Vol. 7(7), pp. 513-529, November 2019
DOI: 10.14662/ARJASR2019.185

ISSN: 2360-7874
http://www.academicresearchjournals.org/ARJASR/Index.htm

Academic Research Journal of Agricultural Science and Research

Full Length Research

Review on Camel Brucellosis: Public health importance and status in Ethiopia.

Fekadu Gutema [*1] and Juhar Tesfaye [*2]

[1]Ethiopian Institute of Agricultural research, Addis Ababa P.O. BOX 2003.
[2] Ethiopian Institute of Agricultural research, Addis Ababa P.O.BOX 2003

Corresponding Author: fikadu881@gmail.com

Accepted 11 November 2019

In many developing countries of Africa and Asia, camels are the most important source of income for the Pastoral population through the provision of milk, meat, and transportation service. The chief role of the camel relates directly to its remarkable adaptation to extremely harsh conditions. However, these contributions of camels to the human welfare of developing countries are generally obscured by several factors, among which disease is the basic one. Brucellosis is one of the common bacterial diseases of a camel which is caused by genus Brucella resulting in substantial loss of production. Currently, ten species of brucella are recognized among which six of them are classical species. In dromedary camels, brucellosis can be caused by *B. abortus*; *B. melitensis* and *B. ovis* which results in significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production. The disease is found widespread in camel rearing regions of the world whereas age; sex; management and husbandry practice are considered as a major risk factor. The prevalence of camel brucellosis which was reported from different pastoral areas of Ethiopia is quite varying. Additionally; research conducted on camel brucellosis is scarce and is limited only to serological study with no confirmed isolation of Brucella bacteria. In humans, brucellosis is a debilitating disease with nonspecific symptoms and infertility being the common sequelae. The disease is transmitted by contact with infected animals; consumption of unpasteurized dairy products and undercooked meat. To treat brucellosis in humans, several conventional antibiotics are used in clinics and can also be controlled through milk pasteurization and hygienic measures coupled with effective disease surveillance and animal movement control. Generally, to combat the public health and economic significance of camel brucellosis detailed studies for isolation and characterization the causative agent of camel brucellosis should be implemented along with enhancing awareness level of the society.

Key words: Brucellosis, Camel, Ethiopia, zoonosis, Seroprevalence, Pastoralist

Cite this article as: Fekadu G., Juhar T (2019). Review on Camel Brucellosis: Public health importance and status in Ethiopia. Acad. Res. J. Agri. Sci. Res. 7(7): 513-529

INTRODUCTION

Camel (*Camelus dromedarius*) belong to the family of *Camelidae* and have an effective socio-economic role in different parts of the world with dry and semi dry climatic condition (Alamian and Dadar, 2019). The major roles of camel are associated directly to its impressive adaptation to extremely harsh situations due to several anatomical and physiological characteristics (FAO, 2001). However, these contributions of camels to the human welfare of developing countries are generally undermined by several factors (Yaqoob and Nawaz, 2007). Although

camels were considered previously, as resistant to many disease causing agents, it has been proved that they are susceptible to the common disease causing pathogens (Gwida *et al*., 2012) due to severe stress conditions (Ducrotoy *et al*., 2017).

Numerous studies revealed that; camels are highly susceptible to certain bacterial pathogens among which brucellosis is the basic one which substantially minimize their production potential. Brucellosis is caused by Brucella bacteria, and currently ten species are recognized including the better known six classical species(Khamesipour *et al*., 2015). In camel brucellosis is mainly caused by *B. abortus*, *B. melitensis* and *B. ovis*(Musa *et al*., 2008; Alshaikh *et al*., 2007). Based on the reports of different scholars, *B. abortus* and *B. melitensis* are the most frequently isolated *Brucella spp* from milk, aborted fetus and vaginal swabs of diseased camels. Even though camels are not known to be the primary hosts of Brucella, they are susceptible to both *B. abortus* and *B. melitensis* and consequently, the prevalence depends upon the infection rate in primary hosts being in contact with them(Robayo and Esubalew, 2017).

Brucella bacteria can enter into the body of animals through inhalation, ingestion and through mucous membrane or broken skin. In camel brucellosis is characterized by abortion, infertility, placentitis in females, and orchitis and epididimits in males(Jafer, 2018). In Ethiopia, brucellosis is found to be one of the diseases associated with reproductive wastage in camel producing pastoral areas. Significant loss of productivity through delayed first calving age, prolonged calving interval , low herd fertility and comparatively low milk production in camels was reported (Abebe *et al*., 2017).

Additionally, this disease has imposed a restricted to livestock trade and free movement animals. In human, brucellosis is a debilitating disease that lacks pathognomonic symptoms(Ducrotoy *et al*., 2017), representing a major public health hazard which affects social wellbeing and stability in many countries. OIE sketch brucellosis as the second most important zoonotic disease in the world, accounting for the annual occurrence of more than 500,000 human cases(Hull and Schumaker, 2018). The disease spread when people consume unpasteurized contaminated milk and contact with infected tissues and discharge including consumption of raw liver. Brucellosis is characterized by none specific symptoms such fever, chills, headache, pain, fatigue, dementia, and arthritis, which occur within 2-3 weeks of inoculation. Consequently, the associated complications includes:- osteoarticular complication, gastro-intestinal complications, genitourinary complications, neurological complications, cardiovascular complications and others(Zerfu *et al*., 2018). Based on the nature of the disease and ease of transmission, the pastoral society are at great risk due to their close physical contact with susceptible animals(Abbas and Agab, 2002).

Camel brucellosis has a worldwide distribution were camel raring are being practiced and has been reported in different regions of Africa, Asia, Latin America and the middle East(Bamaiyi *et al*., 2014). In Ethiopia, the eastern and southern parts of Ethiopia, namely, Afar, Somali and Borena are the major areas where camel husbandry is widely practiced to insure livelihood of the pastoral communities(Hadush *et al*., 2013). In these regions and others, brucellosis in animals and humans has been reported where the prevalence was quite varying under different agro-ecology(Yilma *et al*., 2016). Consequently, this disease has resulted in significant economic and public health problem in the stated area. So, to effectively control camel brucellosis, it is paramount important to establish diagnostic and surveillance systems, by estimating the cost-benefits of control measures (Bayasgalan *et al*., 2018). For accurate diagnosis of camel brucellosis, serological tests like RBPT are cheap and easy for herd based screening of animals with high sensitivity and low specificity(Ullah, 2015) whereas tests like ELISA and CFT are used for confirmatory test.

