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Epidemiology and Control of Bovine Theileriosis in Ethiopia: Review

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Abstract
Globally haemoprotozoan diseases are causing devastating losses to the livestock industry Bovine Theileriosis is also a tick-borne hemoprotozoan disease in cattle caused by several Theileria species and among them *T. parva* the cause of East Coast fever and *T. annulata* the cause of tropical theileriosis are most pathogenic and economically important. Despite widespread distribution of different Theileria species affecting cattle in Ethiopia the gap of well documented information exist and the paper focus on reviewing the epidemiology and control of bovine Theileriosis and highlighting the disease status in Ethiopia. Theileriae are obligate intracellular protozoan parasites and they have complex life cycles involving both vertebrate and invertebrate hosts. Tropical theileriosis and east Coast fever are disease transmitted through by Ixodid tick of genus Hyalomma and Rhipicephalus respectively. Furthermore, the sporozoites are transmitted to animals in the saliva of the feeding tick. PCR is the most beneficial molecular tool for diagnosis of infection till date than blood and lymph node smear examination and serological tests. Bovine Theileriosis has global economic significance thus prevention is the best method to control loss related with the disease. Among Several control methods the most practical and widely used method is the chemical control of ticks with acaricides. However, tick control practices are not always fully effective and vaccination is the most sustainable option. Since there is difference in breed of cattle to tick resistance the selection of tick resistant cattle breeds is also proposed as a sustainable approach for controlling infection in developing world. Currently occurrence tropical theileriosi is confirmed in Ethiopia thus, more research should be conducted to design and implement appropriate control strategies and prevent disease spreading.
Keywords: Bovine theileriosis, Control, Hemoprotozoan, Theileria

1. INTRODUCTION

Arthropod transmitted hemoparasitic diseases are economically important vector-borne diseases of tropical and subtropical parts of the world including Ethiopia (Sitotaw *et al*., 2014). They are of great economic impact on livestock affecting 80% of the world cattle population and causes economic loss due to morbidity and mortality (Kasozi *et al*., 2014). Haemoprotozoan diseases are causing devastating losses to the livestock industry and thus pose major constraints to the dairy industry throughout the world (Kohli *et al*., 2014; Bhatnagar *et al.*, 2015). Theilerioses is also a tick borne protozoal disease in ruminants caused by hemoprotozoan parasites belonging to the genus Theileria (Demessie and Derso, 2015). The parasites belonging to this genus are distinguished on the basis of a distinct group of unique organelles called apical complex (Gul *et al.,* 2015).

Theileriae are obligate intracellular protozoan parasites that infect both wild and domestic *Bovidae* throughout much of the world (some species also infect small ruminants). They are transmitted by ixodid ticks, and have complex life cycles in both vertebrate and invertebrate hosts (OIE, 2014). There are a number of species of *Theileria* spp. that infect cattle; the two most pathogenic and economically important are *T. parva* and *T. annulta (*OIE, 2014). So globally the most common cause of bovine theileriosis is *Theileria annulata* and *Theileria parva* (Kohli *et al.,* 2014; Gul *et al*., 2015; Bhatnagar *et al*., 2015; Gebrekidan *et al*., 2016). Theileria parva occurs in 14 countries (Tarimo, 2013) in sub-Saharan Africa causing East Coast fever (ECF) and still ranks first among the tick-borne diseases of cattle in sub-Saharan Africa (Nene *et al*., 2016), whilst *T. annulta* occurs in southern Europe as well as North Africa and Asia (OIE, 2014).

Tropical theileriosis, also known as Mediterranean coast fever, is an extremely fatal and debilitating tick-transmitted disease infecting cattle (Gul *et al.,* 2015). Tropical theileriosis is caused by *T. annulata* (Saeed *et al*., 2016) and transmitted through Ixodid tick of genus Hyalomma. About 250 million cattle are at risk to Tropical Theileriosis worldwide (Erdemir *et al*., 2012). *Theileria parva*, is also most pathogenic species in Africa the cause of another commercially important parasitic disease called East coast fever (Gachohi *et al.,* 2012) which is characterized by enlargement of superficial lymph nodes and a sustainable fever (Demessie and Derso, 2015). This infection causes mortality in about one million cattle annually in central, eastern and southern Africa. It threatens almost twenty five million cattle in Africa and also limits the introduction of improved breeds (Gul *et al.,* 2015). Hence the diseases have global serious economic impact in view of mortality, reduced milk yield, weight losses, abortions, and control costs prevention is the best mean to control (Gharbi *et al*., 2015). Several methodologies are currently available for the control of bovine theileriosis the most practical and widely used method is the chemical control of ticks with acaricides. However, tick control practices are not always fully effective and vaccination the most sustainable option (Morrison, 2015).

There are no clinical or serological reports of the presence of East Coast fever (*T. parva*) in Ethiopia but there is uncontrolled movement of livestock from Sudan, where these diseases and their vectors (the brown ear tick) are found, suggests that there is high risk to be introduced (Sileshi, 1996). Some of the past studies confirmed that *T. mutans*, T. velifera, and *T. orientalis* infect cattle in western, eastern, and southern Ethiopia (Solomon et al., 1998; Sileshi *et al*., 2011; Tomassone *et al*., 2012). Gebrekidan *et al*. (2014) also reported a widespread distribution of *Theileria* spp. among domestic ruminants in northern Ethiopia.

