Brucellosis: Zoonotic Importance, Prevention And Control

Yewubnesh Asnake[1], Askale Abrhaley[2] and Fufa Abuna[1]

[1] Addis Ababa University, College Of Veterinary Medicine And Agirculture, Debere Zeit, Addis Ababa, Ethiopia, P.O. Box 34

[2] College Of Veterinary Medicine And Animal Science, Gondar, Ethiopia, P.O. BOX 196

Corresponding Author: Yewubnesh Asnake, Email Address: yewbaasnake@gmail.com

Summary: Brucellosis is especially caused by *Brucella melitensis*. It remains one of the most common zoonotic diseases that can seriously affect the wellbeing of animals and humans. The disease is caused by diverse *Brucella* species of which *Brucella abortus*, *B. melitensis* and *B. suis* are highly pathogenic for humans. The possible sources of infections include all infected tissues, aborted fetuses, fetal fluids, vaginal discharges, cultures and potentially contaminated materials. The natural aspects of the pathogenesis of the diseases lies on the presence of the bacteria in the cells and employing various methods to survive in the phagocytic cells. The diseases can be transmitted from infected host to susceptible animals by direct and indirect contacts. But the most common mode of transmission is sexual contact. Public health importance of brucellosis is much related to the infected animal species from which human transmission occurs. It can cause considerable losses in cattle as a result of abortion and reduction in milk yield. Most literature addresses control of *B. Abortus* infection by vaccinating young female animals. The most rational approach for preventing human brucellosis is the control and elimination of the diseases in animal reservoir and health education of the public working at high risk area.

[Yewubnesh Asnake, Askale Abrhaley and Fufa Abuna. **Brucellosis: Zoonotic Importance, Prevention And Control.** *Nat Sci* 2017;15(6):18-30]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 2. doi:10.7537/marsnsj150617.02.

Keywords: *Brucellosis, Control, Prevention, Zoonotic Importance*

Introduction

Brucellosis is commonly called contagious abortion in animals. This is one of the infectious diseases transmissible between humans and animals. This is also called undulant fever, frenzy fever and Mediterranean fever. This disease occurs in all seasons; however, it is more common in springs and falls during calving and lactation periods in animals. This disease was first discovered by David Bruce in 1887 from spleen of English soldiers killed in war in Malta Island. Therefore, it is called brucellosis disease (AGHA, 2002).

Brucellosis is still common in many countries in Mediterranean domain, Middle East, Arabian Peninsula, Central and South America, Asia and Africa. Only 17 countries such as Norway, Scotland, Switzerland, United Kingdom, and N, Sweden and several other countries were formally declared free of Brucellosis. in United State this disease is primarily considered as an occupational hazard (Gotuzzo *et al*., 1998). In addition, it is one of the most important disease common between animals and humans. This is also one of the most important health problems. Prevalence of Malta fever in humans and animals depend directly on prevalence of brucellosis. Therefore, this disease should be inevitably controlled and eradicated in order to avoid economic losses and health risks caused by this disease (Megid *et al*., 2010).

There are four types of Brucella detected as agent of majority of brucellosis infection in humans. *Brucella melitensis* has three serotypes. Most cases are infected by direct or indirect contact with sheep, goats, cattle, camel, pigs, dogs, *Brucella melitensis* is a major cause of brucellosis in humans. *Brucella abortus* has seven serotypes. Most cases occur due to contact with cattle by means of aborted fetuses, fetal membrans after birth, vaginal discharges, use milk from infected animals, due to contact with camels and yaks. is less virulent than Brucella melitensis in humans. *Brucella Suis* has five serotypes. Occur due to contact with pigs (Cecil, 2000).

This type of Brucella can cause abortion in pigs. Serotypes 1 and 3 cause infection in humans. *Brucella canis*occurs due to contact with dogs. It causes asymptomatic infection in humans. *Brucella Ovis*, *Brucella Maris*, *Brucella neotome* are less common from the above-mentioned four strains. *Brucella melitensis*is the most common pathogen of this disease in humans. Any disease caused by *Brucella Canis* and *Brucella Maris* is extremely rare in humans. *Brucella Ovis* causes testicular swelling in ram while it does not cause disease in humans. It is not proved whether *Brucella neotome* and *Brucella microti* (pathogen of brucellosis in rodents) cause disease in humans (Oxford, 1996).

Therefore, the objective of this seminar paper is

- To highlight the public health importance of brucellosis and its control and prevention measures.

1. Epidemiology

1.1. Etiology

Brucellaspecies are facultative intracellular gram-negative cocco-bacilli, non-spore-forming and non-capsulated. Although Brucellaspecies are described as non-motile, they carry all the genes except the chemotactic system, necessary to assemble a functional flagellum. Nine Brucellaspecies are currently recognized, (Sriranganathan *et al.*, 2010) including the better known six classical species comprised of *B. abortus* (cattle, biovars 1-6, and 9), *B. melitensis*, (biovars 1-3), *B. suis* (biovars 1-5), *B. ovis* (sheep*), B. canis* and *B. neotomae.* More recently, new members to the genus include *B. ceti* and *B. pinnipedialis* (dolphins/porpoises and seals respectively) *B. microti* (voles) and *B. inopinata* (reservoir undetermined) (Godfroid *et al*., 2011).

The Brucellahave no classic virulence genes encoding capsules, plasmids, pili or exotoxins and compared to other bacterial pathogen relatively little is known about the factors contributing to the persistence in the host and multiplication within phagocytic cells. And also, many aspects of interaction between Brucellaand its host remain unclear (Seleem *et al*., 2008).

Table 1: Hosts affected by *Brucella*species

Hosts	*Brucella species*				
	B. abortus	*B. melitensis*	*B. suis*	*B. ovis*	*B. canis*
Cattle	+	+	(+)	-	-
Sheep	(+)	+	+	+	-
Goats	(+)	+	-	-	-
Swine	(+)	(+)	+	-	-
Dogs	+	+	(+)	-	+
Camels	+	+	-	-	-
Humans	+	+	+	-	+
Horse	+	(+)	(+)	-	-

Key: +: can be affected, -: can't be affected, (+): rarely affected
Source: FAO. (2006)

The species of Brucellaand their major hosts are *B. abortus*(cattle), *B. melitensis*(goats), *B. suis*(pigs), *B. canis* (dogs), *B. ovis*(sheep) and *B. neotomae (*desert wood rats*)* as indicated in Table 1 above. Some Brucellaspecies 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; due to this, infected countries are challenged and have been under difficulties to overcome or control brucellosis effectively. In addition to cattle, *B. abortus*can affect other animals like sheep, goats, horses, camels, swine, dogs and humans. *Brucella melitensis*also affects other animals like sheep, horses, swine, camels, dogs and humans. *Brucella suis*also affects different animal species such as cattle, sheep, goats, dogs, camels, horses and humans. *Brucella ovis* affects only ovine while *B.canis*affects dogs and humans (FAO,2006).