Generally, despite the presence of large population of camel in the pastoral areas of Ethiopia(Hadush *et al*., 2013; Tilahun *et al*., 2013), reports of camel brucellosis and studies of management practices are limited. Additionally, even if the disease is one of the oldest recognized diseases of mankind and get controlled in most developed countries(Sprague *et al*., 2012), only little effort has being made to control this disease in developing countries specially in Ethiopia due to the nature of diseases .Therefore this review paper is written with following objectives:

- To review the distribution, zoonotic importance, and current status of camel brucellosis in Ethiopia.
- To indicate and highlight existing research gap concerned with camel brucellosis in Ethiopia

LITERATURE REVIEW

Etiology

Brucellosis is an infectious bacterial disease caused by the genus Brucella, affecting a wide range of warm-blooded land and marine vertebrates. Camels can be infected by either of the main species of the genus Brucella (*B.abortus* and *B.melitensis*)(Abbas and Agab, 2002). Brucella are Gram-negative facultative intracellular cocco-bacilli that are non-encapsulated, non-spore forming and non-motile belonging to the alpha-2 subdivision of the proteobacteria(Seleem *et al*., 2010).

Certain strains of brucella bacteria need about 5% to 10% of CO_2 for growth. Brucella organisms grow slowly,

but can be enhanced by using enriched media. Ten Brucella species are currently recognized, including the better known six classical species comprised of *B. abortus* (biovars 1-6, and 9), *B. melitensis*, (biovars 1-3), *B. suis* (biovars 1-5), *B. ovis*, *B. canis* and *B. neotomae*. The more recently, identified new members brucella species include; *B. ceti* and *B. pinnipedialis* , *B. microti* (voles) and B. inopinata (Godfroid *et al*., 2011) . Brucella species are distinguished based host preference and phenotypic characteristics(Seleem *et al*., 2010;O'Callaghan and Whatmore, 2011) . However, host preference is not absolute and most of the species of Brucella bacteria have been isolated in multiple different hosts(Potter, 2013).

For instance, some Brucella species like *B. abortus, B. melitensis, B. suis* and *B. canis* can affect a range 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(Liu, 2014). In camel *B. abortus* and *B.melitensis* are the major causative agents of brucellosis even though camels are not found to be their primary host. Complete genome sequences of *B. abortus, B.melitensis, B. suis, B. canis*, and *B. ovis* are available showing that, their similarity in size and genetic make-up (Meng *et al*., 2009).

In humans the majority of cases are attributed to *B. melitensis* and is also the most pathogenic and virulent species. *B.melitensis* affects almost all domestic animals and many wild animals species(Benkirane, 2006; Pappas *et al*., 2006). (Table 1)

Genome and morphology of Brucella

Due to its great economic and zoonotic importance, it is useful to identify field isolates of Brucella not only at their species level but also their genotypes. This enables the detection of hidden foci of Brucella and to tract the sources of infection in the population(Jafer, 2018). For instance, genotypic analysis of different *B. abortus* field strains isolated from cattle, bison and elk showed that the cattle isolates are closely related to elk isolates but completely divergent from those of bison(El-Sayed and Awad, 2018).

The genomes sequenced from genus Brucella are 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 3.3 Mbp divided on two chromosomes. Housekeeping genes, including those involved in DNA replication, transcription, translation, core metabolism, and cell wall biosynthesis are distributed on both chromosomes(O'Callaghan and Whatmore, 2011). None of the sequenced members of the Brucella genus have any plasmids reported. *B. melitensis* is the first Brucella species to be sequenced 16M (biovar 1) followed closely by B. suis (biovar 1)(Bohlin *et al*., 2010).

Comparison of *B. suis* with *B. melitensis* indicates that the majority (>90%) of *B. suis* and *B. melitensis* genes share 98-100% identity at the nucleotide level. The more variable genes (<95% identity) consist primarily of hypothetical genes, urease component, and probable surface-exposed genes (e.g. outer-membrane proteins, membrane transporters, a putative invasin, and ShdA-like adhesins). These more variable genes may contribute to the differences in pathogenicity or host preference between these two organisms(Liu, 2014)

Brucella are very small faintly stained coccoid rods, with a microscopic appearance of fine sand. Primary culture of brucella reveals punctate, non-pigmented, and non-hemolytic colonies. Colonies of smooth(S) brucella strain are raised, convex, circular, translucent and 0.5-1 mm in diameter. The colony morphology of brucella may become less convex and more opaque with a dull, dry, yellowish, white granular appearance which is caused by dissociation of brucella from smooth to rough(R) forms(Liu, 2015).

EPIDEMIOLOGY OF CAMEL BRUCELLOSIS

Global distribution of camel brucellosis

Brucellosis is a worldwide bacterial disease affecting both animals and humans which subsequently causes serious human health hazards and economic loss. The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging(Seleem *et al*., 2010). Brucellosis was reported in camels as early as in 1931 by Solonitsiun in Russia. Since then, serological evidence of brucellosis has been reported from the most important camel keeping countries(Bayasgalan *et al*., 2018). Camel brucellosis is a wide spread disease in camel rearing regions of the world such as middle East and the Arabian Gulf, parts of Africa, and Latin America with the exception of Australia (Potter, 2013, Robinson, 2003, Wernery, 2014)

According to Gizaw *et al*., 2017, a seroprevalence of camel brucellosis ranging between 2% to 5% was reported in most countries where camels are still kept by nomadic or transhumant pastoralists and extensive form of husbandry is practiced. Additionally, Gul and Khan, 2007 reported seroprevalence of camel brucellosis ranging from 0.0- 17.20% in Arabian and African countries where the disease also occurs in buffalo and other domestic animals. In Saud Arabia *B. abortus* was detected in 8.98% of camels with diarrhea and concluded that the frequent traveling of camels through different countries in the Arabian Peninsula could constitute a transmission risk across borders(Al-Ruwaili *et al*., 2012).