Bovine tropical Theileriosis is reported in Ethiopia for the first time in recent study by Gebrekidan *et al*. (2014) in which four species of Theileria were detected in cattle: T. velifera, T. mutans, *T. orientalis* complex and *T. annulata*. This is the first report of *T. annulata*, the cause of Tropical Theileriosis in Ethiopia, Humera. In spite of aforementioned situation of bovine Theileriosis and the economic importance of the diseases throughout the world there is paucity of well documented information specifically in Ethiopia. Accordingly, the motivation for this review arises from the recognition of global economic importance of the disease and possibility of the disease occurrence in Ethiopia. Therefore the main objective of this paper is reviewing the epidemiology and control of bovine Theileriosis and highlighting the disease status in Ethiopia.

LITERATURE REVIEW

1.1 Etiologic agent and taxonomy

Theileriosis results from infection with obligate intracellular protozoa parasites in the genus Theileria of the suborder Piroplasmorina (Tarimo, 2013; Eshetu, 2015). Globally, Theileria annulata (cause of tropical theileriosis) and Theileria parva (causes of East Coast fever) are the most economically important tick-transmitted pathogenic species causing bovine theileriosis (Bhatnagar *et al*., 2015; Gebrekidan *et al*., 2016). *T. mutans, T. Orientalis/buffeli, T. velifera,* and *T. taurotragi* can also infect domesticated ruminants (Eshetu, 2015) and they are believed to cause milder and/or nonpathogenic theileriosis. However, recently, T. orientalis complex, transmitted mainly by Haemaphysalis spp. caused significant morbidity, economic losses and/or mortality in cattle in the Asia-Pacific region (Gebrekidan *et al*., 2016).

Bovine theileriosis is caused by the protozoan parasite of Theileria species which are round ovoid rod like or irregular shaped organism found in lymphocytes, histiocytes and erythrocytes (Bhatnagar *et al*., 2015). They are phylogenetically most closely related to members of the Babesia genus and fall in the order Piroplasmida under the phylum Apicomplexa (Nene *et al*., 2016). The Phylum Apicomplexa comprises a large group of complex eukaryotic organisms known to be obligate parasites of vertebrates and invertebrates (mans *et al*., 2015). The phylum is divided into four principal groups; the Coccidia, Gregarinasina (gregarines), Haemospororida (haemosporidians) and the Piroplasmorida (piroplasmids) (Adl *et al*., 2012). The Piroplasmorida comprises two main genera (Babesia and Theileria). The genus Theileria is distinguished by infection of leukocytes by sporozoites, maturation of schizonts into merozoites and subsequent infection of red blood cells to form piroplasms. (mans *et al*., 2015). According to Tarimo (2013) taxonomy of genus Theileria can be summarized as follows. Theileria belong to Kingdom: Protista, Subkingdom: Protozoa, Phylum: Apicomplexa, Class: Sporozea, Subclass: Piroplasmia (piroform, round, rod-shaped parasites), Order: Piroplasmida, and Family: Theileriidae, Genus: Theileria.

1.2 Life cycle

The life cycle of Theileria parasite is complex, involving morphologically distinct phases in two hosts. Sporogony and merogony take place in the bovine host while zygote and kinete are formed in ticks (Gul *et al*., 2015). Theileria sporozoites enter their bovine host during tick feeding and they rapidly invade mononuclear leukocytes, where they mature into macroschizonts and induce proliferation in host cells (Shahnawaz *et al*., 2011). Microschizonts gradually develop into macroschizonts and ultimately into merozoites, which are released from leukocytes. These merozoites invade erythrocytes and develop into piroplasms (Khattak *et al*., 2012).

A generalised lifecycle for the Theileria genus include secretion of infective sporozoites during tick feeding into the feeding site (Figure 1). Sporozoites then infect leukocytes and multiply by merogony, after which merozoites are released, which invade red blood cells thereby establishing the piroplasm stage. During a next feeding cycle, larval or nymphal vector ticks ingest piroplasms and the released parasites undergo syngamy in the tick gut, forming a zygote, the only diploid stage. The zygote divides into motile kinetes that infect the tick gut epithelial cells and migrate to the haemolymph and subsequently infect the salivary glands. After moulting and commencement of feeding by the tick, sporogony results in the multiplication of sporozoites in the salivary gland acini before injection into the feeding site by nymphs or adult ticks (mans *et al*., 215).

1.3 Epidemiology

1.3.1 Geographic Distribution

T. annulata (tropical theileriosis) occurs from southern Europe and the Mediterranean coast through the Middle

East and North Africa, and into parts of Asia (Spickler, 2010). The geographical distribution of tropical theileriosis, is mainly determined by the location and biology of its vector, ticks of the Hyalomma genus (Pieszko, 2015). It is important to emphasize that endemic region of *T. annulata* and *T. parva* do not overlap (OIE, 2014) however, there were reports of coexistence in southern Sudan (Spickler, 2010). Tropical theileriosis is prevalent in the South Eastern Europe Southern Europe (Portugal, Spain, Italy, Bulgaria, Greece, and Turkey) the near and Middle East, India, China, and Central Asia (Bakor, 2008). Tropical Theileriosis has also been reported in Ethiopian cattle by Gebrekidana *et al.* (2014). The distribution is determined by the presence of the tick vectors. Therefore, the incidence of the disease has a seasonal occurrence, which is modulated by the ecology of its vectors (Bakor, 2008).

