widespread (Berhe *et al.*, 2007; Ibrahim *et al.*, 2010).The disease is prevalent in cattle in high land and lowland areas (Eshetu*et al.*, 2008; Kebede *et al.*, 2017; Edao *et al.*, 2018). Even though a large number of studies on bovine brucellosis have been reported in different part of the country,the is no documented review on the disease (Tolosa *et al.*, 2010;Megersa *et al.*, 2011).Therefore, this study was carried out with the objective to

- ✓ Review seroprevalence of brucellosis dairy cows and assess its public health importance in Ethiopia

2.1. Etiology

2.1.1. Taxonomy

The genus *Brucella* resides within the family *Brucellaceae* (familyIII) with *Mycoplana* and*Ochrobactrum*, of the order *Rhizobiales* in the class *Alphaproteobacteria* of the phylum*Proteobacteria*. *Brucella* has ten species including the better known six classical species comprised of *B.abortus, B.melitensis, B.suis, B.ovis, B.canis* and *B.neotomae*. The recently discovered species included in the genus are*B.ceti*,*B.pinnipedialis*, *B.microti* and *B.inopinata*(Godfroid *et al.*, 2005)and about 25 additional *Brucella* strains/species arebeing sequenced(Banai and Corbel, 2010).

2.1.2. Host preferences

Brucella has definite host preferences. Secondary hosts play a minor role in the maintenance and spread of a particular *Brucella* species. *B.abortus* mainly infects cattle and is the main cause of contagious abortion. However, sheep, goats, dogs, camels, buffaloes as well as feral animals may also contract *B.abortus* infections (Radostits *et al.*, 2007). The species of *Brucella* based on preferential host specificity are *B.abortus* (cattle), *B.suis* (swine), *B.canis* (dogs), *B.ovis* (sheep), *B.neotomae* (desert wood rats), *B.cetacea* (cetacean), *B.pinnipedia*(seal), *B.microti* (voles), and *B.inopinata* (unknown) (Godfroidah` *et al.*, 2005)(Table 1).

Table 1: *Brucella* species and their host preferences

Species	Zoonotic importance	Host preference
B abortus	Moderate	Cattle*, sheep, goat, pig,, horse
B.melitensis	High	Sheep*, Goat, cattle
B.suis	Moderate	Pig*
B.canis	Mild	Dog*
B.ovis	Absent	Sheep*
B.neotomae	Absent	Deseret wood rat*
B.ceti	Mild	Ceteceans*
B.Pinnipedials	Mild	Seals*
B.microt	Absent	Common Voles*
B.inopinata	Mild	Undeterminedhost*

*- Represent natural host
Source: (Godfroida *et al.*, 2005)

B.melitensis, *B.abortus*, *B.suis*, and *B.canis* are known to cause human disease. *B.neotomae* and *B.ovis* are not pathogenic to humans. The majority of human cases worldwide are attributed to *B.melitensis*. Some *Brucella* specie like *B.abortus*, *B.melitensis*, *B.suis* and *B.canis* can affect a ranges of hosts in addition to their natural hosts resulting hazards on the health of animals including humans; because of this, countries infected with the disease are challenged and have been under serious difficulties to overcome or control zoonotic brucellosis effectively(Pappas *et al.*, 2006).

2.1.3. Genome and its characteristics of Brucella organism

In 1985, it was proposed that the six *Brucella* species should be grouped as biovars of a single species based on DNA-DNA hybridization studies. The genomes sequenced from genus *Brucella* are also known to be very similar in terms of both base composition and genome size. All sequenced species have a GC content of approximately 57%, and most genomes consist of approximately 3.3Mbp divided on two chromosomes. There were no any plasmid reported in sequenced members of the genus*Brucella*. The first *Brucella* species to be sequenced was *B.melitensis* 16M (biovar 1) followed closely by *B.suis*(biovar 1). Analysis of 16S rRNA sequences places *Brucella* species as members of the alpha-2 *Proteobacteria*(Bohlin *et al.*, 2010).

Pulsed-field gel electrophoresis (PFGE) maps of the classical *Brucella* species genomes are composed of two circular chromosomes of approximately 2.1 and 1.2Mbp, with the exception of *B.suis*biovar 3, which has a single chromosome of 3.1Mbp. PFGE studies revealed other differences, including a 640-kb inversion in the small chromosome of *B.abortus*and a deletion in the small chromosome of *B.ovis*. The two chromosomes of *Brucella* differ in important ways. The origin of replication of the large chromosome (Chr I) is typical of bacterial chromosomes, while that of the small chromosome (Chr II) is plasmid like. Further, most of the essential genes are located on Chr I. The GC content of the two chromosomes is nearly identical, consistent with the assertion that the assimilation and stabilization of a plasmid was an ancient event in *Brucella* (Paulsen *et al.*, 2002).

The genome sequences of *B.melitensis*and *B.suis*have been determined. Comparative analyses revealed both that the two genomes are extremely similar and that they have many similarities to both bacterial plant and animal pathogens and symbionts (Halling *et al.*, 2005). The sequence identity for most open reading frames (ORFs) was 99% or higher. Nevertheless, unique fragments were reported to exist between these two genomes. Prior to sequencing the *B.abortus*genome, a large number of short sequences were available in gene bank. Many of these sequences were derived from analyses of plasmids estimated to cover 20% of the genome from a random shotgun library of *B.abortus*S2308 (Bohlin *et al.*, 2010).

The *Brucella* cell envelope is a three-layered structures namely an inner or cytoplasmic membrane, a periplasmic space and an outer membrane can be differentiated (Lapaque *et al.*, 2005). The outer cell membrane closely resembles that of other Gram-negative bacilli with a dominant lipopolysaccharide (LPS) component and three main groups of proteins. It contains lipopolysaccharide (LPS), proteins, and phospholipids. The major *Brucella* outer membrane proteins (OMPs) are group 2 porin proteins having 36-38kDa; group 3 proteins contain 25-27 kD molecular mass and a lipoprotein covalently linked to peptidoglycan(Paulsen *et al.*, 2002). Group 1 minor proteins have a molecular mass of 88 to 94 kDa(Delvecchio *et al.*, 2002).

2.1.4. Antigenic characteristics

Brucella has lipopolysaccharide (LPS) as a major component of their outer membrane and an important virulence factor like other Gram-negative bacteria. Thus, their colonial morphology is termed as either smooth or rough depending on the LPS structure. Structural variation in the LPS of smooth strains also defines the so called A and M antigens that have some significant role in typing (Godfroid*et al.*, 2010). These antigens reflect differential O-side chain which is linked to α-1, 2 in A dominant strains but with every fifth residue linked α-1, 3 in M dominant strains. The O-polysaccharide (O-PS) is involved in bacterial virulence. It contributes in complement resistance and more importantly critically modulates bacterial entry into cells so that its removal causes attenuation (Godfroid1 *et al.*, 2010).

All smooth *Brucella* cross-react with one another in agglutination tests. This cross-reaction does not occur with non-smooth or the rough *Brucella* strains. Lipopolysaccharide (LPS) comprises the major surface antigens of the corresponding colonial phase involved in agglutination. The (S-LPS) molecules carry the A and M antigens, which have different quantitative distribution among the smooth *Brucella* strains. This is of value in differentiating biovars

of the major species using absorbed monospecific A and M antisera (European Commission, 2001). Serological cross-reaction has been reported between the smooth *Brucella* and various other Gram negative bacteria like, *E. coli*(European Commission, 2001; Corbel, 2006).

2.1.5. Morphology and staining

Brucella species are slow-growing, Gram negative coccobacilli or short rods measuring from 0.6 to 1.5µm long and from 0.5 to 0.7µm wide, non-motile, non-spore forming, non-capsulated, non-flagellated, aerobic, facultative intracellular bacteria capable of invading, survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages. They are usually arranged singly, and less frequently in pairs or small groups. The morphology of *Brucella* is constant, except in old cultures where pleomorphic forms may be evident. Usually do not show bipolar staining, not truly acid-fast, but are resistant to decolorization by weak acids and thus stain red by the Stamp's modification of the Ziehl–Neelsen's method (Gorvel, 2008) (Figure 1). The presence of intracellular, weakly acid-fast organisms of *Brucella* morphology or immuno-specifically stained organisms is presumptive evidence of brucellosis. However, these methods have a low sensitivity in milk and dairy products where *Brucella* is often present in small numbers, and interpretation is frequently impeded by the presence of fat globules (OIE, 2012).

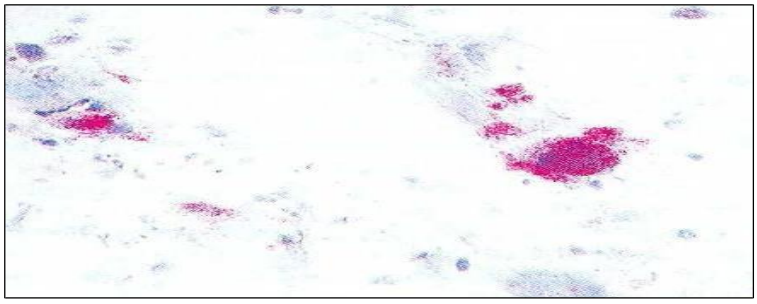

Figure 1:*B.bortus* in an MZN-stained smear of a cotyledon from a case of bovine abortion

Source:(Quinn *et al*., 2013).