The differences in the prevalence of camel brucellosis

Table 1: Species of Brucella isolated from camel in Africa and Middle East

Country	Species isolated	Sample examined	Test employed	Reference
Iran	*B.melitensis*biovar 2	Milk, placenta Vaginal swab	Culture method	(Zowghi *et al*., 2008)
Iran	*B.abortus B.melitensis*	Blood Lymph node	PCR	(Khamesipour *et al*., 2015)
Kuwait	*B.abortus* biovar 1	Aborted fetus	PCR	(Sultan and Abdalla, 1989)
Saud Arabia	*B.abortus*	Serum	PCR	(Alshaikh *et al*., 2007)
Sudan	*B.melitensis,*biovar 3 *B.abortus*biovar 6	Lymph node	PCR	(Musa *et al*., 2008)
Oman	*B.melitensis*	Aborted fetus Vaginal swab Milk	PCR	(Foster *et al*., 2018)
Egypt	*B.melitensis*biovar 3	Milk	PCR	(Ibrahim1 *et al*., 2016)
Iran	*B.abortus*	Milk	PCR	(Alamian and Dadar, 2019)

Notes: PCR (Polymerase chain reaction), RBPT (Rose bengal plate test), CFT (compliment fixation test) S.examined(sample examined), T.employed(Test employed)

from different countries may be attributed to varying husbandry and management practices, the number of susceptible camels, the virulence of the organisms, presence of reactor animals in the region, absence of veterinary service, lack of awareness about the disease in camels and continuous movement of infected camels into a susceptible herd(Gwida *et al*., 2012).

Distribution of camel brucellosis in Africa

The occurrence of camel brucellosis in sub-Saharan Africa (either prevalence or incidence) is not well documented and reports submitted to the World Organization for Animal Health (OIE) are largely confined to serological surveys, which are mainly conducted for cattle, sheep, goats and less for camel. With large pastoral communities, and the demand for meat and livestock products is simulated to double by 2050, brucellosis is expected to poses a major threat to this region*(Racloz et al*., 2013).

According to Ekere *et al*., 2018, the disease has a cosmopolitan distribution, and affects economically important domestic animals such as camel including wild life. Persistent case of brucellosis was observed in most African countries like Tanzania, Nigeria, Uganda, Kenya, Zimbabwe and Somalia reporting brucellosis in humans and domestic animals such as: cattle, camels, goats and sheep(Racloz *et al*., 2013). In East Africa, brucellosis is reported in most member countries of IGAD and endemic with high economic loss and zoonoses(Zewdie and Mamo., 2018).

In countries with more extensive form of husbandry practice, such as Chad and Ethiopia, the seroprevalence of camel brucellosis is 3.8% and 5.5% respectively(Wernery, 2014). In Nigeria, the disease has been reported from nearly all camel producing areas(Salisu *et al*., 2018).

Distribution of camel brucellosis in Ethiopia

Seroprevalence of camel brucellosis in Ethiopia

There are about 50 to 100 million pastoralists globally and the majorities are confined to Africa. Ethiopia has the largest pastoral population of 7 to 8 million where the majorities of these people are living in the Ethiopian Somali and Afar administrative Region(Bekele *et al*., 2013). In Ethiopia, brucellosis has been reported in camels from pastoral areas, where the prevalence was quite vary ranging between 0.73- 11.9% for RBPT and 0.53-9.6% for CFT(Yilma *et al*., 2016).

This variation in seroprevalence of camel brucellosis attributed to the difference in animal husbandry and management systems practiced by pastoral society(Awole *et al*., 2002). In Ethiopia, pastoralists used to consume raw milk, which contributes to the transmission of this disease among human and animals. Above three-quarters of the pastoralists are practicing at least one activity considered to be risky for the transmission and widespread occurrence of zoonotic brucellosis and more than 75% of the animal owners do not know about zoonotic Camel brucellosis(Gwida *et al*., 2010).

A study conducted by Getahun and Belay,(2002) on camel husbandry practice in eastern part of the country indicated abortion rates and stillbirths of 9% and 4.3%, respectively, for which brucellosis is more likely to be incriminated. This is due to a large numbers of different species of animals raised together on communal pastures and watering point which allows close contact of infected and health animals of different species. Compared with other neighboring African countries and middle East, lower seroprevalence of camel brucellosis was recorded by*(*Tilahun *et al*., 2013)in pastoral area of Ethiopia. (Table 2)

Table 2: Seroprevalence of camel brucellosis in Ethiopia

District	N_0.A	Sample taken	Test employed	prevalence	Reference
Afar	768	Serum	RBPT CFT	11.9%(RBPT) 7.6%(CFT	(Zewold and Mekonnen, 2012)
	460	Serum	mRBPT CFT	5.4%	(Bekele *et al*., 2013)
	1152	Serum	RBPT CFT	58%(RBPT) 47%(CFT)	(Hadush *et al*., 2013)
	813	Serum	RBPT CFT	2.09%	(Gebrezgabher and Mohammed, 2016)
Somali, Afar and Oromia	1442	Serum	RBPT CFT	82%(RBPT) 4.2%(CFT)	(Teshome *et al*., 2003)
Southern Ethiopia	1830	Serum	RBPT ELISA	0.9%	(Gumi, 2013)
Akaki	201	Serum	RBPT CFT	6.5%(RBPT) 4.5%(CFT)	(Abebe *et al*., 2017)
Jigjiga and Babile	822	Serum	RBPT CFT	2.43%	(Tilahun *et al*., 2013)
Dire dawa	646	Serum	RBPT CFT	2%(RBPT) 1.5%(CFT)	(Warsame *et al*., 2012)
Borana	756	Serum	RBPT CFT	2.2%	(Megersa et al., 2011)
	1073	Serum	RBPT CFT	1.8%	(Megersa *et al*., 2012)
Bale and Borana	1500	Serum	RBPT CFT	0.53%	(Tesfaye *et al*., 2014)
Yabello	384	Serum	RBPT CFT	3.6% 3.1%	(Admasu and Kaynata, 2017)