1.2. Source of Infection and Mode of Transmission

Both vertical and horizontal transmissions of brucellosis exist in animals. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking or by licking the discharge of an animal, newborn calf or retained fetal membrane. Fetus can be infected in uterus or suckling of infected dams. Congenital infection that happens during parturition is frequently cleared and only few animals remained infected as adult (Radostits *et al.*, 2000).

Venereal infections can also occur and mainly seen with *B. suis*infections. The importance of venereal transmission varies with the species; it is the primary route of transmission for *B. ovis*. *Brucella suis*and *B. canis*are also spread frequently by this route. *Brucella abortus*and *B. melitensis*can be found in semen, but venereal transmission of these organisms is uncommon. species Some *Brucella*have also been detected in other secretions and excretions including urine, feces, hygroma fluids, saliva, and nasal and ocular secretions. In most cases, these sources seem to be relatively unimportant in transmission; however, some could help account for direct non-venereal transmission of *B. ovis* between rams *(*OIE, 2009 and Teferi *et al.,* 2011).

The transmission ways of brucellosis to human is through, ingestion of unpasteurized dairy foods produced from unlicensed family owned flocks whose products are sold door-to-door at low prices is one of the known ways. Dairy products are the main source of infection for people who do not have direct contact with animals, through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas. Occupational aerosol infection in laboratories andabattoirs has also been documented. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B.melitensis* Rev.1) can also occur, resulting in human infections. There are also case reports of venereal and congenital infection; and it can be transmitted through transplacental transfer and breast feeding even though rarely (FAO, 200, and Kulkarni *et al*., 2009).

1.3. Occurrence

Worldwide, especially in Mediterranean countries, the Middle East, Africa, Asia, Central and South America, India and Mexico. The disease is often unrecognized and under-reported. Predominantly an occupational disease of those who work with infected animals or their tissues, especially farm workers, veterinarians, meat inspectors and abattoir workers. Infection is more common in those who consume raw meat, raw milk or raw milk products. There have been reports of isolated cases of infection with *B. canis* occurring in animal handlers from contact with dogs, and *B. suis* occurring in those with contact with feral swine (Heymann *et al*.,2008).

1.4. Immunity Risk of Factors

1.4.1. Environment

The survival of the organism in the environment may play a role in the epidemiology of the disease under unsanitary condition where aborted fetuses are simply left everywhere where livestock, carnivorous animals and humans reach. Bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and because of the extensive environmental contamination that even single abortions or infected births can produce. Temperature, humidity and PH influence the organism's ability to survive in the environment. *Brucella* is sensitive to direct sun light, disinfectant and pasteurization. The congregation of a large number of mixed ruminants at water points facilitates disease spread (Radostits *et al.*, 2007).

1.4.2. Reservoirs

Carrier animals facilitate transmission of brucellosis highly by contaminating the environment and also being site of multiplication for the *Brucella* organisms in their body and execrating such agents and again the execrated organisms infect animals and humans then bring hazards on health and economy of the country (Radostits, 2006).

The carriers are dogs, cats and wild carnivores, such as foxes and wolves, which may be important as mechanical disseminators of infection by carrying away infected material such as fetuses or fetal membranes enhances the viability of the organisms in the environment, thus increasing the chances of infecting susceptible animals (FAO, 2006).

It should be remembered that wild carnivorous like dogs and cats can acquire infection with *B. abortus, B. melitensis*or *B.suis*from aborted ruminants or swine, usually by ingesting fetal or placental material that left freely in the environment. These animals can then excrete these bacteria and contaminate the environment where other animals and human live and this may present a serious hazard to humans and domestic livestock; hence poor management of wastes disposal and lack of controlling pet animals plays a great role in the spread of brucellosis in animals and humans (Bekele, 2004).

1.4.3. Host factors

The host factors, which are associated with spread of the disease brucellosis within a herd, include unvaccinated animals in infected herds, herd size, population density, age, sexual maturity and use of maternity pens. Large herd sizes are often maintained by the purchase of replacement cattle which may be infected. Population density (number of cattle to land area) is attributed to increased contact between susceptible and infected animals. Health status of the animals may also play a great role in acquiring the infection, hence vaccinated and disease free animals are less susceptible than unvaccinated and immune compromised diseased animals (Radostits *et al*., 2007).

The antibody against *Brucella* appears to be associated with age, as low prevalence in young stock has been reported than the adults. This low prevalence in young animals may be explained on the basis that the animal may harbor the organism without expressing any detectable antibodies until their first parturition or abortion (Jergefa *et al.,* 2009). It may be possible that after entry, the organism localizes itself in the regional lymph nodes and enjoy there without provoking antibody production until the animal is conceived and start secreting erythritol, which stimulates and supports the growth of *Brucella* organisms. This is related to the fact that sex hormones and meso-erythritol (in male testicles and seminal vesicles) and erythritol in female, allantoic fluid stimulate the growth and multiplication of *Brucella* organisms and tend to increase in concentration with age and sexual maturity (Radostits *et al.,* 2007; Wadood *et al*., 2009,).

In dairy farm, a higher seroprevalence of bovine brucellosis in females than males was reported as the result of that males are kept for relatively shorter time duration in breeding herd than females and thus the chance of exposure is lower for males and the spread of disease under natural condition is also not important. Moreover, females experience comparatively greater physiological stress during pregnancy and lactation due to which theyare more susceptible to infection (Wadood *et al*., 2009). In animals, a higher prevalence was encountered on farms that used artificial insemination due to poor hygiene practices before and after insemination and inappropriate techniques of using equipments and inseminating (Radostits *et al*., 2000; Jergefa *et al.,* 2009).

1.4.4. Management

The spread of the disease from one herd to another and from one area to another is almost always due to the movement of infected animals from an infected herd into a non infected susceptible herd. Hence, lack of strict movement control of animal from one area to another, lack of proper hygienic practices and good husbandry management play a great role in increament of the prevalence of brucellosis The source of replacement stock was found to affect the prevalence of brucellosis as a matter of a fact that the reproductive and health status of these replacement animals may be under the risk of Brucellosis. The main risk for introducing the disease into a previously non-infected area is by purchase of infected animals (Tigist *et al*., 2011).