2.1.6. Growth requirement and cultural characteristics

Selective media

Brucella species are slow growing and the use of enriched selective media is recommended for primary isolation from most clinical specimens because of the high numbers of over growing contaminants may inhibit the isolation. Such selective media are prepared by incorporating antibiotics and bacteriostatic dyes onto basic enriched media such as *Brucella* selective medium base. Prepared by adding six antibiotics; bacitracin, vancomycin, nalidixic acid, polymixin B, nystatin and cycloheximide onto sucrose dextrose agar for the isolation of *Brucella* species from the clinical sample (Mari´n *et al*., 1996).

These antibiotic supplements of the Farrell's medium are commonly used, in different combinations and proportions onto any one of the basal media such as *Brucella* medium base (Oxoid), Tryptone soya agar (Oxoid), Serum dextrose agar (Oxoid), Columbia blood agar (Bio Merieux) and other medium bases, for the formulation of selective media for isolation of *Brucella* species.Moyer and Holocomb, (2005) reported the use of chocolate agar containing selective supplements for the isolation of *Brucella* spp. Similarly, the use of new media such as rifampin *Brucella* medium and malachite *Brucella* medium (MBM), together with Tryptose Soya Agar (TSA), was found to enhance the recovery of *B.abortus* RB 51 (Hornsby *et al*., 2000). For the isolation of *Brucella* species from milk samples although solid media have been used successfully, the use of enrichment media such as serum dextrose, tryptone soya or *Brucella* broth containing selective supplements of at least amphotericin B and vancomycin should be used because the microorganisms are usually present in too low numbers to be detected on solid media (OIE, 2004).

Growth temperature

The optimum growth temperature for *Brucella* organisms is 36-38°C, but most strains can grow between 20°C and 40°C (European Commission, 2001).

Colonial behavior

Brucella requires biotin, thiamin and nicotinamide and the growth are improved by serum or blood. On suitable solid media *Brucella* colonies are visible after 3 days incubation. After four days, *Brucella* colonies become round, 1-2mm in diameter, with smooth (S) margins, transparent and pale honey color when plates are viewed in transmitted light and have a bluish translucent appearance in reflected light. When viewed from above, colonies appear convex and fairly white. Later, colonies become larger and slightly darker. Rough *Brucella* isolates produce similar colony size and shape but are more opaque off-white in color with a rather granular surface(Alton *et al.*, 1988). Growth on blood agar is slower than on Serum Dextrose Agar (SDA) with the production of non haemolytic, greyish-white glistening colonies after 72 hours incubation. Growth in liquid media is usually poor unless the culture is vigorously shaken. On semi-solid media, CO2- independent *Brucella* strains produce uniform turbidity from surface down to 3mm depth while CO2-dependent strains produce a disk of growth 2mm below the surface of the medium. Little or slow growth is produced by many *Brucella* strains on MacConkey agar, even after five days at 37°C. The growth of most *Brucella* strains is inhibited by media containing bile salts, tellurite or selenite and does not require haeme (V-factor) and nicotinamide-adeninedinucleotide (X-factor) (Corbel, 2006).

Smooth *Brucella* cultures, especially *B.melitensis* cultures, have atendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoid (M) forms. Colonies are then much less transparent with more granular, dull surface (R) or a sticky gelatinous texture (M), and range in colour from matt white to brown in reflected or transmitted light. Intermediate (I) forms between S, R and M forms may occur in cultures undergoing dissociation to the non-smooth state. Checking for dissociation is easily tested by crystal violet staining: rough colonies stain red/violet and smooth colonies do not uptake dye or stain pale yellow. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity (OIE, 2009).

Carbon dioxide and pH requirements

B.abortus does require 5 to 10% CO_2 and can be isolated on containing *Brucella* selective supplement solid media under aerobic and anaerobic conditions at 37°C. The optimum pH for the growth of *Brucella* species is from 6.6-7.4 and culture media should be adequately buffered near pH 6.8 for optimum growth (Alton *et al.*, 1988).

2.1.7. Biochemical characteristics

Brucella metabolism is oxidative and cultures show no ability to acidify carbohydrate media in conventional tests. *Brucella* species are usually catalyzed and oxidase positive and they reduce nitrate to nitrite except *B.canis*strains. The production of H_2S from Sulphur containing amino acid varies (European Commission, 2001). Urease activity of *Brucella* species varies from fast to very slow. Indole and acetyl methyl carbinol are not produced from tryptophan and glucoserespectively.Methyl red and Voges-Proskauer tests are negative and *Brucella* neither liquefies gelatine nor lyses red blood cells (European Commission, 2001).

A summary of the differential characteristics and biochemical tests used to identify Brucella species from other bacteria is given in Tables 2 and 3.

Table 2: Differential characteristics of Brucella species

Test	*Brucella*	*Bordetella Bronchoseptica*	*Campylobacter fetus*	*Moraxella Species*	*Acinetobacter species*	*Yersinia Enterocolitica*
Morphology	S.coccobacilli	Smallcoccobacilli	Coma shaped	Diplococcoid	Diplococcoid	Rods
Motility at 37°C	-	+	+	-	-	-
Motility at 20°C	-	-	-	-	-	+
Lactose fer.	-	-	-	V	V	-
Acid production	-b	-	-	-	V	+
Haemolysis on Blood Agar	-	+	-	V	V	-
Catalase	+	+	+	V	-	-
Oxidase	+c	+	+	+	-	-
Urease	+d	+	-	V	V	+
Nitrate reduction	+e	+	+	V	-	+
Citrate utilization	-	+	-	**-**	**V**	-

Source: (Alton et al., 1988)

Table 3: Differentiation of the species and biovars of the genus Brucella

Characterstic	*B.melitensis*biovars *B.suis*biovars	*B.* *B.ovis*	*abortus*biovars
	1 2 3	1 2 3 4 5 6 7 9	1 2 3 4 5
Catalase	+ + +	+ + + + + + + +	+ + + ++
Oxidase	+ + +	+ + + + + + + +	+e + +
++	–		
Urease	+ + +	+ + +f+ + + + +	+ + +
++	–		
CO_2req.	– – –	[+] [+] [+] [+] – – – –	– – – –
–	+		
H_2Sprod.	– – –	+ + + + – [–][+] +	+ – – –
–	–		
Growth on Media containing Dyes			
Thionin	+ + +	– – + – ++ + +	+++ ++
Basicfuchsin	+ + +	+ – + + ++ + +	[–] – +

[–] –	[–]		
Agglutination With monospecific Anti-sera			
A	– + +	+ + + – – + + –	+ + +
+ –	–		
M	+ – +	– – – + + – + +	– – –
++	–		
R	– – –	– – – – – – – –	– – –
–	–	+	

(a), Symbols: +, positive; [+], positive for most strains, [-], negative for most strains, -, negative, (b), For more certain differentiation of biovar 3 and 6, thionine at 1:25, 000 (w/v) is used; biovar 3 gives a positive growth response, biovar 6, (c),Dye concentration, 1:50, 000 (w/v). (d), Growth will occur in the presence of thionine at a concentration of 1:150, 000 (w/v). (e), Rapid reaction, most strains of B. suistest positive within 5 minutes. (f), Some field strains of B. abortusmay be negative.
Source: (OIE, 2004; Garritty et al., 2005).

Susceptibility to dyes

The effect of the dyes thionin and basic fuchsin on various *Brucella* species and biovars varies (European Commission, 2001)). *Brucella abortus*grows in presence of basic fuchsin but does not grow in presence of thionin at the mentioned concentrations (Alton *et al*., 1988).

Susceptibility to antibiotics

Brucella species are sensitive to a wide range of antibiotics. Penicillin is used for the routine differentiation of the vaccinal strain of *B.abortus*species biovar 1 strain 19, used for the immunization of cattle from its respective field strain. This is because the S19 vaccine strain is sensitive to penicillin while the field virulent strain is resistant. Rev.1 vaccine is sensitive to streptomycin while the field virulent strain is resistant to streptomycin (Alton *et al*., 1988). On primary isolation, *Brucellae* are usually susceptible *in vitro* to gentamicin, tetracyclineand rifampicin. Most strains are also susceptible to ampicillin, chloramphenicol, cotrimoxazole, erythromycin, spectinomycin and streptomycin. Most strains of *Brucella* are resistant to β lactans, cephalosporins, polymixin B,bacitracin, cycloheximide, clindamycin, linomycin, nystatin and vancomycin at therapeutic concentrations (European Commission, 2001).

2.2. Epidemiology of Brucellosis

The epidemiology of brucellosis is complex and it changes from time to time. Wide host range and resistance of *Brucella* to environment and host immune system facilitate its survival in the populations. Since cattle are found throughout the world, prevalence of brucellosis in cattle has been reported from a wide range of countries.

2.2.1. World distribution

Distribution of the disease is worldwide.These countries include Australia, Canada, Cyprus, Denmark, Finland, Netherlands, New Zealand, Norway, Sweden and the United Kingdom. It remains endemic among Mediterranean countries of Europe,Northern and Eastern Africa, Near East countries, India, Central Asia, Mexico and Centraland South America (FAO, 2003) (Figure 2). While *B.melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants in any country(Robinson, 2003). The disease is under reported, although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority. Furthermore, it is also considered as a re-emergingdisease in many countries of the glob (Cutler *et al*., 2005).