Notes: N_0. A= (Number of animals examined)

RISK FACTORS

Animal risk factors

Age

Age has been referred to as one of the intrinsic factors associated with brucellosis in animals. According to Bekele *et al*., 2011 report, brucellosis has traditionally been considered as a disease of adult animals since susceptibility increases after sexual maturity and pregnancy. This is due to the fact that, *Brucella spp*. presents tropism to the reproductive tract due to the production of erythritol sugar in the foetal tissues(Paridah *et al*., 2016). Long time contact with infected animals or with the environment also contributes to the higher prevalence of brucellosis in adults animals which is significantly seen in those herd without culling of positive animals(Megersa *et al*., 2012)

Sex

The influence of sex in the prevalence of brucellosis has been studied in domestic and wild animals(Muñoz *et al*., 2010). In camels, females are more susceptible to brucellosis than male. This relatively higher susceptibility of female camels could be due to the fact that they have more physiological stresses than the males(Salisu *et al*., 2018). According to Hirsh and Zee, 1999, male animals are less susceptible to Brucella infection due to the absence of erythritol sugar which is found in the uterus. Also female camels are kept longer in herds for breeding purposes than male camels which are fattened and sold off except for a few that are kept to service the females, for haulage, transport and other such purposes(Salisu *et al*., 2018).

Environmental and Management risk factors

Brucellosis can occur in any season of a year. However,

February to July is the season of the year when peak epidemics of brucellosis occurs and is closely related to the months associated with delivery and abortion in animals (Deqiu *et al*.,2002). Uncontrolled trade of clinically inconspicuous animals leads to high individual animal and herd prevalence which do not only pose a continuous risk for human infection, but also increase the spread of infection through several risk factors. Habitat, herd size, cohabitation with other ruminants, and contact with other camels, leads to an inter-camel cycle of the disease(Ghanem *et al*., 2009)

Further risk factors are the increase in species composition at household level, and the wet season. Due to this, camels appear to become infected via spill-over from small ruminants and cattle. This observation is supported by the fact that all *Brucella* spp. and biovars infecting other ruminants have also been isolated from camels. According to Musa *et al*., 2008 report, the higher prevalence of brucellosis (23.8%) from camel kept mixed with ruminant species was recorded. The epidemiology of camel brucellosis can also be influenced by management system where the higher prevalence of the disease was recorded in camels kept under intensive management system (Abbas and Omer, 2005).

Pathogen risk factors

B. abortus and B.melitensis are the etiological agents of camel brucellosis and responsible for an economically important cause of abortions. *B. abortus* also affects other species such as bison, buffalo or elks representing an important risk for the maintenance of the agent in the animal population with special importance in areas where wildlife and domestic animals live together. Moreover, infections in wildlife can hinder eradication efforts in domestic animals. *B. abortus* is still a human pathogen and outbreaks arise from contact with infected animals and ingestion of contaminated dairy products represent an important risk of infection(Paridah *et al*., 2016)

TRANSMISSION OF CAMEL BRUCELLOSIS

The primary shedding routes of Brucella organisms remain uterine fluids and placenta expelled from infected animals. Due to this, both domestic and wild animals can contract brucellosis through direct contact with infected animals and their excreta. Many placental mammals, including herbivores, participate in placentophagy, with camel as a noted exception, which may contribute to the spread of Brucella bacteria through wind. Although parturition in camels is generally occurred in a laying or standing position without extra help, they may deliver or abort on the pasture and the aborted material may spread over a wide area of the pasture by stray dogs and foxes. This play an important role for the transmission of the disease to other health animals(Gwida et al., 2012).

On the other hand, a close contact between infected and susceptible camels in a herd promotes the spread of diseases. The camels share the same watering points and pastures with other livestock and so it is not surprising to find a higher incidence of the disease among camels (Teshome et al., 2003). Many researchers disclosed that, survival of the organisms in the environment is enhanced by cool temperatures and humidity which allows maintenance of the bacterial in the environment for fairly long period of time where many susceptible animals can be exposed(Wernery, 2014). However, it was proven that two dromedaries in a Brucella negative dromedary herd were infected with *B. melitensis* through contaminated dust particles from aborted camel fetuses 500 m apart, indicating that organisms can also survive in a hot desert environment(Wernery, 2014).

Generally, Animals become infected through feed, water, colostrum, contaminated milk and, especially, by licking or sniffing at placentas and aborted fetuses. In human, brucellosis is transmitted by contact with infected animal, consumption of unpasteurized dairy products and undercooked meat, drinking camel urine(Salisu et al., 2018)including aerosol transmission (Minogue *et al*., 2014). For instance, consumption of traditional delicacies such as raw liver can cause human infection(Gwida *et al*., 2010). (Figure 1&2)

Wajir County in the northern Kenya is mainly inhibited by Somali community. Residents of this county used to drink camel urine because they believe that it eliminate all the illness in the body which is the predisposing factor for the zoonotic transmission of camel brucellosis. This condition started to be practice in Gawane district of Afar Region in Ethiopia (personal observation).

PATHOGENESIS

Brucella organisms are pathogens that ultimate goal is to propagate in their preferred niche, the cell. The ability of *Brucella spp*. to cause disease requires a few critical steps during infection. Although the mechanisms that allow host cell invasion by *Brucella spp*. are not completely clear, internalization of *Brucella* into host cells requires cytoskeletal changes. *Brucella spp*. can invade epithelial cells of the host, allowing infection through mucosal surfaces: M cells in the intestine have been identified as a portal of entry for *Brucella spp*(Poester *et al*., 2013). Upon cell contact, the bacteria are internalized via receptor molecules by activating small GTPases of the Rho subfamily and by a moderate recruitment of actin filaments(Gorvel and Moreno, 2002).