There are many risk factors for occurrence of brucellosis in human beings and from these factors some of them are food consumption behavior, hygienic practices, occupational exposure, seasons, health status of the veterinary professionals and lack of practicing bio security. Feeding behavior such as Consumption of unpasteurized milk and milk products from cows, small ruminants or camels is considered to be the risk factor of infection in human brucellosis. Occupational exposure is one of the risk factors that affect risk groups like veterinarians, laboratory workers, food processors and farmers who handle infected animals and aborted fetuses or placenta (OIE, 2009).

2. Zoonotic Importance

Five out of the nine known Brucella species can infect humans and the most pathogenic and invasive species for human is *B. melitensis*, followed in descending order by *B. suis*, *B. abortus* and *B. canis.* The zoonotic nature of the marine brucellae (*B. ceti*) has been documented (Mc Donald *et al*., 2006; Sohn *et al*., 2003). *B. melitensis*, *B. suis* and *B. abortus* are listed as potential bio-weapons by the Centers for Disease Control and Prevention in the USA. This is due to the highly infectious nature of all three species, as they can be readily aerosolized (Acha *et al*., 2003).

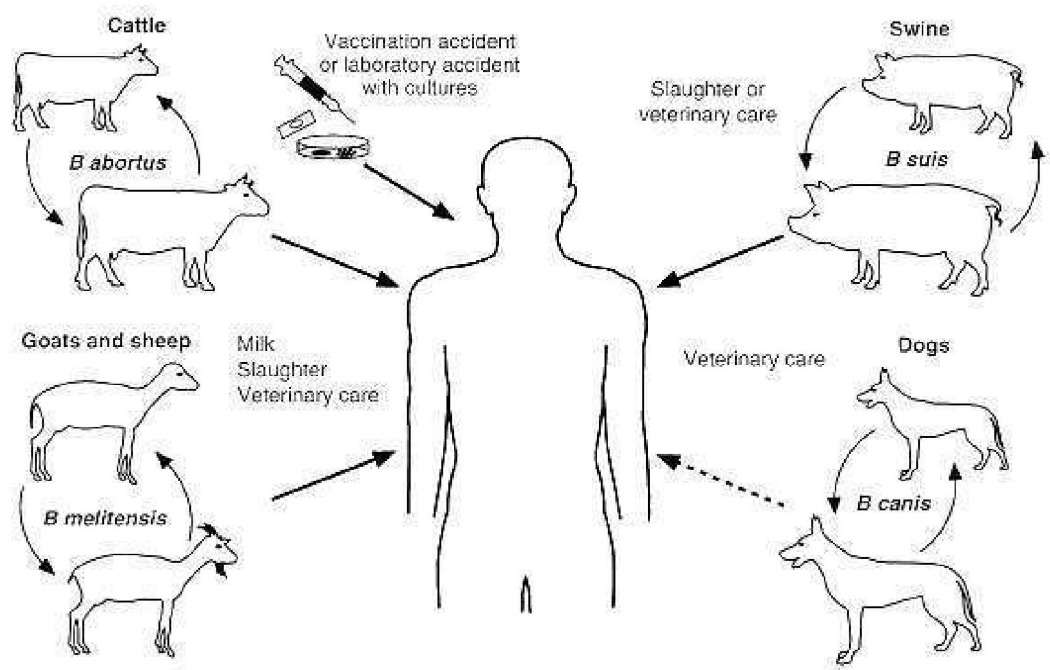

Figure 1: Different source of brucellosis in human (Mangiamele *et al*.,1994).

2.1. Risk Factors for Human Exposure

2.1.1. Occupational exposure

Brucellosis is an occupational hazard with those particularly at risk either living in close proximity with animals or handling them. These include people who work with farm animals, especially cattle, sheep, goats and pigs; farmers, farm labourers, 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. Infection may occur by inhalation, conjunctiva contamination, accidental ingestion, skin contamination especially via cuts or abrasions, and accidental self-inoculation with live vaccines. Also humans get infected by direct contact with infected animal products, ingestion of contaminated food, and inhalation of contaminated aerosols during laboratory works (Ngenzi, 2011). It is important tonote that *B. canis* in culture, like all Brucellae, poses a significant occupational risk of infection to laboratory staff (FAO,2003).

Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughtermen, butchers, meat packers, collectors of fetal calf serum, processors of hides, skins and wool, renderers and dairy workers. The abattoir workers have high chance to be under risk of Brucellosis case and this may be due to high proximity they have to the Brucella microorganisms from that of infected animals organs and parts especially uterus and udder, which come to the abattoir to be slaughtered (Ngenzi, 2011).

Under absence of strict safety precautions in laboratory, the workers become infected seriously by Brucellaagents that found in the infected sample like discharge from the reproductive organs, sample from aborted fetus, milk sample taken from infected dairy animal and any potentially contaminated materials. Inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) accidentally can also occur, resulting in human infections (FAO, 2003).

2.1.2. Feeding behavior

The majority of human brucellosis cases ingestion of unpasteurized dairy foods produced from unlicensed family owned flocks whose products are sold door-to-door at low prices. Camel milk is a known source of infection for humans those who consume unpasteurized raw camel milk. cheese made from sheep and goat milk is preferably prepared from untreated milk and by the use of rennet from lambs and kids that may have come from *Brucella* infected animals. During the course of cheese manufacture, any Brucella present in the milk become trapped in the clot and thus concentrated in the cheese, although bacteria may subsequently be inactivated by manufacturing or ripening processes (SCAHAW, 2001).

Brucella dies off fairly rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5. Equipment used in the transport or processing of infected milk or other raw material may contaminate uninfected products unless good hygienic practice is observed. Meat products are less frequently associated with infection, mainly because they are not usually eaten raw. However, this is a not unknown practice among butchers and abattoir workers (SCAHAW, 2001; Acha *et al*., 2003).

2.1.3. Age and sex

Majority of cases are males between the ages of 20 and 45 years. In these situations, the disease is usually caused by *B. abortus*or *B. suis*. In countries or areas where *B. melitensis*is prevalent, the practices followed in marketing and distributing sheep and goat milk products in particular make the enforcement of hygienic measures very difficult. In this situation the whole population is at risk and many cases occur in women and children. In nomadic societies, the adults have often been exposed to infection at an early age and do not manifest acute disease, although many may have sequelae from chronic infection. Under such conditions children account for a high proportion of acute cases and brucellosis is largely a pediatric problem (FAO, 2006).