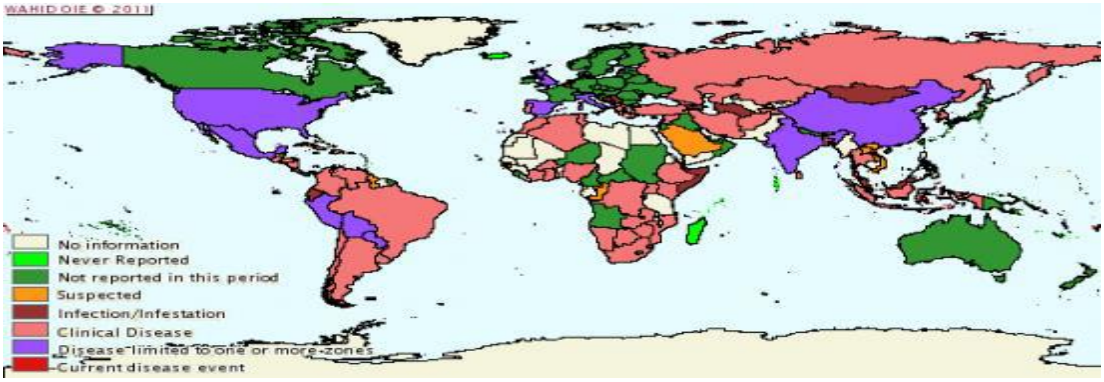

Figure 2: Worldwide distribution of bovine brucellosis (*B. abortus*) report

Source: (OIE, 2011)

2.2.2. Distribution in Africa

Brucellosis is a disease that has been known in Africa for a very long time, in both animals and humans. Bovine brucellosis was first recorded in Zimbabwe in 1906, Kenya in 1914 and inOrange Free State of South Africa in 1915 (Chukwu, 1985). The surveillance and control of brucellosis in this region was rarelyimplemented outside South Africa. In dairy production, the disease is a major obstacle tothe importation of high yielding breeds and represents a significant constraint to theimprovement of milk production through cross breeding (Mustafa and Nicoletti, 1995).Here is the summary of the seroprevalence of bovine brucellosis in some African countries in (Table 4).

Table 2: Seroprevalence of bovine brucellosis in some African countries

Country	No of cattletested	Prevalence (%)	Test applied	Reference
Zambia	1245	14.1	RBPT,c-ELISA	(Muma *et al.*, 2006)
Kenya	393	1	c-ELISA,CFT	(Kang'Ethe *et al.*, 2007)
Sudan	574	24.5	c-ELISA	(Angara *et al.*,2004)
Ghana	444	2.9	RBPT	(Folitse *et al.*, 2014)
Nigeria	-	24.0	RBT+ELI	(Mai *et al.* 2012)
Uganda	-	14	SA RBPT	(Miller *et al.*, 2016)
Uganda	-	5	ELISA	(Bertu *et al.*, 2010)
Zimbabewe	1291	5.5	RBPT, c- ELISA	(Matope *et al.*, 2011)
Algeria	1032	9.7	BPAT	(Gwida, 2010)
Egypt	1966	5.4	BPAT	(Samah*et al.* 2008)
South Africa	5 059	1.5	RBPT, CFT	(Bishop *et al.*, 1994)
Eritrea	1294	8.5	RBPT, CFT	(Omer et al., 2002)
Ghana	183	6.6	RBPT	(Kubuafor *et al.*, 2000)

2.2.3. Status of brucellosis in Ethiopia

In Ethiopia, brucellosis in animals and humans has been reported from different localities of the country, particularly associated with cattle in both intensive and extensive management systems (Jilo, 2017). These prevalence studies in animals and human were largely confined to serological surveys and commonly targeted bovine brucellosis. In intensively managed cattle higher individual bovine brucellosis seroprevalence has been recorded as compared to those in the extensive management system. These studies were conducted in local, pure and cross breed of cattle. In these studies, seroprevalence of brucellosis in cattle ranging from 0.06-11.2% were reported by CFT as depicted in Table 5.

Table 3: Seroprevalence of brucellosis in cattle in some part of Ethiopia

Location	Prevalence			Reference
	RBPT (%)	CFT (%)	c-ELISA(%)	
Addis Ababa	2.77	-	0.06	(Edao *et al.*, 2018)
Tigray Region	3.3	3.19		(Berhe *et al.*, 2007)
Sidama Zone	-	1.66		(Asmare *et al.*, 2010)
West Tigray	-	4.9		(Haileselassie *et al.*, 2010)
Jimma zone	-	0.77		(Tolosa *et al.*, 2008)
Debrebirhan and Ambo	0.7	0.2		(Bashitu *et al.*, 2015)
Adami Tulu	4.5	4.3		(Gebawo et al., 2014)
Debre-Zeit	3.3	2		(Alemu *et al.*, 2014)
Somali and Oromia	-	0.9		(Gumi *et al.*, 2013)
BenishangulGumuz	1.2	1		(Adugna *et al.*, 2013)
East Wollega Zone	2.96	1.97		(Yohannes *et al.*, 2012)
East Showa Zone	11.2	-		(Dinka and Chala, 2009)
Wuchale-Jida district	12.5	11.0		(Kebede *et al.*, 2008)
Central Oromia	4.9	2.9		(Jergefa *et al.*, 2009)
ArsiNegele District	2.6	-		(Amenu *et al.*, 2010)
Jimma zone	-	3.1		(Ibrahim *et al.*, 2010)
Jijjiga zone	1.84	1.38		(Degefu *et al.*, 2011)
WesternTigray	-	6.1		(Haileselassie *et al.*, 2011)
Alage	2.28	2.4		(Asgedom *et al.*,2016)
Central Ethiopia	-	1.40		(Geresu *et al.*, 2016)

2.2.4. Sources of infection

The risk associated with exposure of susceptible animals to the disease following parturition or abortion of infected cattle depends on three factors:- the number of organisms excreted, the survival of these organisms under the existing environmental condition and the probability of susceptible animals being exposed to enough organisms to establish infection. *B.abortus* achieves its greatest concentration in the contents of the pregnant uterus, the fetus and the fetal membranes after birth (Radostits *et al.*, 2006). In addition, vaginal discharge and to a lesser extent, farm areas contaminated by fecal matter of calves fed on contaminated milk could be considered as main source of

infection. Infected animals also shed organisms in the milk. Therefore, raw milk or raw milk products of bovine origin are ready sources for infections in humans. There can be also accidental self-inoculation with live Brucella vaccine strains that result in the disease(PAHO/ WHO, 2001).

2.2.5. Mode of transmission and route of infection

The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water (Figure 3). Moreover, cows customarily lick after birth, fetuses, and newborn calves and all may contain the organism in large number and this constitutes a very important source of infection. Bulls do not usually transmit infection from infected cows to non-infected mechanically. The use of infected bulls for AI constitutes an important risk, since the infection can be spread to many herds (PAHO/ WHO, 2001).

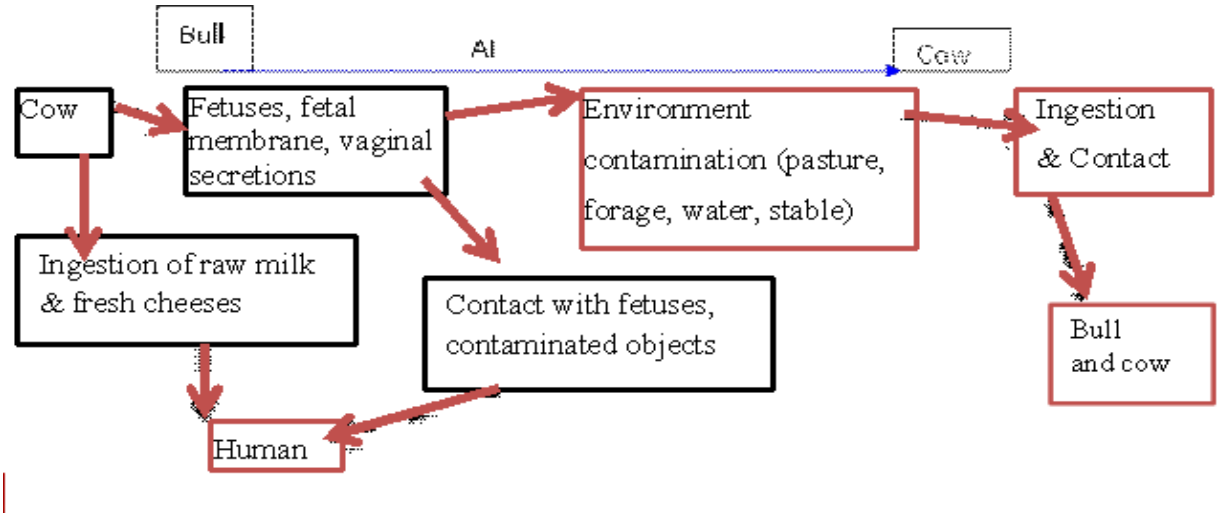

Figure 3: Bovine brucellosis mode of transmission

Source:(PAHO/WHO, 2001)

2.2.6. Reservoirs/ Carriers of Brucella species

Domestic animals

Domestic animals such as cattle, goats, sheep, pigs, camel, buffalo and dogs serve as a reservoir hosts (Moreno *et al.*, 2002). *Brucella* species can survive in proper environmental condition, damp soil and seawater and can be a source of infection. Notably abortion materials such as fetal parts, and fetal membranes, amniotic fluid and vaginal discharges of infected animals may contain high amounts of the bacterium and act as source of brucellosis (Henk and Kadri, 2005).