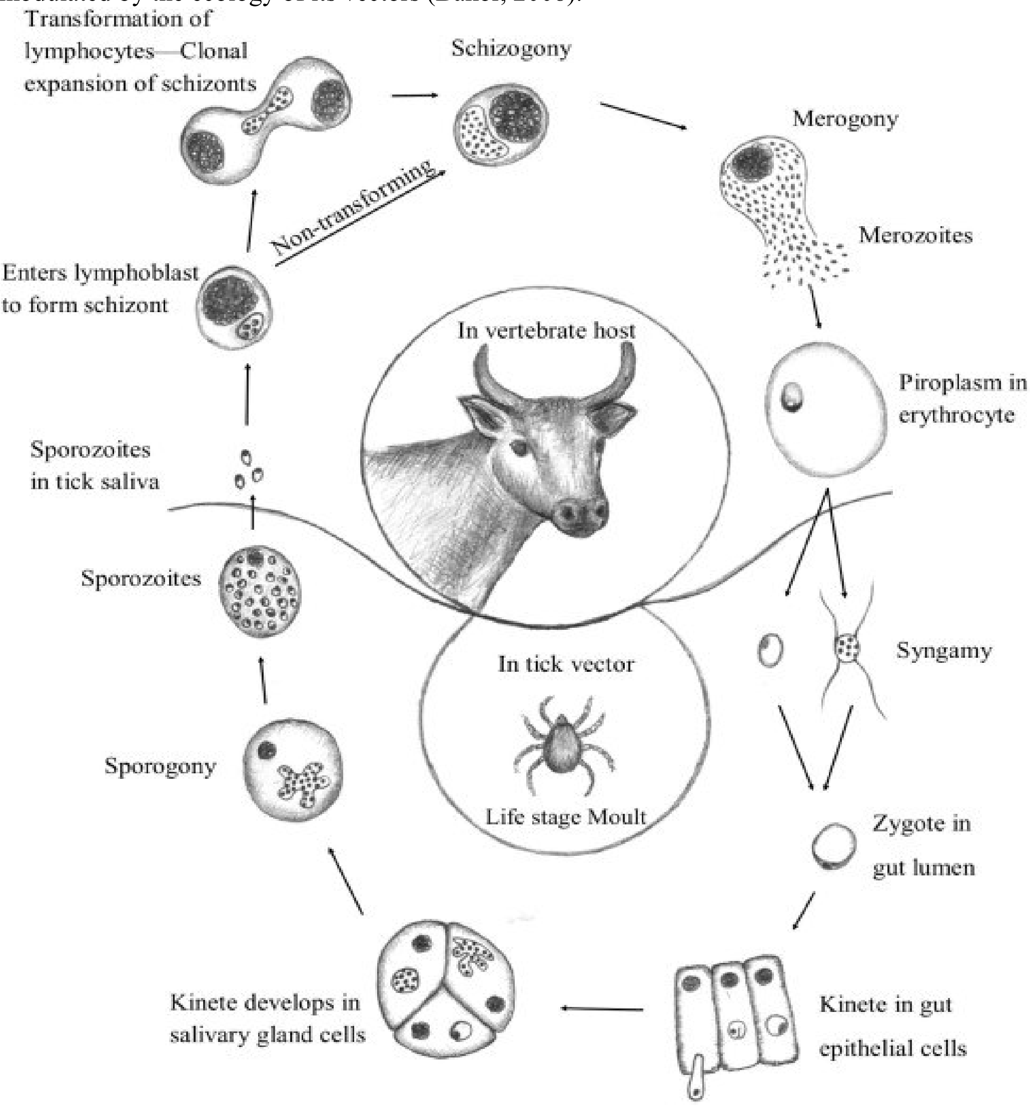

Figure 2: A generalized lifecycle for the Theileria using *T. parva* as example.
Source: (mans *et al*., 2015)

T. parva (East Coast fever) is found in sub-Saharan Africa (table 1) and is prevalent in fourteen countries in Southern, Central and Eastern Africa (Tarimo, 2013). The affected countries are Kenya, Sudan, Burundi, Tanzania, Malawi, Rwanda, Zaire, Mozambique, Zambia, Uganda and Zimbabwe (Gachohi *et al.,* 2012). *T. mutans* has been found in African and on some Caribbean islands, and was reported from the U.S. in 1950 and 1975. *T. velifera* and *T. taurotragi* occur in Africa (Spickler, 2010).

1.3.2 Host range

The Theileria species infect a wide range of both domestic and wild animals and are transmitted by ixodid ticks of the genera *Amblyomma, Haemaphysalis, Hyalomma* and *Rhipicephalus* (mans *et al*., 2015). *T. parva* can infect cattle, African buffalo (Syncerus caffer), water buffalo and waterbucks. Symptomatic infections are common only in cattle and water buffalo (Spickler, 2010). *T. parva* is highly virulent for European dairy cattle, however, the indigenous cattle breeds and African buffaloes in endemic areas have a natural resistance to this Theileria species (Radostits, *et al*., 2007). The introduction of *T. parva* infection into a previously unexposed cattle population results in an epidemic situation with mortality up to 95% in all age categories of cattle (Demessie and derso, 2015).