Interestingly, invasion through the digestive tract does not elicit any inflammatory response from the host and therefore, *Brucella spp*. invade silently or unnoticed by the innate immune system of the host(Barquero-Calvo *et*

Figure 1: Unhygienic milking and drinking of raw camel milk
Source: (Own photo, MOA, 2017)

Figure 2: Pastoralist collecting and drinking Camel milk
Source: (K24 TV, 2018).

al., 2007). Once *Brucella spp*. have invaded, usually through the digestive or respiratory tract, they move to regional lymph nodes and are capable of surviving intracellularly within phagocytic or non-phagocytic host cells with the help of enzyme called cytochrome oxidase(Seleem *et al*., 2008). On the other hand, Acidification of the Brucella containing vacuole during early steps of infection is also required for intracellular survival since acidified environment induces changes in the profile of bacterial gene expression favoring intracellular survival(Neta *et al*., 2010).

So, the pathogenicity of Brucella is due to its ability to adapt to the environmental conditions encountered in its intracellular replicative niche including low levels of nutrients and oxygen, acidic pH and reactive oxygen intermediates(Seleem *et al*., 2008). Inside the cells, *Brucella* has the ability to interfere with intracellular trafficking, preventing fusion of the *Brucella* containing microphages(phagosomes) with lysosome markers, and directing the vacuole toward a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of *Brucella* (Marchesini *et al*., 2011).

Then*, Brucella spp* disseminate throughout the body and induces suppression of the transcription of pro-inflammatory mediators in trophoblastic cells at very early stages of infection in female(Neta *et al*., 2008). After an initial suppression of pro-inflammatory transcripts, brucella bacteria induces expression of pro-inflammatory chemokines which finally results in abortion in female animals*(*Neta *et al*., 2008)

The outcome of Brucella infection depends on the animal species infected, age, immune status of the host, pregnancy status, and the virulence and the number of

invading organism. When the bacteria prevail over the host's defenses of susceptible pregnant animal; bacteremia often leads to the invasion of the uterus. Generally, Localization of *Brucella* bacteria within the female and male reproductive tracts accounts for the most common clinical signs of infection: abortion and male infertility(Poester *et al*., 2013).

CLINICAL SIGN

The clinical picture of brucellosis in camels can vary from asymptomatic to abortion(Musa *et al*., 2008). According to various researchers, the clinical signs of brucellosis in breeding camels are the same as those in bovines and small ruminants, although infection in breeding camel causes fewer abortions than it does in bovines and small ruminants. Abortion in camel due to brucellosis usually occurs only once. Dams can develop ovario-bursal adhesions, hydrobursitis, and granulomatous endometritis. Placental retention, infertility, and delayed sexual maturity have also been reported(Rafieipour and Ziaei, 2011). Males may suffer from orchitis, infection of the accessory sex glands, arthritis accompanied by acute lameness(Sprague *et al*., 2012).

Some authorities feel that the most significant result of infection may be premature birth. Brucellosis also causes fetal death and mummification and reduced milk yield. It was reported that delayed service age and fertility are also another complication associated with brucellosis. However, placental retention is rare in camel due to the difference in the placental attachment as they possess a diffuse like placenta (Fowler *et al*., 2010).

DIAGNOSIS

Establishment of adequate control programs against brucellosis in a population depends on the presumptive diagnosis of the infection. Brucellosis may be suspected based on clinical signs such as abortion, but confirmation can be made through serological tests. Since 1897, a considerable number of serological tests have been developed. A number of these tests were modified in various ways to increase performance(Nielsen, 2011). Serological tests offer best alternatives to culture and isolation method of diagnosis since the tests are easy to perform, less risky and provide result within a short period. On the other hand, brucellosis can be diagnosed definitively by isolation and identification of the causative organism. This was first reported by Bruce and co-workers in 1887 when they isolated *B. melitensis* from military personnel in Malta(Nielsen, 2011).

The diagnosis of brucellosis by culture and isolation of organisms from clinical samples is the gold standard method. But this method is laborious, time consuming, and risky, whereas the outcome of the test depends on the competence of the laboratory personnel. In clinical brucellosis, valid samples to diagnosis the disease include aborted fetuses (stomach, spleen, and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm, and fluid collected from arthritis or hygroma(Godfroid *et al*., 2010).

At slaughter, in order to confirm suspected cases of acute or chronic brucellosis, the preferred tissues are the genital and oropharyngeal lymph nodes, the spleen, and the mammary gland and associated lymph nodes(Godfroid *et al*., 2010). The presence of anti-Brucella antibodies suggests exposure to *Brucella* spp. But it does not indicate which *Brucella* spp induced production of those antibodies(Godfroid *et al*., 2010).

Conventional methods

Bacteriological diagnosis

This refers to isolation and identification of *Brucella* from clinical samples. The morphology of the Brucella bacterial colonies is associated with the presence of lipopolysaccharides (LPS) in the external membrane of the bacterium. Smooth (S-LPS) and rough (R-LPS) phenotypes are differentiated. The S-LPS phenotype is found in most Brucella species, and only B. canis and B. ovis possess the R-LPS(Wernery, 2014).

Brucellosis is usually diagnosed in the laboratory by the culture of blood, milk or tissue or the detection of antibodies in sera. Brucella organisms can be recovered from the placenta, but, more conveniently, in pure culture from the stomach and lungs of aborted fetuses. For isolation of brucella, the recommended medium is Farrell's medium, which contains six antibiotics. But other selective Brucella media are also in use for the growth of this pathogen from fresh Camel milk and other tissue samples (Radwan *et al*., 1995).

Serological diagnosis

The majority of studies on camel brucellosis use serological methods for diagnosis. But none of the serological tests are validated for use in camels yet, as acknowledged by OIE. Similarly, none of the tests have been validated for the diagnosis of human brucellosis according to (Yohannes *et al*., 2012). However, it was found that a combination of different serological tests can increase diagnostic efficacy in camels, although none of the serological tests can differentiate between a *B. abortus* or *B. melitensis* or *B. suis* infection. On the other hand, false-positive or unspecific reactions with various other bacterial species such as *Yersinia enterocolitica* serotype O: 9 can occur(Wernery, 2014).