Wild animals

*Brucella abortus*and *B.suis*have been isolated from a great variety of wildlife species (Godfroid, 2002). Wild ruminants have been suggested as brucellosis carriers, but they are probably not true reservoirs (Godfroid *et al.*, 2013). Other works showed that wild ruminants do not play a relevant role in the maintenance of *B.abortus*and *B.melitensis* infections since limited cases of brucellosis have been reported in wild ruminants (Godfroidah` *et al.*, 2005).However, a potential risk for brucellosis infection of livestock by wild animals could be associated when artificial management such as winter feeding increases aggregation (Godfroid, 2002;Gortázar *et al.*, 2007).

2.2.7. Risk factors for infection

The risk factors that influence the initiation, spread, maintenance and/or control of bovine brucellosis are related to the animal population, management and to biology of the disease (Radostits *et al.*, 2006).

Agent risk factors

Brucella species are a facultative intracellular pathogen, which is capable of multiplication and survival within host phagocytes. The organisms are phagocytosed by polymorph nuclear leukocytes in which some survive and multiply. These are then transported to lymphoid tissues and fetal placenta. The inability of the leukocytes to effectively kill virulent *Brucella* at the primary site of infection is a key factor in the dissemination to regional lymph nodes and other sites such as the reticuloendothelial system and organs such as the uterus and udder. The organism is also able to survive within macrophages because it has the ability to survive phagolysosome. *Brucella* are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanism during the period of haematogenous spread (Radostits *et al*., 2006).

Risk factors associated with host

Age has been referred to as one of the intrinsic factors associated with brucellosis. Higher seroprevalence of brucellosis has been observed in older animals. Since susceptibility increases after sexual maturity and pregnancy,the disease brucellosis has traditionally been considered a disease of adult animals. Tropism to the reproductive tract of*Brucella species*is due to the production of erythritol, a 4-carbon sugar produced in the foetal tissues of ruminants that stimulates the growth of *Brucella*. Thus, it may also explain the higher prevalence in adult animals than in young(Bekele *et al*., 2011).

Female ruminants presented a higher odd of brucellosis infection. It could be associated with the intrinsic biology of the microorganisms and its tropism to the foetal tissue. 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 (Coelho *et al*., 2013).

Management risk factors

The spread of the disease from one herd to another and from one area to another is usually due to the movement of an infected animal from an infected herd into a non-exposed herd. Whether a herd raises its own replacement animals or purchases replacement animals affects the potential for introduction into the herd. The unregulated movement of cattle from infected herds or areas to brucellosis free herds or areas is the major cause of breakdowns in brucellosis eradication programs. Once the herds are infected, the time required to become free of brucellosis is increased by large herd size, by active abortion, and by loose housing (Radostits *et al*., 2006). A contaminated environment or equipment used for milking or artificial insemination is further sources of infection. Permanent calving camps and lush pastures, particularly if they are wet and muddy, may play a very important role in the spread of the disease (Bishop *et al*., 1994).

2.3. Pathogenesis

Brucella may enter the host via ingestion or inhalation, or through conjunctiva or skin abrasions. *Brucella* specie can induce infection through mucosal surfaces by invading epithelial cells of the host, allowing M cells in the intestine have been identified as a portal of entry for *Brucella* species (Köhler *et al*., 2002). Once *Brucella* species has invaded, usually through the digestive or respiratory tract, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells (Pizarro-Cerdá *et al*., 2000). *Brucella* has the ability to interfere with intracellular trafficking, preventing fusion of the *Brucella*-containing vacuole (BCV) with lysosome markers, and directing the vacuole towards a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of *Brucella* (Pizarro-Cerdá *et al*., 2000).

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 into the blood. Smooth *Brucella* inhibit host cell apoptosis, favoring bacterial intracellular survival by escaping host immune surveillance, while rough *Brucella* mutants (*B.canis*and *B.ovis*are two exceptions) induce necrosis in macrophage. However, the mechanisms and virulence factors that mediate macrophage cell death have not been identified(Pei *et al*., 2006).

In contrast to other pathogenic bacteria, no classical virulence factors, such as exotoxins, cytolysins, capsules, fimbria, plasmids, lysogenic phages,endotoxiclipopolysaccaride (LPS) have been described in *Brucella* (Moreno and Moriyo, 2002). *Brucella* uses a number of mechanisms for avoiding or suppressing bactericidal responses inside macrophages. The smooth lipopolysaccharides that cover the bacterium and proteins involved in signaling, gene regulation, and transmembrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al*., 2005).The smooth phenotype of *Brucella* is due to the presence in the outer cell membrane of a complete LPS, which is composed of lipid A, a core oligosaccharide, and an O side chain polysaccharide. Rough (vaccine) strains (i.e, strains with lipopolysaccharide lacking the O side chain) are less virulent because of their inability to overcome the host defense system (Lapaque *et al*., 2005).

*Brucella*display strong tissue tropism and replicate within vacuoles of macrophages, dendritic cells (DCs), and placental trophoblasts. However, the pathogen has the ability to replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells. The intracellular lifestyle of *Brucella* limits exposure to the host innate and adaptive immune responses, sequesters the organism from the effects of some antibiotics, and drives the unique features of pathology in infected hosts, which is typically divided into three distinct phases: the incubation phase before clinical symptoms are evident, the acute phase during which time the pathogen invades and disseminates in host tissue, and the chronic phase that can eventually result in severe organ damage and death of the host organism. Chronic infection results from the ability of the organism to persist in the cells of the host in which *Brucella*are distributed by way of the lymphoreticular system to eventually cause cardiovascular, hepatic, lymphoreticular, neurologic, and osteoarticular disease (Baud and Greub, 2011).

2.3.1. Intracellular survival of Brucella

Brucella species are facultative intracellular bacteria and can become secluded within the endoplasmic reticulum of cells and thereby avoid lysosome fusion. By controlling the maturation of the brucellosome (*Brucella*-containing vacuole) at the onset of infection, unopsonized*Brucella* can enter, survive and replicate in a variety of cells, including dendritic cells and macrophages to evade the host innate immune response before activation of anti- *Brucella* mechanisms by adaptive immunity. To restrict long-term protective immunity, the organism first avoids the innate immune response by stealthy entry into host cells. From there, the organism controls aspects of protein secretion, intracellular trafficking, and bacterial replication, ultimately altering the course of the innate and adaptive immune responses (Xavier *et al*., 2013).

The two-component BvrR/BvrS gene sensing system that also acts through a cascade of protein phosphorylation to modulate bacterial gene expression is thought to be one of the key factors involved in the modulation of cell binding and penetration. In *Brucella,* VirB is thought to be essential for intracellular survival. In these brucellosome, *Brucella* organisms are able to produce virulence genes (*VirB*) which promote multiplication of the organisms in such environments. The VirB pumping system is built from a series of proteins encoded by the VirB operon. Many attenuated *Brucella* strains show mutations within the VirB operon, indicating that an intact VirB is essential for virulence. VirB seems to have a role in adherence of the bacterium to the host cell, cell entry, and it modulates the intracellular trafficking and replication of the bacterium (Boschiroli *et al*., 2001) (Figure 4).

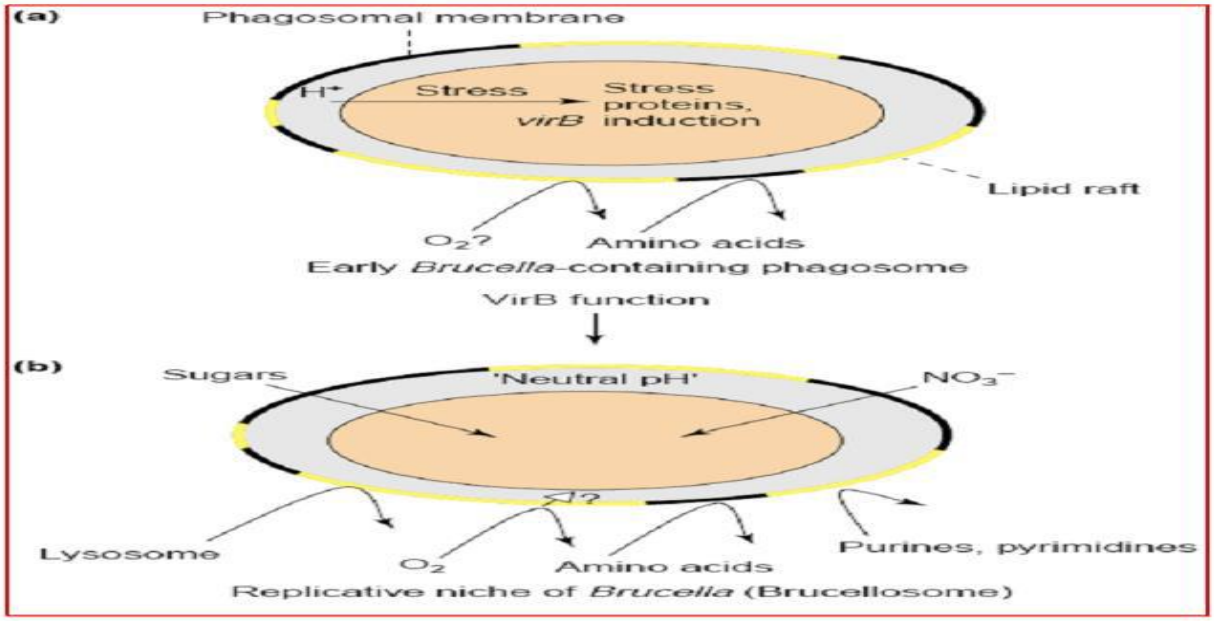

Figure 4: Characteristic properties of an early vacuole and the replicative niche of *Brucella* spp.