T. annulata occurs in cattle (Bos taurus and Bos indicus), yaks, water buffalo and camels (Pieszko, 2015). Mildly pathogenic and nonpathogenic species found in cattle include *T. mutans*, *T. buffeli*, *T. velifera*, *T. taurotragi* and *T. sergenti* has also been recognized. Theileria spp. has also been found in most wild Bovidae in Africa and reported in wild animals in other continents. *T. lestoquardi*, *T. separata*, *T. ovis* and other species occur in sheep and goats (Spickler, 2010). *Theileria annulata* sporozoites can be transmitted to goat and sheep and cause mild febrile response, however limited experimental studies indicate that schizonts and piroplasms are not produced in these host species (Pieszko, 2015).

1.3.3 Risk Factor

The prevalence of theileriosis depends upon geographical region and several other factors like tick density, climatic conditions, age, gender, management practices and immunity, either passive or active (Gul *et al*., 2015). Prevalence is also influenced by cattle breed as cattle usually differ in tick resistance and innate susceptibility to infection (Muhammad *et al*., 2008). Tropical theileriosis is more severe in exotic and cross-bred cattle (Bos taurus) than indigenous animals (e.g., Bosindicus). For example, the disease became significant in India when a program was launched to increase milk production by introducing exotic breeds. Mostly, the disease occurs in its subclinical form, leading to significant economic losses; without treatment or control, case fatality rates can reach 80 % in exotic breeds, compared with ~ 20 % in indigenous breed (Jabbar *et al*., 2015).

Table 3: Different species of Theileria affecting domestic animals and their vectors and Distributions

Species	Host	Vector	Disease	Distribution
T. parva	Cattle and Buffalo	*R. appendiculatus* *R. zambezienesis*	East Coast Fever	East, Central and southern Africa
T. annulata	Cattle	*Hyalomma*	Tropical Theileriosis	Southern Europe, North Africa, Middle East, Sudan, central Asia, and Indian subcontinent
T. mutans	Cattle	*A. variegatum*	Benign bovine theileriosis	Sub-Sahara Africa and possibly the Caribbean
T. hirci	Sheep and goat	*Hyalomma*	Malignant theileriosis of Sheep and goats.	North Africa, South Europe, Middle East. Asia

Source: (Mohammed, 2007)

Environmental Factor is one risk factor for bovine Theileriosis. The disease occurs when there is much tick activity, mainly during summer but a single tick can cause fatal infection (Hassan, 2010). The presence of ticks on animals an important risk factor for the spread of Theileriosis (khattak *et al*., 2012). Saeed *et al*. (2016) reported as there is higher prevalence of *T. annulata* in hot dry summer. High ambient temperature in this season provides an environment conducive to growth and multiplication of ticks and ultimately increases the transmission of theileriosis. The pattern of seasonal occurrence of Rhipicephalus (R) appendiculatus (Vector of *T. parva*) is determined by climate *R. appendiculatus* is most active following onset of rain, outbreak of ECF may be seasonal or, where rainfall is relatively constant, may occur at any time (Demessie and Derso, 2015). Another risk factor is host Factor for example increase age is associated with increased *T. parva* sero prevalence (Gachohi *et al*., 2012). However, in the recent study by Saeed *et al*. (2016) the prevalence of tropical theileriosis in young (23.4%) animals showed a higher prevalence than did adults (15%). Innate immunity in calves is not developed enough to combat *T. annulata.* Furthermore, in the result of reviewed study prevalence was found to be higher in females (24.6%) than male (13.1%).

1.3.4 Transmission

Knowledge about tick vectors, their intensity and abundance is crucial studying epidemiology of Theileriosis (Gul *et al*., 2015). Almost 80% of the cattle are exposed to tick infestation worldwide (Anim *et al.,* 2013) and ticks are responsible for severe economic losses both through the direct effects of blood sucking and indirectly as vectors of pathogens and toxins. Feeding by large numbers of ticks causes reduction in live weight gain and anaemia among domestic animals (Nejash, 2016). Economically important Theileria species that infect cattle and small ruminants are transmitted by ixodid ticks of the genera Rhipicephalus, Amblyomma, Hyalomma and Haemaphysali. Theileria sporozoites are transmitted to animals in the saliva of the feeding tick (Demessie and Derso, 2015). Iatrogenic transmission can also occur via blood (e.g., on re-used needles) (Spickler, 2010).Developmental stages of the parasite occur in the tick and they pass trans-stadially through the stages of larva, nymph and adult, but there is no transovarian transmission. Consequently, larvae or nymphs become infected and transmit infection as nymphs or adults. Adults are more efficient vectors than nymphs (Radosite *et al*., 2007)

Rhipicephalus appendiculatus is the most important vector for *T. parva,* but *R. zembeziensis* and *R.*

duttoni carry this organism in parts of Africa (Spickler, 2010. These are three host ticks because nymph, larvae and adult may not necessarily feed on the same host. The nymph and larval instars of tick acquire infection through blood meal and leave the host before molting to the next stage. Both nymph and larvae are responsible for further transmission of infection by attaching to the new host. (Gul *et al*., 2015). *T. annulata* is transmitted by ticks in the genus *Hyalomma* (Saeed *et al*., 2016). These are two host ticks because the larva molt to nymph on the same cattle. The nymph detaches and drops off of the ground to molt into an adult and seeks a new host (Gul *et al.,* 2015). *T mutans and T. velifera are transmitted by Amblyomma spp. Ticks in the genus Rhipicephalus spread T. taurotragi* (Spickler, 2010).