Rose Bengal plate test (RBPT)

Among many types of serological test employed for diagnosis of brucellosis in camel and other domestic animals, RBPT is a widely used screening test for regulatory control and export requirements. Rose Bengal Plate Test (RBPT) 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(Hotam Singh Chaudhary, 2011).

RBPT is a very sensitive test and is suitable for screening herds for brucellosis, but it can give false positive results due to vaccination with *B.abortus* strain 19 vaccine or cross reactions with other bacteria(Omer *et al*., 2010). The RBPT has been reported to have high sensitivity; therefore false negative responses are reported to occur less frequently than false positive responses(Omer *et al*., 2010). It was reported by (Chachra *et al*., 2009) that, among the commonly used conventional serodiagnostic tests for brucellosis, RBPT and STAT may not be absolutely reliable. RBPT detected antibody in the sera of 50% of the animals suspected for brucellosis whereas, STAT could detect only 5.55% cases according to (Chachra *et al*., 2009)report.

Complement fixation test (CFT)

The Complement Fixation Test (CFT) allows the detection of anti-Brucella antibodies that are able to activate complement. Many authors regarded the CFT as being the most sensitive and specific test for brucellosis diagnosis. Because CFT antibodies remain in the serum for longer period of time than SAT antibodies(Njeru *et al*., 2016). On the contrary, some authors disclosed that this test is not highly sensitive but shows an excellent specificity. In the recent year CFT is progressively being replace by ELISAs since it is difficult to be standardized. Nevertheless, CFT is a “prescribed test for trade” by the OIE(Godfroid *et al*., 2010).

Enzyme Linked Immuno sorbent Assay (ELISA)

ELISAs are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs). Most iELISAs use purified smooth LPS as antigen and detect mainly IgGs or IgG sub-classes. Their main quality is their high sensitivity but they are also more vulnerable to non-specific reactions, notably those due to YO9 infection(Godfroid *et al*., 2010). ELISA was first developed for the diagnosis of human brucellosis. The ELISA tests offer an 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. A comparison with the SAT, ELISA yields higher sensitivity and specificity. ELISA is also reported to be the most sensitive test for the diagnosis of neurobrucellosis(Miguel *et al*., 2006) . The omp28 protein is now being used in an indirect plate ELISA system and has been evaluated with good sensitivity and specificity on large number of clinical samples(Hotam Singh Chaudhary, 2011).

Molecular methods

Polymerase Chain reaction (PCR)

The isolation of Brucella organisms is still the preferred method of diagnosis. But, PCR method allows typing of the isolated strains. PCR based assays have been developed for brucellosis diagnosis and are based on the detection of specific gene sequences of the pathogens. One of the first PCR assays to differentiate among *Brucella spp* was called AMOS- PCR, developed by Bricker and Halling in 1994. This PCR uses a single reverse primer, targeting the *Brucella* specific insertion element IS711(Ewalt and Bricker, 2000) Even though PCRs can discriminate between Brucella species and between wild and vaccine strains, but it does not discriminate between Brucella biovars. In recent time new PCR techniques are being implemented for both identification and phenotypic biotyping(Ron-Román *et al*., 2019).

Multiple Locus Variable Number tandem repeat Analysis (MLVA)

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms which is developed by Le Fleche and co-workers(Le Flèche *et al*., 2006). It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms and is the current gold-standard of Brucella typing(Georgi *et al*., 2017).

This method has been used to type various species and strains of *Brucella* with fine scale resolution of closely related isolates (Gyuranecz *et al*., 2016). Because of its rapidity, highly discriminatory power and reproducibility, it has been suggested that MLVA assay can be useful in epidemiological trace-back analysis of *Brucella* infections with the potential to advance surveillance and control of brucellosis(Al Dahouk *et al*., 2007).

PUBLIC HEALTH IMPORTANCE OF CAMEL BRUCELLOSIS

Significance and source of infection

Brucellosis is a systemic infection that can involve any

organ or organ system of the body. Since many cases of brucellosis go unrecognized, the true incidence of the disease is unknown. In human, the disease is common in rural and pastoral areas, because farmers or pastoralists live in close contact with their animals and often consume fresh unpasteurized dairy products. In addition, pastoralist handle aborted cases with bare hand which is the main predisposing factor of the disease in the area(Zewdie and Mamo, 2018). Food producing animals such as cattle, sheep, goats, pigs and camel are also the main sources of brucellosis to human being(Potter, 2013). The type of Brucella to which an individual exposed is a significant determinant factor of the risk of disease and its severity in humans. This will be influenced by the species of host animal acting as source of infection(Corbel, 2006).

Interpersonal and occupational transmission

Person to person transmission of brucellosis can rarely occur among innocent camel herders through close personal or sexual contact while occupational exposure usually resulting from direct contact with infected animals, and food borne transmission (Zewdie and Mamo, 2018). Blood donation/tissue transplantation and bone marrow transfer are the prominent interpersonal transmission ways of brucellosis. Even though, *B.abortus, B. suis* and *B. canis* are considered as potential causative agents of brucellosis in human, *B. melitensis* is the most virulent brucella with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection(Xavier *et al*., 2014).

Manifestation of brucellosis in human

Brucellosis may present with acute or insidious onset, with continued, intermittent or irregular fever of variable duration, profuse sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching. Abscess formation is a rare complication(Seleem *et al*., 2010). Brucella endocarditis and neurobrucellosis cause most deaths(Pendela *et al*., 2017). Sometimes, the manifestations of brucellosis are more pronounced in a specific organ system. The most common local manifestations are: spondylitis, peripheral arthritis (especially of the hip, knee and shoulder) and epididymo-orchitis(Colmenero *et al*., 1996). Arthritis and joint pain are common and usually migratory in character, affecting mostly the large joints, with unilateral joint involvement being more common among the younger age group(Memish and Balkhy, 2004). (Figure 3)

In human's brucellosis essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of raw contaminated milk constitutes the main risk to the general public where the disease is endemic. Though camel milk ingestion is a known mechanism for brucellosis acquisition, only a few reports of sporadic cases have been published in the medical literature(Shimol *et al*., 2012).