Source: (Köhler *et al*., 2003).

2.4. Immunity Against *Brucella* Infection

2.4.1. Humoral immune response

Naturally infected animals and those vaccinated as adults with strain 19 remain positive tothe serum and other agglutination tests for long periods. The serum of infected cattlecontains high levels of IgG1, IgG2, IgM and IgA isotypes of antibody. Similar isotypes atdifferent relative concentrations occur in milk, although most of the IgA is present insecretory form. The first isotype produced after an initial heavy infection or strain 19immunization is IgM and is soon followed by IgG antibody. IgG1 immunoglobulin is themost abundant in serum and exceeds the concentration of IgG2. The magnitude and durationof the antibody response following immunization is directlyrelated to the age atimmunization and the number of organisms administered (Tegegne and Crawford, 2000).

Residual antibody if present, is usually predominantly of the IgM class. Following exposure to virulent *B.abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and route of entry of the inoculums and the stage of pregnancy of the animal. Antibodies of IgM, IgG1 and IgG2 isotypes can all react in the tube agglutination but those of the IgM class are by far the most efficient (Tegegne and Crawford, 2000).

2.4.2. Cellular immune response

Brucella species are readily phagocytized by macrophages and polymorph nuclear leukocytes and, in the case of virulent strains, are capable of surviving within these cells and phagocytosis is promoted by antibody. However, since virulent *Brucella* can survive within normal macrophages for long periods, recovery from infection is likely to be dependent acquisition of increased bactericidal activity by phagocytic cells. Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines(Bekele *et al*., 2011) (Figure 5).

The release of these activating factors is dependent upon recognition of the appropriate antigen by the T-lymphocyte and is subject to regulation through the major histocompatibility complex. Live organisms capable of establishing persistent intracellular infection and certain types of antigen, with or without adjuvant, are the most effective inducers of cell-mediated immunity (Bekele *et al*., 2011).

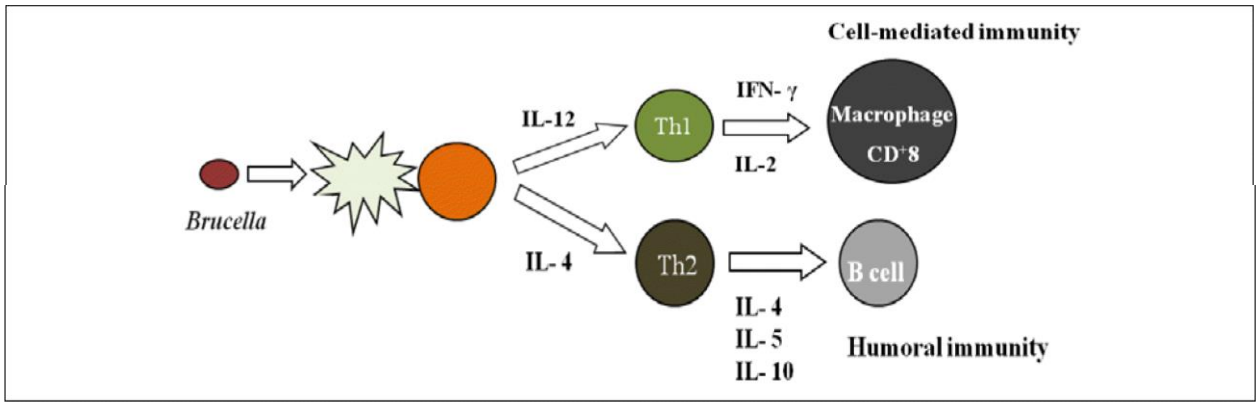

Figure 5: Humoral and cellular immune response of host body to Brucellae

Source: (Tegegne and Crawford, 2000)

2.5. Clinical Manifestation

The incubation period varies between 14 and 120 days. The major clinical sign in the first stage of the disease is abortion, but other signs due to localization of the organism may be observed. These signs include Orchitis, epididymitis, hygroma, arthritis, metritis and subclinical mastitis among others (Radostits *et al.*, 2007)(Figure 6). However, numerous animals develop self-limiting infection or they may become asymptomatic latent carriers and potential excretors. The second stage is characterized by either elimination of *Brucella* or more frequently, by persistent inflammation of mammary gland and supra mammary and genital lymph nodes, with constant or intermittent shedding of the organisms in milk and genital secretions (Poester *et al.*, 2010).

The cardinal feature of the disease in highly susceptible non-vaccinated pregnant cattle is abortion after the 5th month of pregnancy is(Radostits *et al.*, 2006) and other clinical signs are mainly the calving-associated problems and breeding-associated problems such as repeat breeding, a retained placenta and metritis(Acha and Szyfres, 2003). The infected cows usually abort only once after which a degree of immunity develops and the animals remain infected. At subsequent calving, the previously infected cows excrete huge numbers of *Brucella* in the fetal fluids. Brucellosis does not usually result gross organic lesions, but sometimes a mild interstitial inflammatory reaction in the mammary gland may be observed,which is associated with elimination of bacteria in the milk (Xavier *et al.*, 2013).

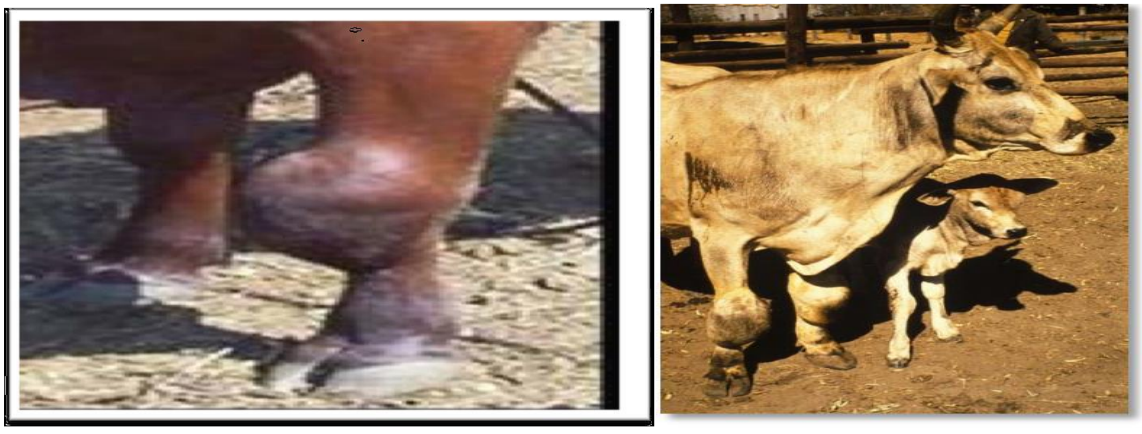

Figure 6: Unilateral and bilateral *Brucella abortus*-induced hygroma in cattle

Source: (Mantur *et al.*, 2019)

2.6. Public Health Importance of Brucellosis

Brucellosis in humans is known as "undulant fever" or "Mediterranean fever", "Malta fever" or "Bangs disease" (Corbel, 2006). Brucellosis remains amongst the most normally disregarded zoonotic diseases worldwide. The true incidence of brucellosis in human and animals worldwide is obscure and the occurrence is expanding in low and middle income nations. 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 (Sriranganathan *et al*., 2010).

Human brucellosis is caused by four species of *Brucella*, namely, *B.melitensis*, *B.abortus*, *B.suis*, and *B.canis*, with the majority of cases of the disease in humans being attributed to *B.melitensis*, although there is a possibility that human infection by the other three species is underappreciates. *B.melitensis* is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. In humans, brucellosis is described as a chronic febrile debilitating disease with an incubation period of 2 to 24 weeks, which leads to significant socioeconomic losses owing to long-term treatment and inability of the affected individuals to provide for their families (Atluri *et al*., 2011).

2.6.1. Transmission of brucellosis to humans

The possible means of acquisition of brucellosis include: person-to-person transmission, infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals, and foodborne transmission.

Person-to-person transmission

This is extremely rare. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission. Of more potential significance is transmission through blood donation or tissue transplantation. Bone marrow transfer in particular carries a significant risk. It is advisable that blood and tissue donors to be screened for evidence of brucellosis and positive reactors with a history of recent infection be excluded. Transmission to attendants of brucellosis patients is most unlikely but basic precautions should be taken. Laboratory workers processing sample from *Brucella* patients have a much greater risk (WHO, 2004;WHO, 2006).

Infection from a contaminated environment

Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, especially if abortions occur. Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc., (WHO, 2004). Contact infection may also result from contamination of skin or conjunctivae from soiled surfaces. Water sources, such as wells, may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas. *Brucella* spp. in dung,dust, water, aborted fetuses, soil, slurry, meat and dairy productscan survive for long periods(WHO, 2006).

Occupational exposure

Certain occupations are associated with a high risk of infection with brucellosis are people who work with farm animals, farmers, farm laborers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment. The families of farmers and animal breeders may also be at risk as domestic exposure may be inseparable from occupational exposure when animals are kept in close proximity to living accommodation. Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughter men, butchers, meat packers, collectors of fetal calf serum, processors of hides, skins and wool, renderers and dairy workers (WHO, 2004). The preparation and use of live vaccines is also hazardous as strains such as *B.abortus*S19 and *B.melitensis* Rev 1 are not completely avirulent for humans. The rough vaccine strain *B.abortus*RB 51 appears to be of low pathogenicity but still presents a potential hazard through accidental injection and is rifampicin-resistant (WHO, 2006).