Ticks are mostly found in the inguinal/groin region and external genitals as these body parts are richly supplied with blood and the thinner and short hair skin is usually preferred by tick for infestation because mouth parts can easily penetrate the vascular region for feeding (Gul *et al*., 2015). Warm and moist climate is conducive for rapid growth and development of ticks (Kohli *et al.,* 2014).

1.3.5 Morbidity and Mortality

Morbidity and mortality vary with the host's susceptibility, and the strain and dose of the parasite. The case fatality rate for untreated East Coast fever can be as high as 100% in taurine, zebu or sanga cattle from non–endemic areas. In contrast, the morbidity rate approaches 100% among indigenous cattle, but the mortality rate is usually low. Similarly, tropical theileriosis is more severe in introduced breeds, with a mortality rate of 40-90%, while the mortality rate in indigenous cattle can be as low as 3%. Breeds of cattle that are relatively resistant to experimental infection with *T. annulata* include the Sahiwal breed of *Bos indicus* and the Kenana breed of *B. taurus*. Infections with *Theileri*a spp. other than *T. parva* and *T.annulata* are rarely fatal in cattle (Spickler, 2010).

1.4 Pathogenesis

The *Theileria* spp. can be grouped into schizont "transforming" and "non-transforming" species. Non-transforming Theileria are regarded as being but still able to cause disease as a result of anaemia induced by the piroplasm stage (man *et al*., 2015). pathogenesis of various forms of Theileriosis is dependent on the production of schizonts in lymphocytes and piroplasms in erythrocytes (Radosites *et al*., 207). The severity of infection depends upon virulence of the causative strain, the quantum of infection, the susceptibility status, age and health of the host (Bakor, 2008). Thus, *T. parva*, *T. annulata*, and *T. hirci* produce numerous schizonts and piroplasms and are very pathogenic; *T. mutans*, *T. buffeli*, and *T. avis* rarely produce schizonts but may cause varying degrees of anemia when piroplasms are many in red blood cells; and with *T. velifera* and *T. separata*, no schizonts have been described, the parasitemia is usually scanty and the infection is mild or subclinical (Radosites *et al*., 2007).

Sporozoites of *T. parva* are injected into the bovine host by the tick in its saliva. The sporozoites then enter lymphocytes and develop into schizonts in the lymph node draining the area of attachment of the tick, usually the parotid node. Infected lymphocytes are transformed to lymphoblasts which continue to divide synchronously with the schizonts so that each daughter cell is also infected (Taylor *et al.*, 2007). Eventually, infected lymphoblasts are disseminated throughout the lymphoid system and in non lymphoid organs where they continue to proliferate. Later, some schizonts differentiate into merozoites, are released from the lymphoblasts and invade erythrocytes which lead to development of anemia (man *et al*., 2015).

The pathological damage is induced in cattle by schizont stage of *T. annulata* and *T. parva*. The cells infected by schizonts induce massive and uncontrolled proliferation of both specific and nonspecific T lymphocyte resulting in enlarged lymph nodes (Gul *et al*., 2015). This is followed later by necrosis of infected lymphoblasts induced by cytotoxic T-Iymphocytes. The severe lymphocytolysis often leads to immunosuppression. Terminally, the animal develops severe pulmonary edema, probably due to release of vasoactive substances from lymphocytes disintegrating in the lungs. Erythrocytic indices are usually unchanged, but there may be terminal anemia in January disease. Affected lymph nodes show reactive follicular hyperplasia, reticulo-endothelial hyperplasia, enlarged germinal centers and slight increase of interfollicular lymphoid tissue within the paracortical and cortical regions (Hassan *et al.,* 2012).

1.5 Clinical sign

The occurrence of the disease varies depending on the parasite strain, the host's susceptibility furthermore the quantity of sporozoites inoculated and the severity of the disease is directly proportional to the initial inoculum of sporozoites injected (Bakor, 2008). *T. annulata* infection (Tropical theileriosis) is characterized by high fever, weakness, weight loss, inappropriate appetite, conjunctival petechia, enlarged lymph nodes, and anemia. Lateral recumbency, diarrhea and dysentery are also associated with later stages of infection (Radostits *et al.,* 2007 and Muhanguzi *et al*., 2014). Cattle may also develop an extremely fatal condition referred to as turning sickness. In this disease, capillaries of central nervous system are blocked by infected cells and leads to neurologic symptoms (Gul *et al.,* 2015). The incubation period varies from 4 to 14 days after attachment of the infected ticks to the host. The disease may last as little as three to four days in the acute form or may be prolonged for about 20 days

(Bakor, 2008).

Mahmmod *et al*. (2011) reported different degrees of the characteristic clinical signs of tropical theileriosis like fever (>40 °C), enlargement of the superficial lymph nodes (acute form), anorexia, pale or congestion of the visible mucous membranes, conjunctivitis, severe congestion of the eyes, excessive lacrimation, corneal opacity, respiratory signs from serous nasal discharge to cough, purulent nasal discharge, and dyspnoea (chronic form). Unlike *T. parva*, which causes only a small reduction in circulating erythrocytes, mild to moderate anaemia is observed in tropical theileriosis, although pathology produced by the schizont stage is usually the primary cause of mortality (Morrison, 2015).