In Nigeria pastoralist believes that camel milk and urine, when consumed, serve as cure for various diseases including HIV/AIDS, epilepsy and various cancers(Salisu et al., 2018).Cheese made from camel milk plays an important role of transmitting Brucella bacteria from infected camels (Salisu *et al*., 2017).There is an occupational risk to veterinarians, abattoir workers and farmers who handle infected camel carcasses and aborted fetuses or placentas. Brucellosis is also one of the most easily acquired laboratory infections, and all laboratory manipulations with live cultures or potentially infected/contaminated material must be performed at an appropriate biosafety and containment level determined by biorisk analysis(OIE, 2018).

Public health importance of camel brucellosis in Ethiopia

As it was stated above, pastoral community of Ethiopia, mainly depends on camel and other domestic animals milk and milk product to fulfill their dietary requirement which is the well-known transmission route of brucellosis from camel to human(Cossins and Upton, 1987). On the other hand, traditional type of food animal slaughtering in non-hygienic methods are common practices which definitely downgrade the hygiene, safeness and wholesomeness of food of animal origin. Consumption of such contaminated food which may contain Brucella bacteria has the potential to cause an adverse health effect(Desta, 2016)

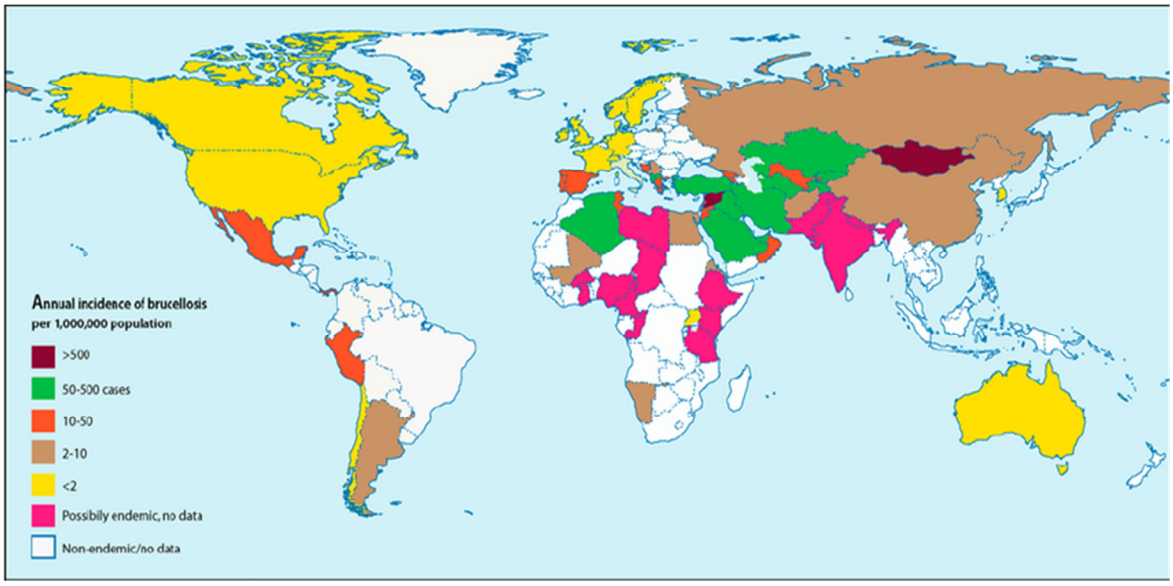
Somali regional state and Afar pastoralists do not use any protective materials during handling parturient camels, removing placenta and/or other aborted materials since most of the people had poor knowledge about brucellosis. So, these practices could potentially facilitate the transmission of zoonotic Brucella pathogens from camel to human. They also believed that, camel milk to possess superior shelf life, medicinal properties (against dropsy, jaundice, diabetes and glycaemia) (Bekele *et al*., 2013).

Generally, human brucellosis is increasing in Ethiopia like many other developing countries due to various sanitary, socioeconomic, and political factors (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 camel keepers is required(Catley *et al*., 2005). (Table 3)

TREATMENT

Treatment of brucellosis in Animals

As a general rule, treatment of infected livestock is not



Source:(Ariza *et al*., 2007)
Figure 3: Global incidence of human brucellosis

Table 3: Sero prevalence of Human Brucellosis in Pastoral an abattoir workers of Ethiopia

District	N_0.examined	Sample taken	Test employed	prevalence	Reference
Fafan zone	211	serum	CFT	0.4%	(Lakew *et al*., 2019)
Afar	200	serum	RBPT	16%	(Zewolda and Mekonnen, 2012)
			CFT	15%	
	630	Serum	RBPT	12.7%	(Zerfu *et al*., 2018)
	80	Serum	CFT	35%	
Bishoftu	149	serum	RBPT	4.7%	(Tsegay *et al*., 2017)
Modjo			CFT	1.3%	
Addis	360	serum	RBPT	-	(Kassahun *et al*., 2006)
Ababa			2-MET	4.8%	

Notes: N0. examined (Number of humans examined).

attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs(Yousefi-Nooraie *et al*., 2012). In developed countries, treatment of infected animal is not a common practice. However, the infected animals are isolated, culled or slaughtered to prevent the spreading of infection to another herd. Even though the complex nature of brucellosis makes it difficult to treat, long term treatment with an antibiotic is thought to be beneficial to care for economically valuable breeding male animal and must be instituted before irreparable damage to the epididymis has occurred(Alemneh and Akeberegn, 2018).