Foodborne transmission

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unboiled milk is the main source of infection for most populations. Cow contaminated with *B.melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk (Young, 1990). The cheese-making process may actually concentrate the *Brucella* organisms, which can survive for up to several months in this type of product. Such cheeses should be stored in cool conditions for at least six months before consumption. Hard cheeses prepared by lactic and propionic fermentation presents a much smaller risk. Similarly, yoghurt and sour milk are less hazardous. *Brucella* dies off fairly rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5. Raw vegetables may be contaminated by infected animals and present a hazard. In endemic areas, tourists consuming "ethnic" food products may be particularly at risk (Young and Corbel, 1989).

Travel-acquired brucellosis

Business travelers or touriststo endemic areas can acquire brucellosis, usually by consumption of unpasteurized milk or other dairy products. Travelers may also import infected cheeses or other dairy products into their own countries and infect their families or social contacts by this means. Imported cases now account for most of the acute brucellosis cases seen in North America and Northern Europe (WHO, 2004).

2.6.2. Clinical manifestations in human

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as many signs referable to almost any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome (WHO, 2004;WHO, 2006).

Osteoarticular complications with bone and joint involvement are the most frequent complications of brucellosis, occurring in up to 40% of cases. A variety of syndromes have been reported, including sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis. *Brucella* sacroiliitis is especially common. Patients present with fever and back pain, often radiating down the legs (sciatica). Children may refuse to walk and bear weight on an extremity (WHO, 2004).

Foodborne brucellosis resembles typhoid fever, in that systemic symptoms predominate over gastrointestinal complaints. Nevertheless, some patients with the disease experience nausea, vomiting, and abdominal discomfort. Rare cases of ileitis, colitis and spontaneous bacterial peritonitis have been reported (Yong and Corbel, 1989).

Hepatobiliary complications often occur in brucellosis, although liver function tests can be normal or only mildly elevated. The histological changes in the liver are variable, but disease caused by *B.abortus*may show epithelioid granulomas that are indistinguishable from sarcoidosis lesions. A spectrum of hepatic lesions has been described in cases due to *B.melitensis,* including scattered small foci of inflammation resembling viral hepatitis. Occasionally larger aggregates of inflammatory cells are found within the liver parenchyma with areas of hepatocellular necrosis. In other cases, small, loosely formed epithelioid granulomas with giant cells can be found (WHO, 2004).

Aerosol inhalation of *Brucella* specie cause pulmonary complications, including hilar and paratracheal lymphadenopathy, interstitial pneumonitis, bronchopneumonia, lung nodules, pleural effusions, and empyema (WHO, 2006).

Orchitis and epididymitis are the most frequent genitourinary complications of brucellosis in men. Usually unilateral, *Brucella* Orchitis can mimic testicular cancer. Although *Brucella* organisms have been recovered from banked human spermatozoa, there have been a few reports implicating sexual transmission. In women, rare cases of pelvic abscesses and salpingitis have been reported (Young, 1990).

Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to

be associated with erythritol, a growth stimulant for *B.abortus*. Although erythritol is not present in human placental tissue, *Brucella* bacteremia can result in abortion, especially during the early trimesters (WHO, 2004).

Cardiovascular complications with endocarditis are the most common cardiovascular manifestation, and it is said to be the most common cause of death from brucellosis. Endocarditis is reported in about 2% of cases, and can involve both native and prosthetic heart valves. The aortic valve is involved more often than the mitral valve. Direct invasion of the central nervous system occurs in about 5% of cases of *B.melitensis* infection, and meningitis or meningoencephalitis is the most common manifestations (WHO, 2006).

Cutaneous complications with a variety of skin lesions have been reported in patients with brucellosis, including rashes, nodules, papules, erythema nodosum, petechiae, and purpura. Although uncommon, a variety of ocular lesions have been reported in patients with brucellosis (WHO, 2006).

2.6.3. Public health importance of brucellosis in Ethiopia

In Ethiopia, mixed cropping farmers and pastoral and agro pastoral peoples depends on domestic animals, milk and milk product to fulfill their dietary requirement which is the well-known transmission route of brucellosis from animals to human. On the other hand, common practices such as traditional type of food animal slaughtering in non-hygienic systems and areas which definitely reduce the safeness,hygiene and wholesomeness of food of animal origin. Animal owners who consume such contaminated food which may contain *Brucella* bacteria has may got an adverse health effect(Desta, 2016). Majority of mixed cropping farmers and pastoral and agro pastoral peoples do not use any protective materials during handling parturient animals, removing placenta and/or other aborted materials since most of the people had poor knowledge about brucellosis(Desta, 2016).

So, these practices could potentially facilitate the transmission of zoonotic *Brucella*pathogens from domestic animals to humans (Bekele *et al*., 2013). Generally, human brucellosis is increasing in Ethiopia like many other developing countries due to various sanitaryand socioeconomic (Pappas *et al.,* 2006). Thus, collaborative work of different stakeholders to prevent and control the disease as well as to enhance public awareness level of livestock keepers is required(Catley *et al*., 2005) (Table 6).

Table 4: Seroprevalence of human brucellosis in some part of Ethiopia

District	N_o: examined	Sample taken	Test employed	prevalence	Reference
Afar	200	serum	RBPT	16%	(Zewolda and Wereta, 2012)
			CFT	15%	
	630	Serum	RBPT	12.7%	(Zerfu *et al*., 2018)
	80	Serum	CFT	35%	
Fafan zone	211	serum	CFT	0.4%	(Lakew *et al*., 2019)
Addis Ababa	360	serum	RBPT	-	(Kassahun *et al*., 2006)
			2-MET	4.8%	
Bishoftu Modjo	149	serum	RBPT	4.7%	(Tuli *et al*., 2017)
			CFT	1.3%	

2.7. Diagnostic Techniques of Brucellosis

Brucellosis signs are non-pathognomonic in livestock and human and definitive diagnosis depends on laboratory testing. Laboratory diagnosis includes indirect tests that can be applied to milk or blood, as well as direct tests (classical bacteriology and direct polymerase chain reaction or PCR based methods). The choice of a particular testing strategy depends on the prevailing epidemiological situation of brucellosis in susceptible animals (livestock and wildlife) within a country or region (Godfroid *et al*., 2013).

There is no single test by which a bacterium can be identified unequivocally as *Brucella*.Accordingly, for a definitive identification, a combination of growth characteristics,serological, bacteriological or molecular methods is required

(Alton *et al*., 1988;FAO, 2003). The existence of different *Brucella* biotypes among the *Brucella* species and theiridentification is important to confirm the infection. Because of the complications involved in the diagnosis of the disease,including the difficulties in distinguishing between infected and vaccinated animals byconventional serological tests, bacteriological isolation and identification of biotypes of theetiological agent are necessary steps in the design of epidemiological and eradicationprograms (Zinsstag *et al*., 2005).

2.7.1. Bacteriological diagnosis

The "gold standard" of the brucellosis diagnosis is the direct bacteriological testing: cultivation of *Brucella*, isolated from body fluids (blood, cerebrospinal fluid, urine and others) or tissues (Smirnova *et al*., 2013). It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing (Corbel, 2006). Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view. However, in spite of its high specificity, culture of *Brucella* species is challenging. *Brucella* species is a fastidious bacterium and requires rich media for primary cultures. Furthermore, its isolation requires a large number of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory and it requires biosafety level 3 facilities which are not available in most developing countries. However, the cultivation and isolation of the bacteria are also necessary preliminary steps for staining and biotyping of *Brucella* spp.(Al Dahouk *et al*., 2013).

Staining

Stamp staining is still often used, even though this technique is not specific: other abortive agents such as *Chlamydophilaabortus*(formerly *Chlamydia psittaci)* or *Coxiellaburnetii*are also stained red (Corbel, 2006). They are not truly acid fast; however, they are resistant to decolonization by weak acids, and stain red against a blue background with the Stamp's modification of the Ziehl-Neelsen method. *Brucella* species is a coccobacillus measuring 0.6-1.5µm long and 0.5-0.7µm wide. They generally occur singly and are observed in clusters of two or more. Smears fromvaginal discharge,placental cotyledon or fetal stomach contents can be stained with modified Ziehl-Neelsenmethod. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis(Alton *et al*., 1988).
Culture

Bacterial isolation is always required for the biotyping of strains. For the definitive diagnosis of brucellosis, the choice of samples depends on the clinical signs observed. Valid samples in clinical brucellosis include, aborted fetuses (spleen, stomach, and lung), vaginal secretions,fetal membranes, milk,sperm, colostrum,and fluid collected from hygroma or arthritis. During post mortem, to confirm suspected cases of acute or chronic brucellosis, the preferred tissues are the genital and oropharyngeal lymph nodes, the mammary gland and the spleen, and associated lymph nodes(Corbel, 2006). Direct isolation and culture of *Brucella* are usually performed on solid media which is most satisfactory method as it enables the developing colonies to be isolated and recognized clearly. A wide range of commercial dehydrated basal media is available, such Tryptose Soya Agar (TSA), blood agar base (Oxoid), Columbia agar, serum dextrose agar (SDA) or glycerol dextrose agar can be used (Alton *et al*., 1988).