2.1.4. Pregnancy and breast feeding

Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for Brucella organisms. Although erythritol is not present in human placental tissue, Brucella bacteremia can result in abortion, especially during the early trimesters. Whether the rate of abortions from brucellosis exceeds rates associated with bacteremia from other bacterial causes is unclear. In any event, prompt diagnosis and treatment of brucellosis during pregnancy can be lifesaving for the fetus. Very rare human-to-human transmission from lactating mothers to their breastfed infants has been reported (FAO, 2006)

2.1.5. Seasons

In humans, prevalence of the disease is high in summer season. Notifications of human brucellosis, which are mandatory in Italy, reach a peak between April and June. However, considering the standard incubation period of 2-4 weeks, and the fact that lamb slaughter is traditionally at a peak during the Easter period, it might be expected that occupational exposure would result in a peak of human cases between March and May. The observed peak between April and June could be related to the production and consumption of fresh cheese, starting just after lamb slaughter (Gul and Khan, 2007).

2.1.6. Bioterrorism

Brucella could be used to attack human and animal populations. The organism can be obtained from natural sources in many parts of the world. *Brucella melitensis* and *B.suis* have been developed experimentally as biological weapons by state sponsored programmes. Their relative stability in aerosol form combined with low infectious dose make them suitable agents for this purpose (FAO, 2006).

2.1.7. Travel-acquired brucellosis

Tourists or business travellers to endemic areas may acquire brucellosis, usually by consumption of unpasteurized milk or other dairy products. Travellers 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 (Glynn and Lynn, 200).

3. Clinical Signs

The main symptom in pregnant female is abortion (premature or full term birth of dead or weak calves) usually in the second half of gestation with retention of placenta and metritis (Kahler, 2000). There is an estimated 25% reduction in milk production in infected cows (Acha *et al.,* 2003). The brucellae localize in the supra-mammary lymph nodes and mammary glands of 80% of the infected animals and thus continue to secrete the pathogen in milk throughout their lives (Hamdy and Amin, 2002). Most infected cows abort only once although the placenta will be heavily infected at subsequent apparently normal calvings (Morgan, 1969).

Brucellosis in goats is characterized by late abortion, stillbirths, decreased fertility and low milk production (Lilenbaum *et al.,* 2007). Sheep brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and constitutes a major public health threat equal to goat brucellosis (Acha *et al*., 2003). Besides the abortion, swine may also develop orchitis, lameness, hind limb paralysis, or spondylitis; occasionally, metritis or abscesses (Musa *et al.,* 2008).

Camels can be infected by *B. abortus* and *B. melitensis* when they are pastured together with infected sheep, goats and cattle. The main etiologic agent for dog brucellosis is *B. canis*, but sporadic cases of brucellosis in dogs caused by *B. abortus*, *B. suis* and *B. melitensis* have been reported. Dogs infected with *B. canis* may have reproductive related conditions (abortions during the last third of a pregnancy, stillbirths, or conception failures) and/or nonreproductive tract related conditions (including ocular, musculoskeletal, or dermatologic lesions) (Wanke, 2004).

4. Diagnostic Methods

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk. Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens (Corbel, 2006).

4.1. Bacteriological Methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme. 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).

4.1.1. Stained smear

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Kosters' methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as Coxiellaburnetii or Chlamydia may superficially resemble *Brucella* (Corbel, 2006).

4.1.2. Culture

Brucella may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem. Brucellas are excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media. It is of the utmost importance that faecal and environmental contamination of the material is kept to a minimum to give the greatest chance of successfully isolating Brucella. If other material is unavailable or grossly contaminated, the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of *Brucella*. In some circumstances it may be appropriate to attempt the isolation of *Brucella* post-mortem. Suitable material includes supramammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus (Corbel, 2006).

4.2. Serological Methods

4.2.1. Rose bengal test

The RBT is one of a group of tests known as the buffered *Brucella* antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The RBT is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones (Corbel, 2006).

4.2.2. Enzyme linked immune sorbent Assays (ELISA)

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal 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. 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 RBT, sometimes they do not detect infected animals which are RBT positive. It is also important to note that ELISAs are only marginally more specific than RBT or CFT (Corbel, 2006).

4.2.3. Complement fixation test (CFT)

The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff. If these are available and the test is carried out regularly with good attention to quality assurance, then it can be very satisfactory. It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody isotypes competing for binding to the antigen. At higher dilutions these are diluted out and complement is fixed. Such positive samples will be missed if they are only screened at a single dilution. In other cases, contaminating bacteria or other factors in serum samples fix or destroy complement causing a positive reaction in the test, even in the absence of antigen. Such “anti-complementary” reactions make the test void and a CFT result cannot be obtained (Corbel, 2006).

4.3. Molecular Methods

PCR assays differentiating between Brucella species and/or biovars tend to be more complex and consequently more difficult to perform because appropriate target sites are rare in *Brucella* due to the remarkable homogeneity of the genus. Discrimination of multiple species simultaneously utilises one of two approaches. The first approach includes complex reaction mixtures containing multiple primer pairs, each targeting a unique species-specific DNA sequence polymorphism. The second approach uses a single primer pair to amplify a DNA sequence containing internal species-specific polymorphism. Subsequently, the internal polymorphism is confirmed by some other method downstream. Based on these two approaches, multiplex PCR assays for identification and differentiation of Brucella species and/or biovars such as AMOS and BaSS were developed (Jamba, 2008).

More recently, promising results in the typing of *Brucella* strains for epidemiological trace-back were obtained using variable number of tandem repeats analysis (VNTR), the methods being multilocus VNTR analysis and the hyper variable octameric oligonucleotide finger-prints (HOOF-Prints) as its variant (Bricker *et al.,* 2003; Jamba, 2008).

5. Economic Importance

Brucellosis is a major importance in economy of affected countriesdue to veterinary and human health impact. Among thegenus *Brucella*, *B. melitensis*, *B. abortus*, *B. suis*, and *B. ovis* which preferentially infect sheep and goats, cattle, pigs and sheep, respectively are the most important from a socio-economic standpoint. In addition to decreasing productivity in animals, the first three species are the main ones responsible for brucellosis in human beings (Miguel *et al*., 2011).

Costs include production loss associated with infection in animals, preventive program, and in human diseasecost of treatment and absenteeism from work brings many economical impacts. Losses in animal production dueto brucellosis disease can be of major important, primarily because of the decreased milk production by abortingdairy animals; the common sequel of infertility increases the period between lactation, and in an infected herdthe average inter calving period may be prolonged by several months. This is of greatest importance in beef herds where the calves represent the sole source of income (Smits, 2007).

A high incidence of temporary and permanent infertility results in heavy culling of valuable and some deaths occur as the result of acute metritis following retention of the placenta. The effect of the disease on ram’s fertility can influence the number of rams that are required in a flock; the required ram to ewe ratio is significantly reduced in *B. ovis* free flocks. The percentage of lambs born early and within the first three weeks of the lambing period is also markedly increased (Radostits *et al*., 2000).