Studies in Japan by Chaisi *et al*. (2014) indicate that some *T. orientalis* parasites can cause transient anaemia, with clinical signs in up to 2.5% of animals and occasional mortalities (<0.1%) The main clinical manifestations are fever, haemolytic anaemia of variable severity and mortality in some animals; infection is also associated with an increased incidence of abortion and stillbirths and significant reductions in milk yields in affected herds (Morrison, 2015). *T. mutans* infection can result in mild clinical signs, but pathogenic strains in eastern Africa cause severe anemia, icterus and sometimes death (Tomassone *et al*., 2012). In general benign theileriosis is characterized by moderate to severe anemia in heavily parasitized cattle and moderate enlargement of lymph nodes (Radostits *et al.,* 2007).

1.6 Diagnosis

For routine diagnosis of bovine Theileriosis, conventional methods are used, whereas serological and molecular methods are utilized for research purposes and epidemiological studies. Conventional methods involve microscopic examination of Giemsa stained thin/thick blood films for detection of piroplasms and lymph node biopsy smears for detection of schizonts. The mostly used serology tests are Indirect Immunofluorescent Atibody Test (IFAT) and Enzyme Linked Immunosorbent Assay (ELISA) (Tarimo, 2013). Diagnosis of acute theileriosis can also be based on clinical signs (rise in body temperature, enlarged superficial lymph nodes), knowledge of disease, and vector distribution as well as examination of Giemsa-stained (either in smears of needle aspirates from enlarged lymph nodes or blood smears) (OIE, 2014). Definitive identification of the *Theileria* species involved sometimes requires the application of species-specific PCR assays (Morrison, 2015).

1.6.1 Microscopic examination

Traditional diagnosis of bovine theileriosis is mainly based on the microscopic examination of blood smears for the presence of the merozoites stage of Theileria (Junlong *et al*., 2015). This method is frequently used for detection as it is comparatively inexpensive. However, the method is insensitive and not suitable for carrier animals because the pathogen level is usually low in the blood stream making it an unreliable technique for accurate results (Gul *et al*., 2015). Morphological differentiation of *T. annulata* and *T. parva* is also difficult, but both species are geographically separated (Hoghooghi-Rad *et al*., 2011). One of important character of Theileriosis is that once the animal recovered from the primary infection, the animal will become a carrier for a long time. At this stage, the animal has very low parasitemia which is difficult to detect by a microscope (Junlong *et al*., 2015). Therefore, high sensitivity and specific assays for detection of these pathogens from the subclinical animals are needed (Gul *et al*., 2015).

1.6.2 Serological tests

Serological tests are reliable methods for detection of low grade or previous infections where measurement of antibody levels of a cattle herd is used for assessing the response to natural infection, and also to vaccination for the purpose of disease control (Tarimo, 2013). Serologica method depends on antigens and antibodies reaction. Antibodies can be detected by different serological tests but IFAT remains the gold standard assay recommended by the OIE for most economically important parasites (OIE, 2014). However, the biggest problem with the IFAT is the significant cross-reactivity observed between closely related species. Cross-reactivity between *T. parva* and *T. taurotragi* antigen and anti-sera has been observed (man *et al*., 2015).

ELISA can also be used in diagnosis of bovine theileriosis and the test is easy to perform, can diagnose a large number of samples in a short time and it is less laborious (Mohammed, 2007). However, these methods are also not reliable due to their limitations. There are chances of cross reactivity, and may confront false positive and false negative results. Theileria piroplasm may occasionally be present in the erythrocytes of long-term carriers whereas antibodies have a tendency to disappear. The animals may still be infected despite of negative serological test. Precise identification of carrier cattle is of crucial importance as they are capable of transmitting infection to non-endemic regions (Gul *et al*., 2015).

1.6.3 Polymerase Chain Reaction

Molecular tools can be used to differentiate *theileria* specie. The tests have proved to be highly sensitive and specific for detecting parasite DNA in blood (Tarimo, 2013). Polymerase chain reaction (PCR) has largely superseded other methods and is widely used specie-specific molecular diagnostic assay in veterinary parasitology to determine piroplasm carrier animals. However, these methods are laborious, expensive; require specialized equipment and technical skills (Gulr *et al.,* 2015; Junlong *et al*., 2015). PCR could detect parasites at

0.000001% parasitaemia, allows direct, specific and sensitive detection of parasite and differentiation of different piroplasms infecting animals (Mohammed, 2007). Kohli *et al*. (2014) reported 27.2% prevalence of theileriosis by blood smear examination while using PCR, prevalence was reported to be 32.5 %. Similarly, most recent research by Saeed *et al*. (2016) they used blood smear examination and PCR and Prevalence was 1.9 % and 19.3% respectively indicating that PCR is more sensitive than blood smear examination.

1.7 Preventions and controls

1.7.1 Prevention

Due to the high costs of Theilericidal drugs, the high prevalence of carrier state infection and the high costs of treatment, prevention is the best mean to control Thelerial infection; it consists of two types of action: (i) control of the vector tick through one or more control options and (ii) vaccination (Gharbi and Darghouth, 2015). Animals can be protected from both East Coast fever and tropical theileriosis by vaccination. Attenuated vaccines are used to control tropical theileriosis in some countries. Vaccination against East Coast fever is done by simultaneously injecting virulent *T. parva* and an antibiotic (usually a long-acting tetracycline). Considerations in *T. parva* vaccination include the possibility of introducing live organisms into areas where they are not currently endemic (Spickler, 2010). Control of the vector tick is one of the widely used methods to prevent outbreaks. The control option for vector and vaccination will be discussed in detail in the following topics.