The most widely used selective medium is the Farrell's medium, which is prepared by the addition of antibiotics to a basal medium. Farrell's medium, have inhibitory effect on some *B.abortus*and *B.melitensis* strains. Therefore, the sensitivity of culture increases significantly by the simultaneous use of both Farrell's and the modified Thayer Martin medium (OIE, 2012). Some *Brucella* species, like *B.abortus*wild type (biovars 1-4), need CO 2 for growth, while others, like *B.abortus*wild type (biovars 5, 6, 9), *B.abortus*S19 vaccine strain, *B.melitensis*, and *B.suis,* do not(Alton *et al*., 1988).

For liquid samples (milk or blood), sensitivity is increased by the use of a biphasic medium like the Castaneda medium, originally described for use with human blood cultures. Growth may appear after 2-3 days, but cultures are usually considered negative after 2-3 weeks of incubation (Alton *et al*., 1988). The identification of *Brucella* species is based on morphology, staining and metabolic profile (catalase, oxidase, and urease) (Corbel, 2006).

All culture media should be subject to quality control and should support the growth of *Brucella* strains from small inocula or fastidious strains, such as *B.abortus*biovar 2. On suitable solid media, *Brucella* colonies can be visible after a

2–3 days incubation period. After 4 days incubation, *Brucella* colonies are round, 1–2mm in diameter, with smooth margins. They are translucent and a pale honey color when plates are viewed in the daylight through a transparent medium (OIE, 2012).

Smooth (S) *Brucella* cultures have a tendency to undergo variation during growth, especially with subcultures, and to dissociate to rough (R) forms. Colonies are then much less transparent, have a more granular, dull surface, and range in color from matt white to brown in reflected or transmitted light. Checking for dissociation has been easily tested by crystal violet staining: rough colonies stain red/violet and smooth colonies do not uptake dye or stain pale yellow (OIE, 2012).

Biochemical test

Identification of *Brucella* strains using different biochemical tests like oxidase activity, urease activity, H2S production, Dye tolerance (basic fuchsin and thionin) and sero-agglutination. It has been also recommended that Gram stain morphology and modified ZN staining, coupled with the urease test, for rapid identification of *Brucella* to the level of genus where facilities for further identification are not available (Mantur *et al.*, 2019).

2.7.2. Serological diagnosis

Serology is the mainstay of diagnosis for brucellosis because the diagnostic material is relatively easily accessible, and the tests are relatively cheap, available and sensitive. Since most of control and eradication programs rely on these methods serology, those tests are crucial for laboratory diagnosis of brucellosis. Inactivated whole bacteria or purified fractions (i.e. lipopolysaccaride or membrane proteins) are used as antigens for detecting antibodies generated by the host during the infection. Antibodies against smooth *Brucella* species (e.g. *B. abortus*, *B. melitensis*, and *B. suis*) cross react with antigen preparations from *B. abortus*, whereas antibodies against rough *Brucella* species (e.g. *B. ovis*and *B. canis*) cross react with antigen preparations from *B. ovis*(Nielsen, 2002).

Although several serological methods are currently available, these tests can be classified as screening tests (e.g. buffered antigen plate agglutination - BPAT), monitoring or epidemiological surveillance tests (e.g. milk ring test), and complementary or confirmatory tests (e.g. 2-mercaptoethanol, complement fixation, ELISAs, and fluorescence polarization assay).

Rose Bengal Plate test (RBPT)

The RBPT is a rapid, slide-type agglutination assay performed with a stained *B.abortus*suspension at pH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources. The drawbacks of RBPT include: low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative in RBPT. The overall sensitivity is 92.9%, so the use of RPBT should be considered carefully in endemic areas, particularly in individuals exposed to brucellosis and those having history of *Brucella* infection (Ruiz-Mesa *et al.*, 2005).RBPT is an agglutination test that is based on reactivity of antibodies against smooth lipopolysaccharide (LPS). As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution [1:2 through 1:64] of the serum samples. The present World Health Organization (WHO) guidelines recommend the confirmation of the RBPT by other assays such as serum agglutination tests(Ruiz-Mesa *et al.*, 2005).

The RBPT is based on the detection of specific antibodies of the IgM and IgG types but more effective in detecting antibodies of the IgG1 type than the IgG2 and IgM types. Also the low pH (3.65) of the antigen enhances the specificity of the test by inhibiting nonspecific agglutinins. The temperature of the antigen and the ambient temperature at which the reaction takes place may influence sensitivity and specificity (Díaz *et al.*, 2011).

Complement Fixation test (CFT)

Complement fixation test (CFT) 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 its specificity 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, as the latter are partially destroyed during inactivation. Since antibodies of the IgG1 type usually appear after antibodies of the IgM type, control and surveillance for brucellosis is best done by CFT (Perrett *et al*., 2010).

Complement fixation test is used as confirmatory test for *B.abortus*, *B.melitensis*, and *B.ovis*infections due to its high accuracy, and it is the reference test recommended by the OIE for international transit of animals (OIE, 2009). However, this method has some disadvantages such as high cost, complexity for execution, and requirement for special equipment and trained laboratory personnel. In addition, the test presents limitations with hemolysed serum samples or serum with anti-complement activity of some sera, and the occurrence of prozone phenomena (OIE, 2009). Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100%(Perrett *et al*., 2010).

Serum Agglutination Test (SAT):

Serum agglutination test measures the total quantity of agglutinating antibodies IgM and IgG. The quantity of specific IgG is determined by treatment of the serum with 0.05M 2-mercaptoethanol (2ME), which inactivates the agglutinability of IgM. SAT titers above 1:160 have been considered diagnostic in conjunction with a compatible clinical presentation. However, in areas of endemic disease, using a titer of 1:320 as cut off may make the test more specific. The differentiation in the type of antibody is also important, as IgG antibodies are considered a better indicator of active infection than IgM and the rapid fall in the level of IgG antibodies is said to be prognostic of successful therapy (Buchanan and Faber, 1980).

Enzyme linked immune sorbent assays test (ELISA)

ELISAs are divided into two categories, the indirect ELISA (iELISA) and the competitive ELISA (c-ELISAs) (Saegerman *et al*., 2004). They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range ofanimal and human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Competitive ELISA (c-ELISA) and Indirect ELISA (iELISA) tests can be used as supplementary tests to CFT. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted, however, that although the ELISAs are more sensitive than the RBPT, sometimes they do not detect infected animals which are RBPT positive (McGiven, 2013).

i. Indirect ELISA (i-ELISA)

The method is based on the specific binding of antibodies present in the test sample with immobilized antigen. The binding event is visualized using chemically or enzymatically derived fluorescent, luminescent or colorimetric reaction. Many iELISA tests are available on the market (Poester *et al*., 2010). It has been used for diagnosis using serum or milk from cattle. i-ELISA has been usually used for smooth LPS *Brucella* species, and it is sensitive and specific for *B.abortus*or *B.melitensis*, but it is not capable of differentiating antibodies induced by the vaccine strains S19 or Rev1. Sensitivity of i-ELISA varies from 96 to 100%, and its specificity from 93.8% and 100% (Gall *et al*., 2001).

ii. Competitive ELISA (c-ELISA)

With smooth *Brucella* LPS as antigen is used for detection of anti-*Brucella* in serum samples from cattle, sheep, goats, and pigs. This test is capable of differentiating vaccine antibody response from actual infections, and its sensitivity varies from 92 to 100%, whereas the specificity ranges from 90 and 99% (Perrett *et al*., 2010). It can also be used both for screening and confirmatory tests (FAO, 2003). Antibodies against smooth LPS are used in all the above mentioned tests. They have a common significant disadvantage: O-polysaccharides of *Brucella* are similar to that of *Yersinia enterocolitica*and other bacteria. It leads to the false positive results and thus reduces the specificity of the test (Nielsen *et al*., 2004). Partly this problem is solved in the competitive ELISA (cELISA), where the specific epitopes of *Brucella* O-polysaccharides are used as antigens, but the sensitivity of c-ELISA is significantly lower than the iELISA.

2.7.3. Molecular methods

In order to avoid difficulties of bacteriological testing the molecular biological techniques, often based on the polymerase chain reaction (PCR) amplification, are successfully used for *Brucella* identification and typing (Smirnova *et al*., 2013). Molecular methods for *Brucella* species genotyping molecular techniques are important tools for diagnosis, providing relevant information for identification of species and biotypes of *Brucella* species, allowing differentiation between virulent and vaccine strains (Lopez-Goñi *etal*., 2008). Initially, PCR based identification has been developed for the determination of bacterial isolates but now these methods are also used for detection of *Brucella* species in clinical samples of human and animals without previous isolation of the organism(Smirnova *et al*., 2013). In addition, these techniques can be used to complement results obtained from phenotypic tests (Bricker, 2002).

PCR DNA-based methods such as gene probes and PCR utilize primers derived from different polymorphic regions in the genomes of *Brucella* species. Different PCR methods for the detection of *Brucella* species that utilize primers derived from different polymorphic regions in the genomes of *Brucella* species as i.e. (1) a gene encoding a 31kDa *B.abortus*antigen which is conserved in all *Brucella* species (primers B4/B5) (Baily *et al*., 1992), (2) a sequence +16S rRNA of *B.abortus*(primers F4/R2), (3). a gene encoding an outer membrane protein of 26kDa (omp-2) (primers JPF/JPR and primers P1/P2), (4) outer membrane proteins (omp 2b, omp2a and omp31), (5) proteins of the omp25/omp31 family of *Brucella* spp. (Vizcaíno *et al*., 2004), the entire bp26 gene of *B. melitensis*16M, encoding the BP26 protein (omp 28) (primers 26A/26B) (Cloeckaert *et al*., 2000) were described.