6. Status Of Brucellosis In Ethiopia

In Ethiopia higher brucellosis seroprevalence has been recorded in Borena Zone of Oromia Region, the highest seroprevalence (50%) was documented using ELISA (Alem and Solomon, 2002). A seroprevalence of 39% was also recorded at the Institute of Agricultural Research in Western Ethiopia, 22% in Dairy Farm in Northeastern Ethiopia 11 to 15% in dairy farms and ranches in Southeastern Ethiopia (Bekele *et al.,* 2000), 8.2% in Arsi area 8.1% in dairy farms in and around Addis Ababa (Asfaw *et al*., 1998), and 7.7% in Tigray region (Haileselassie *et al*., 2010).

In Ethiopia, Brucellosis is highly prevalent in traditional pastural communities such as Borana, Metema and Hamer. Risk factors for brucellosis in these communities are living in close proximity of livestock, milking livestock, consumption of raw meat, milk and fresh cheese. The blood sample collected from (34.1%) patients from Borana, (29.4%) from Hamer and (3%) from Metema tested in the *Brucella* of the 38 seroposetive patients (61.7%) were acute (14.7%) sub-acute had persistent disease *(*Ragassa *et al*., 2009).

Studies on the prevalence of brucellosis have been carried out in many parts of Ethiopia by different persons. These studies were conducted in local and cross breed animals. In these studies prevalence of brucellosis in cattle ranging from 0.2-22% were recorded.

Table 2: Summary on prevalence of brucellosis in Ethiopia

Area	**Breed**	**n***	**Prevalence**	**Authors**
Tigray	Cross	816	3.19%	Gebretsadik(2005)
Bahir Dar	Local Cross	1135 811	0.26% 2.5%	Mussie(2005) Kassahun(2004)
Sidama Zone	Local Cross	1627 805	1.7% 0.8%	Tadele(2004)
Jimma Zone	Local Cross	1305 4243	0.2% 22%	Mekonen(2002)

n*=Total number of animal tested

Source: (Teshager *et al*., 2014)

7. Prevention And Control

7.1. Prevention

7.1.1. Animal

Prevention of brucellosis can be adopted realistically through understanding of local and regional variations in animal husbandry practices, social customs, infrastructures and epidemiological patterns of the disease. The common approaches used to control brucellosis include, quarantine of imported stock, hygienic disposal of aborted fetuses, fetal membrane and discharges with subsequent disinfection of contaminated area. Animals which are in advanced pregnancy should be kept in isolation until parturition (Bshop *et a*l., 1994). Moreover replacement stock should be purchased from herd free of brucellosis, and decide for or against immunization of negative animals. Eradication by test and slaughter of positive reactors is also possible (Walker, 1999).

7.1.2. Human

The most rational approach for preventing human brucellosis is control and eradication of the infection in animal reservoirs. In addition there is a need to educate the farmers to take care in handling and disposing of aborted fetus, fetal membrane and discharges as well as not to drink unpasteurized milk and abattoir workers in transmission of infection especially via skin abrasion (Walker, 1999). The drug recommended is rifampcin at dosage of 600 -900 mg daily combined with doxicycline at 200 mg daily. Both drugs are given in the morning as a single dose and relapse is unusual after a course of treatment continued for at least 5 weeks (WHO, 1997).

Muscle tissue is unlikely to contain more than low concentrations of *Brucella* organisms and their numbers are further reduced if the meat is stored correctly before consumption. Kidney, liver, spleen, udder and testes may contain much larger numbers. None of them present a serious hazard from brucellosis if thoroughly cooked. However, in some cultures, raw or undercooked meat may be eaten through choice. This practice and the consumption of fresh blood, either alone or mixed with milk, should be discouraged (Blasco *et al.,*1998).

The handling and preparation of infected meat and offal without proper hygienic precautions may be also lead to the contamination of other foods. Drying, salting and smoking are not reliable methods for killing Brucella. Similarly, the organisms survive well under refrigeration or deep freeze conditions. It is strongly recommended that all meat products are thoroughly cooked before consumption(Almuneef and Memish, 2003).

7.2. Control

An effective control of animal brucellosis requires the following elements: surveillance to identify infected animal herds, prevention of transmission to non-infected animal herds, and 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. 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 eliminated. 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 (Giannacopoulos *et al,* 2002*).*

7.2.1. Treatment

It is mostly not successful because of intracellular sequestration of the organisms in the lymph nodes, mammary glands and reproductive organs. If it is necessary the treatments often given are, sulphadiazine, streptomycin, chlortetracycline and chloramphenicol (Radostits *et al.,*2006).

The optimal treatment for brucellosis is a combination regimen using two antibiotics since monotherapies with single antibiotics have been associated with high relapse rates (Pappas *et al.,* 2006; Seleem *et al.,* 2009). 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 (Alp *et al*., 2006; Seleem *et al*., 2009).

Neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *brucellae* (Shasha *et al.,* 1994). Although the DS regimen is considered as the goldstandard treatment, it is less practical because the streptomycin must be administered parenterally for 3 weeks. A combination of doxycycline treatment (6 weeks duration) with parenterally administered gentamicin (5 mg/kg) for 7 days is considered an acceptable alternate regimen (Solera *et al.,* 1995). Although DS combinations had been considered by the WHO to be the standard therapy against brucellosis for years, in 1986 the Joint FAO/ WHO Expert Committee on Brucellosis changed their recommendations for treatment of adult acute brucellosis to rifampicin (600–900 mg/day orally) plus doxycycline (200 mg/day orally) DR for 6 weeks as the regimen of choice. However, the studies that compared the effectiveness of DR regimen with the traditional DS combinationconcluded that DR regimen is less effective than the DS regimen especially in patients with acute brucellosis (Glynn and Lynn, 2008).

7.2.2. Test and isolation/slaughter

The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered. In most cases, test and slaughter of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (e.g. 2%). Retention of positive animals is less hazardous if the remaining animals have been vaccinated but should only be considered as a last resort. The isolation of test-positive animals is essential, especially during and after parturition (Seleem *et al*., 2008).

The immediate slaughter of test-positive animals is expensive and requires animal owner cooperation. Compensation is usually necessary. Furthermore, the application of test and slaughter policies is unlikely to be successful with brucellosis of sheep and goats where the diagnostic tests are less reliable than in cattle. Test and slaughter is also unlikely to be successful in cattle if the remainder of the herd is unvaccinated, especially in large populations. Repeated herd or flock tests are necessary to further reduce the incidence of brucellosis and to confirm elimination (Moriyon *et al*., 2004).