Treatment of brucellosis in Human

Humans are treated with antibiotics (doxicycline with rifamipicine) even though relapses are possible (Solis and Solera, 2012). Several conventional antibiotics including tetracyclines, trimethoprim, sulfamethoxazole, amino-glycosides, rifampicin, quinolones, chloramphenicol, doxycycline, and streptomycin are commonly used in clinics (Saltoglu *et al*., 2002). The World Health Organization recommends that acute brucellosis cases should be treated with oral doxycycline and rifampicin (600 mg for six weeks) (Ersoy *et al*., 2005). However, rifampicin monotherapy is in common practice for treating brucellosis in pregnant women, and combined therapy of sulphamethoxazole and trimethoprim is recommended for children(Karabay *et al*., 2004).

PREVENTION AND CONTROL OF CAMEL BRUCELLOSIS

Although brucellosis has been controlled in most industrialized nations, the disease has become a neglected zoonosis in some tropical or developing

countries due to lack of sustainability in the disease prevention and control programs(Ekere *et al*., 2018). According to Zhang *et al.,* 2018 report, brucellosis control or eradication programs, the implementation of the programs, and the control measures in different countries vary greatly depending on their own national conditions. Even with the high economic burden of the disease in many low-income countries, the disease does not attract the appropriate attention of national health systems(Godfroid *et al*., 2013).

In the developed world, the control of animal brucellosis has been approached with a combination of procedures such as: vaccination, test and slaughter programs whereas human brucellosis through milk pasteurization and hygienic measures coupled with effective disease surveillance and animal movement control(Godfroid *et al*., 2011). Control of camel brucellosis should be tailored to suit conditions in the particular countries where camels are raised. Most of these countries are poor and camels are raised by nomadic tribes. So control of camel brucellosis can be achieved through extending veterinary services to pastoral areas(Abbas and Agab, 2002).

It was suggested that, the preferred control strategy of camel brucellosis in high camel keeping country should be based on whole herd vaccination using S19 or Rev 1 vaccinal strains preceded by blood testing using the SAT or card test on the field. Seropositive animals should be identified by branding or special ear-mark and subjected to retesting. This marking will restrict the sale of seropositive animals. Camel calves should be vaccinated at 4-8 months of age, using a full adult dose of vaccine(Abbas and Agab, 2002, Dorneles *et al*., 2015)

Vaccination

Animal brucellosis control strategies differ in the developed and developing world. In developed world, most emphasis is given to eradication and risk analysis to avoid the re-introduction of *Brucella* while information related to the prevalence of brucellosis is still scarce and control programs are rarely implemented in developing world (Franc *et al*., 2018). However, vaccination is the cornerstone of control programs to prevent brucellosis in livestock in both developed and developing world. So, serious efforts of vaccination have been made to prevent the infection through the use of vaccines(Wernery, 2014).

Before vaccination is started in camels, thorough investigations are paramount important, in order to find out whether the animals are naturally infected by *B. abortus* or *B. melitensis* and this can only be determined by culture or PCR. An eradication campaign in camel may also be based on vaccination (Wernery, 2014). According to (Radwan *et al*., 1995), vaccination of camel with Rev 1 found to be effective, safe, successful and economically acceptable methods of controlling brucellosis in Saud Arabia.

Generally, the main approach in a long term control strategy of brucellosis is to vaccinate only female replacement camels less than 1-year-old (maturity in OWCs begins with 4 years). After several years, this strategy will establish an immunized herd and will not induce abortions. It will also protect these herds from brucellosis threat by surrounding positive livestock(Castillo *et al*., 2016).

Test and slaughter

Test and slaughtering of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (Luelseged, 2019) which is feasible only in developed world. The decision about slaughtering of test positive animals is made after regulatory, economic and prevalence factors are considered. In developing countries, the isolation of test positive animals is essential, especially during and after parturition since immediate slaughtering of test-positive animals is expensive and requires animal owner cooperation(Luelseged, 2019).

This indicates that, camel producers in developing countries cannot afford the traditional test and slaughter approach especially when expensive animals with high genetic potential are involved(Radwan *et al*., 1995). Furthermore, the application of test and slaughter policies work well only under reliable diagnostic tests to avoid unnecessary decision due to false positivity(Alp *et al*., 2006).

Hygienic prophylaxis

Application of hygiene measures to the control of brucellosis become successful through the reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g., commingling of herds or flocks), patterns of commerce, type of facilities, and degree of dedication of the owners of animals, will also determine success. However, owners have poor understanding about the transmission route of brucellosis in camels so that, separation of parturient animals, can be difficult or even impossible to implement(Glynn and Lynn, 2008) which is a conspicuous existing gap

CONCLUSION AND RECOMMENDATION

Camels play a paramount role to feed large population of the pastoral community especially in Middle East, sub-Saharan Africa including Ethiopia. On the contrary, this animal can act as a reservoir for different infectious agent and contributes a crucial role for the persistence of zoonotic disease in the Environment. Since pastoral

community mainly uses Camel milk to feed themselves, they are the number one victim to zoonotic diseases such as brucellosis and tuberculosis. Brucellosis is a zoonotic bacterial disease which results in significant economic lose and affect public health at large. In camel brucellosis can be transmitted by direct contact with infected animals and liking of the aborted fetus or new born calf which results in delayed first calving age, and reduced milk yield including still birth and abortion. In human; brucellosis can be acquired through drinking contaminated raw milk from infected camel and consumption of under cooked meat, direct contact with infected animals and probably through aerosol transmission. This is due to the fact that, more than 75% of the animal owners in pastoral area have no information about zoonotic camel brucellosis. In Ethiopia, research conducted on the camel brucellosis is scarce and is limited only to serological study with no confirmed isolation of *Brucella* bacteria. Age; sex; management and husbandry practice are considered as major risk factors whereas information related to vaccinating camel against brucellosis is not available. So, to combat the public health and economic significance of camel brucellosis

- Further studies for isolation and molecular characterization of the causative agent of camel brucellosis shall be proposed
- Detailed and comprehensive study plan to access the major risk factors aggravating the widespread occurrence and zoonotic transmission of the disease shall be designed
- Working to enhance the awareness level of the society about zoonotic disease. For instance, informing not to touch aborted fetal material without using protective wearing's in addition to abstaining them themselves from drinking raw milk.
- Collaborative work among human and animal health professionals.

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