Multiplex PCR typing

Multiplex PCR typing is more effective method of diagnosis and identification of *Brucella*. Several multiplex PCRs which identify the genus *Brucella* at the species level and partly at the biovar level using different primer combinations have been reported. It provides identification of all known *Brucella* species at the species or even biovars level by using certain combinations of primer pairs. The first multiplex PCR based test for *Brucella* detection was developed in 1994 (Bricker and Halling, 1994), it is also called AMOS PCR assay. It allowed identification of the four *Brucella* species (*Brucella abortus, Brucella melitensis, Brucella ovis*and *Brucella suis*) and was named AMOS PCR (AMOS is an acronym from abortus-melitensis-ovis-suis) for the first letters of species names. It comprised five oligonucleotide primers for the identification of selected biovars of four species of *Brucella*.The assay exploited the polymorphism arising from species-specific localization of the genetic element IS711 in the *Brucella* chromosome (Smirnova *et al*., 2013).

Real-Time PCR

It is more rapid and more sensitive than conventional PCR. It does not require post amplification handling of PCR products, thereby reducing the risk of laboratory contamination and false-positive results. Real-time PCR assays have been recently described in order to test *Brucella* cells (Redkar *et al*., 2001), urine (Queipo-Ortuño *et al*., 2005), blood, paraffin-embedded tissues (Kattar *et al*., 2007), serum, and other tissues (Smirnova *et al*., 2013). Three separate real-time PCRs were developed to specifically identify seven biovars of *B. abortus*, three biovars of *B. melitensis*and biovar one of *B. suis*using fluorescence resonance energy transfer. The upstream primers used in these real-time PCRs derived from the insertion element, IS711 whereas the reverse primer is selected from unique species or biovar-specific chromosomal loci. Sensitivity of *B.abortus*-specific assay was as low as 0.25 pg DNA corresponding to 16-25 genome copies and similar detection levels were also observed *for B. melitensis*and *B. suis*-specific assays (Redkar *et al*., 2001).

2.8. Treatment, Prevention and Control

2.8.1. Treatment

Treatment of brucellosis in domestic animals is not indicated (Kassahun, 2003). In human, due to intracellular localization of *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage (Sriranganathan *et al*., 2010), treatment failure and relapse rates are high and depend on the drug combination and patient compliance. The optimal treatment for brucellosis is a combination regimen using two antibiotics since mono therapies with single antibiotics have been associated with high relapse rates (Sriranganathan

et al., 2010). The combination of doxycycline with streptomycin (DS) is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis. Neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *Brucella.* Acombination of doxycycline treatment (6 weeks duration) with parenterally administered gentamicin (5mg/kg) for 7 days is considered an acceptable alternate regimen(Sriranganathan *et al*., 2010).

2.8.2. Prevention and control

Brucellosis is an infectious disease which has been controlled and eradicated in some countries in the world (Godfroid *et al*., 2005). In sub-Saharan Africa, animal health services delivered bythe public sector have greatly decreased over the last 20 years due to various factors such as decreasing government budgets, particularly for operational costs of disease control. Thus, programs that require coordinated surveillance, information exchange and application of control measure are not implemented in many sub-Saharan countries (Mcdermott and Arimi, 2002). The general strategies proposed in FAO, (2003) by the WHO including Mediterranean Zoonoses Control Program to eradicate animal brucellosis were: prevention of spread between animals and monitoring of brucellosis-free herds and zones, elimination of infected animals by test and slaughter programs to obtain brucellosis-free herds and regions, and vaccination to reduce the prevalence (FAO, 2003).

Immunization

Vaccination is one of the most successful methods for prevention of livestock brucellosis.Both live vaccines, such as *B.abortus*S-19, *B. suis*S-2,rough *B.melitensis* strain M111 *B. melitensis*Rev-1, and *B.abortus*strain RB51 and killed vaccines, such as *B.abortus*45/20 and *B.melitensis*H.38 are available in different parts of the world (Kassahun, 2003).

Use of the RB51 attenuated live vaccine has recently gained popularity for control of brucellosis in cattle. But on a cautionary note, the failure of this strain to induce serological reactivity, coupled with its inherent resistance to rifampicin, might complicate detection and management of zoonotic infection spilling into humans with occupational risk factors for acquiring brucellosis. Currently, despite huge research efforts, no vaccine has been approved for the prevention of human brucellosis (Marzetti *et al*., 2013).

Application of farm Biosafety measures

Implementation of measures to reduce the risk of infection through personal hygiene, adoption of safe working practices, protection of the environment and food hygiene should minimize risks of further infection. Under appropriate conditions, *Brucella* organisms can survive in the environment for prolonged periods. Their ability to withstand inactivation under natural conditions is relatively high compared with most other groups of non-sporing pathogenic bacteria (WHO, 2006). *B.abortus*is inactivated by pasteurization and its survival outside the host is largely dependent on environmental conditions. The pathogen may survive in aborted fetus in the shade for up to eight months, for two to three months in wet soil, one to two months in dry soil, three to four months in faeces, and eight months in liquid manure tanks (OIE, 2004). For example, in nomadic populations where people travel in search of green pasture and water, the proper handling and burying of abortion materials to prevent contamination of water sources and pasture is of paramount importance (OIE, 2004).Brucellae in aqueous suspensions are readily killed by most disinfectants. A 10g/l solution of phenol will kill brucellae in water after less than 15 min exposure at 37°C. Formaldehyde solution is the most effective of the commonly available disinfectants, provided that the ambient temperature is above 15°C (WHO, 2006).

Application of veterinary extension

The development of a national veterinary extension services in the country, is essential to promote awareness about brucellosis, its impact on livestock production and zoonotic risks, would provide a valuable prevention measure. This would help to unify both community/dairy cattle producers to control and eliminate brucellosis. Currently, many dairy cattle producers hide or dispose of animals with a history of abortion, potentially facilitating disease transmission between farms and regions. This seriously undermines efforts of controlling and preventing the disease(OIE, 2004).

2.9. Economic Impact

Food and Agriculture Organization of the United Nations (FAO) and the Organization of Animal Health (OIE) consider brucellosis as has not only direct public health implications, it also poses a barrier to trade of animals and animal products (Fitcht, 2003) and has a wide socioeconomic impacts especially in countries where people in rural areas rely to a large extent on livestock breeding and dairy products as a source of income (Zinsstag *et al.*, 2005). Brucellosis is consistently ranked among the most economically important zoonoses globally. It is a multiple burdens disease with economic impacts attributable to human, livestock and wildlife disease. The epidemiology and economic impact of brucellosis vary by geography and livestock system. In many high-income countries, brucellosis has been successfully controlled or eliminated in livestock populations. Where it persists, wildlife populations have become the main reservoirs (for example, bison and elk in North America). In emerging middle-income countries, the brucellosis picture is much more variable. Middle-income countries tend to report the greatest number of outbreaks and animal losses (ILRI, 2012). Data on the yearly economic impact of brucellosis in the developing world associated with disease in livestock have generally been hard to assess, especially in Africa (Smits *et al.*, 2007).

In countries such as Argentina and Mexico, which depend heavily on the sale of livestock products for both domestic and international markets, these annual costs for control are estimated to be US$60 million and $200 million, respectively. Studies done in developing countries by the United Nations highlight that the need for effective control programmes which have an obvious benefit to the health of both human beings and livestock. If the costs of control programs are shared between the public and private sectors and include international aid, they are likely to be profitable and cost effective (Smits *et al.*, 2007). The economic impact in terms of human disease has been even harder to gauge (Smits *et al.*, 2007).

The economic losses due to bovine brucellosis include: losses of calves due to abortion, reduced milk yield, culling and condemnation of valuable cows because of breeding failure, endangering animal export trading of a nation, loss of man power, medical costs and government cost for research and eradication programs (Chukwu, 1987). In pregnant, abortion occurs during the second half of the pregnancy, often with retention of the placenta and resultant metritis, which may cause permanent infertility. It is estimated that the infection causes a 20% to 25% loss in milk production as a result of interrupted lactation due to abortion and delayed conception (Mcdermott and Arimi, 2002).

6. Conclusion and Recommendations

The present review revealed that the seroprevalence of bovine brucellosis and human brucellosis is low in different part of Ethiopia. The finding of positive serological reactors did not only suggest the presence of the disease in the cattle population, but also indicated the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and humans.This review also provided important information on knowledge, attitude and practice of livestock owners and occupational workers about brucellosis that result in significant zoonotic importance of using raw milk for human consumption. This emphasizes impact of brucellosis in animals, public health and the need to control and prevent brucellosis in the study areas. Based on the above conclusions, the following recommendations are forwarded to curb further spread of the disease in both cattle and human populations:

- ✓ Aborted animals must be isolated, aborted fetusesand fetal membranesmust be disposed properly, preferably, by incineration.
- ✓ Replacement stock should be purchased from herd known to be free of brucellosis.
- ✓ Strict movement control of animal from one area to another in order to prevent the spreadand transmission of the disease from infected cattle to the non-infected ones.
- ✓ The implementation of test and slaughter policy with compensation payment to thefarmers as the prevalence of the disease is low in the study area.
- ✓ Adoption of replacement stock vaccination with the aim of eradicating the diseases and prevention of its impact on the public and economic sector.
- ✓ Awareness creation among farmers, butchery men, abattoir workers and animal health workers about the nature and effect of the disease through formal and informal educational channels is required.

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