7.2.3. Vaccines

A vaccines like *B. abortus* strain 19, which is a live vaccine and is normally given to female calves aged between three and six months as a single subcutaneous dose of 5-8×10^{10} viable organisms. A reduced dose from 3×10^8 to 3×10^9 organisms can be administered subcutaneously to adult cattle. Alternatively, it can be administered to cattle of any age as two doses of 5-10^9 viable organisms, given by the conjunctival route. This reduces the risk of abortion and excretion in milk (OIE, 2009). The protection on a herd basis is much greater due to reduction of clinical symptoms and increased herd resistance (Seifert, 1996).

Strain 19 vaccine leads to the production of antibodies whose persistence depends mainly on the age of the animals at the time of vaccination. Based on test and slaughter coupled with control by vaccination, for a policy of eradication to be successful, there must be rigid control of the age at which strain 19 vaccination is allowed (Briones *et al*., 2001).

B. melitensis strain Rev1 although highly infectious to human, is considered as the best vaccine available for the control of ovine and caprine brucellosis, especially when administrated at the standard dose by the conjunctival route. However, the Rev1 vaccine shows a considerable degree of virulence and induces abortions when administered during pregnancy. Also, the antibody response to vaccination cannot be differentiated from the one observed after field infection, which impedes control programs. Attempts have been made to develop new live attenuated rough *B. melitensis* vaccines, which are devoid of the O-side chain. Those vaccines await further evaluation in field experiments (Adone *et al.,* 2008).

Vaccination alone will not eradicate *Brucella* as the immunity produced by *Brucella* vaccines are not absolute and can be circumvented by increasing the level of infection. It is obvious, therefore, that a policy of vaccination is more likely to succeed if combined with good measures of husbandry Live human vaccines *B. abortus* strain 19-BA and strain 104M are being used only in the former Soviet Union and China, respectively (Acha *et al*., 2003).

7.2.4. Control animal movement

Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests. In practice, it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries (Corbel, 2006).

8. Conculusion And Recommendations

Brucellosis is world wide and has high prevalence in many African countries. The spread of the disease from one herd to another and from one area to another is almost always due to the movement of infected animals from an infected herd into a non-infected susceptible herd. Brucellosis affected both animals and humans, has a very high public health impact. Its impact on Public health is very well related to the infected animal species from which human transmission occurs. The disease transmits from infected animals to human beings through several routes. It causes considerable losses in cattle as a result of abortion and reduction in milk yield. It has high economic impact. Even though the disease is prevalent in Ethiopia, the report does not support evidence at species level.

Based on the above concluding remarks, the following recommendations are forwarded:

❖ Different livestock species should be kept and maintained separately to reduce the risk of transmission of brucellosis among them.

❖ Public education and awareness rising among pastoralists, on the public health hazard of brucellosis should be undertaken.

❖ Further extensive epidemiological studies should be undertaken to investigate the transmission dynamics of brucellosis in human and animals in the study area.

❖ Molecular investigations of brucellosis should be conducted to identify the specific species prevailing in the study area.

Acknowledgements

First of all, I would like to give my special thanks to our Almighty God, with his mother St. Virgin Merry forever for his mercy guiding and helping.

Secondly, my sincere thanks are extended to my advisor, Dr. Fufa Abuna, for his unreserved support and guidance for the accomplishment of this work.

Last but not least, my special thanks goes to my lovely family and friends, Dr.Israle Yared and Mis Kidist Asnake who helped me in the compilation of this critical review work.

References

1. Acha, N. and Szyfres, B. (2003): Zoonoses and Communicable Diseases Common to Man and Animals, 3rd ed. Pan American Health Organization (PAHO), Washington, DC.
2. Adone, R., Francia, M. and Ciuchini, F. (2008): Evaluation of *Brucella melitensis* B115 as rough-phenotype vaccine against B. melitensis and B. ovis infections. *Vaccine*, 26: Pp. 4913–4917.
3. AGHA. (2002): Australian Government, Department of Health and Ageing. Brucellosis Laboratory Case.
4. Alem, W. and Solomon, G. (2002): A retrospective sero-epidemiology study of Bovine Brucellosis in different Production Systems in Ethiopia. In: Proceeding of 16th *Annual Conference*, Pp.53-57.
5. Almuneef, M. and Memish, Z. (2003): Prevalence of Brucella antibodies after acute brucellosis. *Journal of Chemotherapy*, 15: 148–51.
6. Alp, E., Koc, R., Durak, A., Yildiz, O., Aygen, B., Sumerkan, B., Ariza, J., Gudiol, F., Pallares, R., Viladrich, P., Rufi, G., Corredoira, J. and Miravitlles, M. (2006): Doxycycline plus streptomycin versus ciprofloxacin plus rifampicin in spinal brucellosis. *BMC Infect. Dis.,* 6: Pp. 72.
7. Arenas, G., Staskevich, A., Aballay, A. and Mayorga, L. (2000): Intracellular trafficking of *Brucella* abortus in J774 macrophages. *Infect. Immun.,*68: 4255–63.