1.7.2 Control methods

Calves infected with several other bovine tick-borne blood pathogens, including *Babesia* species and *Anaplasma marginale*, show enhanced resistance to disease in the first six months of life, enabling them to acquire immunity to these pathogens in regions where the infections are endemic Such age-related resistance is not seen with *T. parva* or *T. annulata*, Nor is there any evidence that maternally derived antibodies are protective. Because of the absence of such protective mechanisms and the fatal nature of the diseases in susceptible stock, control of disease caused by these highly pathogenic Theileria is particularly challenging (Morrison, 2015). So it is important to design and implement control strategies to prevent outbreaks in endemic and non-endemic regions on a priority basis (Simuunza *et al.,* 2011).

Several methodologies are currently available for the control of bovine theileriosis (Bakor, 2008) and various cost effective prophylactic measures are used to control and minimize economic losses to dairy farms globally, however, all of these need to be integrated in such a manner that they meet the specific requirements of livestock holders in different situations (Gul *et al*., 2015). The most practical and widely used method is the chemical control of ticks with acaricides. However, tick control practices are not always fully effective for a number of reasons, including development of acaricide resistance, the high cost of acaricides, poor management of tick control, and illegal cattle movement in many countries. Vaccination using attenuated schizont-infected cell lines has been widely used (OIE, 2014).

1.7.2.1 Management

Good management is one control option in controlling bovine Theileriosis. Management involves restriction of livestock movement and implementation of quarantine measures to keep the tick free and disease susceptible cattle apart from the tick-infested and infected animals. This is also to ensure that ticks and the pathogens they transmit are not transported to ecologically suitable but currently uninfected areas. The overall objective is to ensure that the target population is entirely free of the disease or there is endemic stability (Bakor, 2008). Newly purchased cattle may first be properly examined before mixing with the existing stock. If the number of ticks or tick infested cattle is small, manual removal of tick is a common practice. Forefingers are used to grasp ticks and twisted counter-clock wise. The removed ticks are, then, put on the smoldering dung cake to kill them (Vahora *et al*., 2012).

1.7.2.2 Vector control

Tick control is one of the most important factors influencing the epidemiology of bovine Theileriosis. It has been achieved mainly by application of acaricides (Bakor, 2008) and acaricides may be applied to kill ticks in both free living as well as parasitic stages. Tick free or acaricide treated cattle have better productivity as compared to tick infested cattle (Sajid, 2007). Acaricides are applied by spraying, injections, spot-on or dipping but human safety is of utmost importance in acaricide application. Prolonged and repeated contacts with skin should be avoided. Hands and face should be properly washed before eating (Vahora *et al*., 2012).

Dipping is considered the most effective method for acaricide application (Bakor, 2008). Dipping tanks are usually covered with a roof to avoid dilution by rain or evaporation. It is important to carefully adjust dip concentration according to the recommendation. Poor or incorrect application of even highly effective acaricide gives unsatisfactory results and develops acaricidal resistance. Dipping of cattle less than 3 months is not recommended. Wounds of cattle must be thoroughly checked before dipping, otherwise, it can cause discomfort and toxicity. The heads of cattle must be dipped once or twice in the solution. Cattle that are thirsty or fatigued shouldn't be dipped (Vahora *et al*., 2012). Despite its effectiveness Dipping become very expensive, and inconsistent due to lack of facilities such as finances for rehabilitation of dip tanks, provisions of acaricides and water (Tarimo, 2013).

Acaricides can also be applied with hand spray which is environmental friendly practice, easy to operate and economical but is suitable for small herds only. For effective control, it is important to moisten the hair as well as skin with spray (Gul *et al*., 2015). There are certain body parts of cattle that escape treatment by spraying and dipping. Such predilection sites include inner fringes of ear, under part of tail and legs and require special attention. Selective application of acaricides to these sites is called hand dressing and is done as a supplement to usual dipping (Vahora *et al.,* 2012). Other options for controlling ticks are Ecological and Biological. Ecological control method is used for habitat and host linked treatment. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks. Biological control, include predators like rodents, birds, ants (Nejash, 2016).

1.7.2.3 Selection of tick resistant cattle breeds

Different breed of cattle are different in their susceptibility to Theileriosi for example exotic cattle and their crossbreds are highly susceptible, while indigenous cattle are relatively resistant to tropical Theileriosis (Saeed *et al*., 2016). Low prevalence of parasite is reported in Sahiwal cattle than European breeds suggesting that Sahiwal cattle are more resistant to tick infestation and tick borne diseases (Sajid *et al*., 2009). It is widely known that *Bos indicus* cattle are more resistant to ectoparasites than are *Bos taurus* animals. There are great differences between these two breeds of cattle in regard to their susceptibility to parasitism by cattle ticks (Nejash, 2016). In general sense rearing disease-resistant breeds play significant role in controlling bovine Theileriosis. Hence selection of cattle breeds with enhanced tick resistance is proposed as a sustainable tactic for controlling infection in developing world (Gul *et al*., 2015).

1.7.2.4 Immunization

Control of the disease by prevention of tick infestation requires essentially continuous application of acaricides and is therefore expensive and difficult to sustain. Furthermore result it can in acaricide resistance. Because of the shortcomings of these control measures, vaccination is seen as the most sustainable option for control of the disease (Morrison, 2015). The attempt of immunization in cattle against tropical theileriosis was first made in Algeria in 1930s. Blood with low virulence strain was donated from infected cattle followed by mechanical passage between healthy cattle. This practice resulted in subsequent loss of parasite's ability to differentiate into merozoites with one year estimated protection in the absence of natural challenge (Gul *et al*., 2015).