8. Asfaw, Y. (1998): The epidemiology of brucellosis in intra and peri urban dairy production system in and around Addis Ababa. Faculty of Veterinary Medicine, Addis Ababa University, DebreZeit, Ethiopia.
9. Bekele, A. Molla, B., Asfaw, Y. andYigezu, L. (2000): Bovine brucellosis in ranches and farms in Southeastern Ethiopia. Bull. *Anim. Hlth. Prod. Afric.,*48: Pp. 13-17.
10. Bekele, M. (2004): Sero-epidemiolgical study of Brucellosis in Camels in Borena Lowland Pastoral Areas, Southern Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. Partial fulfillment of Degree of Master of Science in Tropical Veterinary Epidemiology.
11. Blasco, J., Grayon, M. and Verger, J. (1998): *Brucella melitensis* infection in sheep: present and future. *Veterinary Research*, 29: 255–280.
12. Boschiroli, M., Ouahrani-Bettache, S. and Foulongne, V. (2002): The *Brucella suis* virB operon is induced intracellularly in macrophages. *Proc. Natl. Acad. Sci. USA*, 99: Pp. 1544–49.
13. Bricker, B., Ewalt, D. and Halling, S. (2003): Brucella HOOF-Prints: strain typing by multi-locus analysis of variable number tandem repeats (VNTRs). *BMC. Microbiol.*,11: Pp. 3:15.
14. Briones, G., Inon de Iannino, N., Roset, M., Vigliocco, A., Paulo, P. and Ugalde, R. (2001): Brucella abortus cyclic beta-1,2-glucan mutants have reduced virulence in mice and are defective in intracellular replication in HeLa cells. *Infect. Immun.*, 69: Pp. 4528–4535.
15. Caro-Hernandez, P., Fernandez-Lago, L., De Miguel, M., Martin- Martin, A., Cloeckaert, A., Grillo, M. and Vizcaino, N. (2007): Role of the family in outer membrane properties and virulence of *Brucella* ovis. *Infect. Immun.,*75: Pp. 4050–4061.
16. Cecil Textbook of Medicine. (2000): Saunders, Philadelphia USA, 21: 171-179.
17. Celli, J., Salcedo, S. and Gorvel, J. (2005): *Brucella* coopts the small GTPase Sar1 for intracellular replication. *Proc. Natl. Acad. Sci. USA,* 102: Pp. 1673–1678.
18. Corbel, M. (2006): Brucellosis in humans and animals. Produced by the World Health Organization in collaboration with the Food and Agriculture Organizationof the United Nations and World Organisation for Animal Health.
19. FAO, WHO and OIE. (2006): Brucellosis in Humans and Animals. Produced by the World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organization for Animal Health.
20. FAO. (2003): Guidelines for coordinated human and animal brucellosis surveillance. FAO, Animal Production and Health Paper, Pp. 156.
21. Gebresadik, B. (2005): Seroepidemiological study of bovine brucellosis in Tigray Region, Northern Ethiopia. MSc Thesis, FVM, AAU, DebreZeit, Ethiopia.
22. Giannacopoulos, I., Eliopoulou, M., Ziambaras, T. and Papanastasiou, D. (2002): Transplacentally transmitted congenital brucellosis due to *Brucella abortus. J. Infect.*, 45: Pp. 209-211.
23. Glynn, M. and Lynn, T. (2008): Brucellosis. *J. Am. Vet. Med. Assoc.*, 233: Pp. 900–908.
24. Godfroid, J., Scholz, H. and Barbier, T. (2011): Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Prev. Vet. Med.*, 102: Pp. 118-131.
25. Gotuzzo, E., Gorbach, S., Barttlett, J. and Black-low, N. (1998): Brucellosis Infectious diseases. 2nd edition. Philadelphia: WB Sunders, Pp. 498-503.
26. Gul, T. and Khan, A. (2007): Epidemiology and Epizootology of Brucellosis. *Pakistan Vet. Journal,* 27: Pp. 145-151.
27. Haileselassie, M., Shewit, K. and Moses, K. (2010): Serological survey of bovine brucellosis in barka and arado breeds (*Bosindicus*) of Western Tigray, Ethiopia. *Prev. Vet. Med.*, 94: Pp. 28-35.
28. Hamdy, M. and Amin, A. (2002): Detection of Brucella species in the milk of infected cattle, sheep, goats and camels by PCR. *Vet. J.,* 163: Pp. 299–305.
29. Heymann, D. (2008): Control of communicable diseases manual. 19th ed. Washington, DC: American Public Health Association. *J. Antimicrob. Chemother.*, 33: Pp. 545–551.
30. Jamba, Z. (2008): New Procedures for the Diagnosis of Human Brucellosis in Mongolia. PHD, Dissertation, University of Giessen, Germany.
31. Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson, H. and Kindahl, H. (2009): Epidemiological study of bovine brucellosis in three agro ecological areas of central Oromiya, Ethiopia. *Rev. SciTech Off. Int. Epiz.*, 28: Pp. 933-943.
32. Kadiohire, M., McDermott, J., Shoukri, M. and Kyule, M. (1997): Variation in the Prevalence of antibody to Brucella infection in cattle by farm area and district in Kenya. *Epidemiol. Infect.*, 118: Pp. 35-41.
33. Kahler, S. (2000): Brucella melitensis infection discovered in cattle for first time, goats also infected. *J. Am. Vet. Med. Assoc.,*216: 648-650.

34. Kassahun, A. (2004): Epidemiology of brucellosis in cattle and its seroepidemiology in animal health professionals in Sidama zone, South, *MSc Thesis*, FVM, AAU, Debrezeit, Ethiopia.
35. Kohler, S., Foulongne, V., Ouahrani-Bettache, S., Teyssier, J. and Ramuz, M. (2002): The analysis of the intramacrophagicvirulome of *Brucella* suisdeciphers the environment encountered by the pathogen inside the macrophage host cell. In: Proceedings of the National Academy of Sciences of the United States of America, 99: Pp. 15711-15716.
36. Kulkarni, R., Sneha, K., Chunchanur, A., Shubhada, C. and Pavitra, J. (2009). Presumptive diagnosis of Brucella epididymoorchitis by modified cold ZN staining of pus sample. Indian *Journal Med Res*., 130: Pp. 484-486.
37. Lapaque, N., Moriyon, I. and Gorvel, J. (2005): *Brucella* lipopolysaccharide acts as a virulence factor. *Curr. Opin. Microbio.,* 8: 60–66.
38. Lilenbaum, W., Souza, G., Ristow, P., Moreira, M., Fraguas, S. and Oelemann, W. (2007): A serological study on Brucella abortus, caprine arthritis-encephalitis virus and Leptospira in dairy goats in Rio de Janeiro. *Braz. Vet. J.,* 173: 408–412.
39. Mc Donald, W., Jamaludin, R., Mackereth, G., Hansen, M., Humphrey, S., Short, Whatmore, A. and Simmons, G. (2006): Characterization of a Brucella sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *J. Clin. Microbiol.*,44: 4363–4370.
40. Mangiamele, D., Chomel, B. and DeBess, E. (1994): Changing trends in the epidemiology of human brucellosis in Californiya from 173 to 1992: at shift towards food borne transmission. J. Infect. *Dis*., Pp. 170-1216.
41. Megid, J., Antonio, L. and Carlos, A. (2010): Clinical Manifestations of Brucellosis in Domestic Animals and Humans. *The Open Veterinary Science Journal*, 4: Pp. 119-126.
42. Mekonnen, G. (2002): Sero-epidemological investigation of bovine brucellosis in North Eastern Ethiopia. A report submitted to Agricultural Bureau of Amhara National Regional State Baher Dar, Ethiopia.
43. Miguel, D., Marín, M., Mun, M., Diesteoz, L., Grillo, M. and Blasco, J. (2011): Development of a Selective Culture Medium for Primary Isolation of the Main *Brucella* Species. *Journal of clinical microbiology, Pp.* 1458-1463.
44. Moreno, E. and Moriyon, I. (2002): *Brucella melitensis*: a nasty bug with hidden credentials for virulence. *Proc. Natl. Acad. Sci. U.S.A.,* 99: 1-3.
45. Morgan, W. (1969): Brucellosis in animals: diagnosis and control. *Proc. R. Soc. Med.,* 62:1050-1052.
46. Moriyon, I., Grillo, M., Monreal, D., Gonzalez, D., Marin, C., Lopez-Goni, I., Mainar-Jaime, R., Moreno, E. and Blasco, J. (2004): Rough vaccines in animal brucellosis: structural and genetic basis and present status. *Vet. Res*., 35: 1-38.
47. Musa, M., Eisa, Abdel- Wahab, M. and Perrett, L. (2008): Brucellosis in camels (Camelusdromedarius) in Darfur, Western Sudan. *J. Comp. Pathol.,* 138: 151-155.
48. Muss, H. (2005): Sero-prevalence study of brucellosis in cattle and human in Baher Dar milk shed. MSc Thesis, FVM, AAU, Debre-zeit, Ethiopia.
49. Ngenzi, V. (2011): Seroprevalence of Human Brucellois in Rwanda. National University of Rwanda Faculty of Science Biology Department of Option zoology and Conservation. Memoir submitted for partial fulfillment of the Bachelor's degree in Biology.
50. OIE. (2009): Bovine brucellosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Office *International Des Epizootics, Paris*, Pp. 409-435.
51. Oxford Textbook of Medicine (1996). Oxford, UK.,3: Pp. 619-23.
52. Pappas, G., Panagopoulou, P., Christou, L. and Akritidis, N. (2006): Brucella as a biological weapon. *Cell Mol. Life Sci.*, 63: 2229–2236.
53. Pei, J., Turse, J. and Ficht, T. (2006): *Brucella abortus* rough mutants induce macrophage oncosis that requires bacterial protein synthesis and direct interaction with the macrophage. *Infect. Immun.,* 74: 2667–2675.
54. Porte, F., Naroeni, A. and Liautard, J. (2003): Role of the *Brucella suis* lipopolysaccharide O antigen in phagosomal genesis and in inhibition of phagosome-lysosome fusion in murine macrophages. *Infect. Immun.,*71: Pp.1481–90.
55. Radostits, E., Gay, C. and Inchcliff, K. (2006): Veterinary Medicine Textbook of the disease of cattle, sheep, pigs, goats, and horses, 9th ed., Newyork: W. B. Sounders Company ltd., Pp. 867-882.
56. Radostits, E., Gay, C. and Inchcliff, W. (2000): Veterinary Medicine, Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th ed., New York, W. B. *Saunders Company Ltd.*, Pp. 867-882.
57. Radostits, O., Gay, C., Blood, C. and Hinchclift, W. (2007): Veterinary Medicine, Textbook of

the Diseases of Cattle, Sheep, Pigs, Goats and Horses., 10th ed., London, UK., Pp. 963-994.

58. Ragassa, G., Mekonnen, D., Yamuah, L., Tilahun, H., Guta, T., Gebreyohannes, A. and Aseffa, A. (2009): Human brucellosis in Traditional pastoral communities in Ethiopia. *Int. J. Trop. Med.,* 4: 59-64.
59. SCAHAW, (2001): Brucellosis in Sheep and Goat (*Brucella melitensis*). Health and Consumer protection Directorate General, Europian Commission.
60. Seifert, H.S.H. (1996): Brucellosis In: Tropical-Animal Health. 2nd ed. Klywer Acadamic Publishers, London.
61. Seleem, M., Boyle, S., Sriranganathan, N. (2008): Brucella: a pathogen without classic virulence genes. *Vet. Microbiol.,*129: Pp.1–14.
62. Seleem, M., Jain, N., Pothayee, N., Ranjan, A., Riffle, J. and Sriranganathan, N. (2009): Targeting *Brucella* melitensis with polymeric nano particles containing streptomycin and doxycycline. *Fem. Microbiol. Lett.,*294: Pp. 24–31.
63. Shasha, B., Lang, R. and Rubinstein, E. (1994): Efficacy of combinations of doxycycline and rifampicin in the therapy of experimental mouse brucellosis.
64. Smits, H., Franco, M., Mulder, M. and Gilman, R. (2007): Human brucellosis. *Lancet Infect. Dis.*, 7: Pp. 775–86.
65. Sohn, A., Probert, W., Glaser, C., Gupta, N., Bollen, A., Wong, J., Grace, E. and Mc Donald, W. (2003): Human neurobrucellosiswith intracerebral granuloma caused by a marine mammal Brucella spp. *Emerg. Infect. Dis.*, 9: Pp. 485–488.
66. Solera, J., Martinez-Alfaro, E. and Espinosa, A. (1995): Recognition and optimum treatment of brucellosis. *Drugs*, 53: Pp. 245–256.
67. Sriranganathan, N., Mohamed, N. and Stephen, M. (2010): Brucellosis: A re-emerging zoonosis. *Vet. Microbiol.,*140: Pp. 392–398.
68. Tadele, T. (2004): Sero-prevalence study of bovine brucellosis and its public health significance in Jimma zone, Western Ethiopia.
69. Teferi, D., Asmamaw, D. and Reta, D. (2011): Brucellosis and Some Reproductive Problems of Indigenous Arsi Cattle in Selected Arsi Zone's of Oromia Regional State, Ethiopia. *Global Veterinary,* 7: Pp. 45-53.
70. Teshager, D., Mulie, A., Tesfaye, S. and Yimer. (2014): Global Research Journal of *Public Health and Epidemiology*,1: Pp. 054-064.
71. Tigist, A., Yosefe, D. and Tadele, T. (2011): Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia African *Journal of Microbiology Research,* 5: Pp. 1682-1686.
72. Tumurkhuu, G., Koide, N., Takahashi, K., Hassan, F., Islam, S., Ito, H., Mori, I., Yoshida, T. and Yokochi, T. (2006): Characterization of Biological activities of *Brucella melitensi* lipopolysaccharide. *Microbial. Immunol.,*50: Pp. 421–427.
73. Wadood, M., Ahmad, A., Khan, S., Gul. and Rehman, N. (2009): Seroprevalence of Brucellosis in Horses in and around Faisalabad. *Pakistan Vet. Journal,* 29: 196-198.
74. Walker, L., Dwight, C., Hirsh, D. and Zee, Y. (1999): Brucella. In *Veterinary Microbiology Black GG science.*, Pp. 196-202.
75. Wanke, M. (2004): Canine brucellosis. *Anim. Reprod. Sci.*, Pp. 195–207.
76. WHO. (1997): Emerging and Communicable Disease surveillance and control. The development of new brucellosis. Report of the WHO meetings, Genava, December, Pp. 41-47.

4/15/2017