Successful vaccination against *T. annulata* and *T. parva* has only been achieved using live parasites. A method of vaccination against *T. annulata*, based on the use of parasitised cell lines in which the parasite had been attenuated by up to 200 passages in vitro, was developed in the 1960s (Morrison, 2015). Vaccination using attenuated schizont-infected cell lines has been widely used for *T. annulata*. While, for *T. parva* control vaccination is based on a method of infection and treatment (ITM) in which cattle are given a subcutaneous dose of tick-derived sporozoites and a simultaneous treatment with a long-acting tetracycline formulation. This treatment results in a mild or inapparent East Coast fever reaction followed by recovery. Recovered animals demonstrate a robust immunity to homologous challenge, which usually lasts for the lifetime of an animal (OIE, 2014).

The result most recent study in Kenya by Woolhouse *et al*. (2015) suggested a novel alternative approach, inoculation of young calves with more benign *T. mutans* or *T. velifera*, without the need for treatment and helping calves to survive their first exposure to *T. parva* and develop immunity. This Successful ECF control would benefit an estimated 30 million cattle in sub-Saharan Africa, reducing the costs of treatment as well as reducing demand for both antibiotics and acaricides.

1.7.2.5 Chemotherapy

Acording to OIE (2014) chemotherapeutic agents such as parvaquone, buparvaquone and halofuginone are available to treat *T. annulata* and *T. parva* infections. These best Theilericidal drugs belong to the hydroxynaphtoquinones family (Gharbi and Darghouth, 2015). Naphthoquinone compounds were discovered in 1970 with a wide therapeutic index (Gachohi *et al*., 2012). These naphthoquinone compounds are not only effective for curing Theileriosis but can also be used as a remarkable prophylactic measure against the disease (Gul *et al*., 2015). However, according to OIE (2014) treatments with chemotherapeutic agents do not completely eradicate Theilerial infections leading to the development of carrier states in their hosts. Parvaquone (Parvexon ND, Bimeda) is mainly active drug against schizontes; it should be injected intramuscularly at the dose of 20 mg/kg. buparvaquone is active against both schizontes and piroplasmes; it is injected intramuscularly at the dose of 2.5 mg/kg. Its efficacy after a single injection was estimated to 92%, which is higher than parvaquone (Gharbi and Darghouth, 2015).

However, these naphthoquinone compounds are not used by cattle breeders due to their high price (Gachohi *et al*., 2012) and drugs infiltrate the muscles and are not easily eliminated from the cattle's body (Gul *et al*., 2015). The meat and milk products may be contaminated with drug residues leading to health hazards (Sonenshine *et al*., 2006). Drug resistance is also reported in Tunisia recently. 4 out of 7 cattle died of acute tropical theileriosis in spite of buparvaquone injections (Mhadhbi *et al*., 2010). Similarly, 7 out of 8 cattle died in southern Iran, though buparvaquone treatment was given (Sharifiyazdi *et al*., 2012). Mechanism of buparvaquone action has not been fully elucidated. However, products belonging to the hydroxynaphtoquinones probably acts by binding to cytochrome b (cyt b) inhibiting the electron transport chain in the parasite (Hostettler *et al*., 2014). *T. annulata* and *T. parva* show similar disease symptoms and the symptoms include immune-depression and secondary bacterial infection e.g. pneumonia and enteritis. Antibiotic treatment is usually recommended to limit such secondary infections (Gul et al., 2015). Tetracycline antibiotic was probably the first chemotherapeutic compound used against ECF in 1953. This antibiotic is effective only at the early stages and can't be used at later stages of infection (Gachohi *et al*., 2012).

Plant called Calotropis procera locally named as Thophiya (Tobia) are reported to have killing or repealing effects on tick in North Gondar, Ethiopia (Melaku, 2013). This plant is found as wild plant in Asia and Africa and has multipurpose chemotherapeutic activities and can be effectively used to treat bovine theileriosis (Farah *et al*., 2014; Gul *et al*., 2015).

1.8 Economic Importance bovine Theileriosis

Theileriosis causes major constraints on livestock development in Africa, Asia and Middle East. The disease causes high morbidity and mortality in exotic cattle, thus inhibiting the introduction of improved cattle into endemic areas. The consequence is that the quality of cattle in endemic areas remains low, therefore impeding the development of the cattle industry and the wellbeing of producers and their families (Demessie and Derso, 2015). *T. annulata* causes severe financial losses due to live weight decrease, a drop of milk yield, abortions and in some cases deaths. Moreover, the treatment of this disease is very expensive (Gharbi and Darghouth, 2015).

In Eastern, Central and Southern Africa due to East Coast fever, about one million cattle per year die, with a further 28 million of the 47 million cattle in the region being at risk of contracting the disease. The disease causes high morbidity and mortality, and is considered as the important restriction to the improvement of the livestock industry in Africa. Apart from death, farmers face a lot of loses such as impaired weight gain, weak calves, low grade meat, decreased milk production, and enhanced costs of veterinary services (drugs, laboratory diagnosis, surveillance, vaccinations, administration, training, prophylaxis, dipping and others). It is estimated that losses of more than US$300 million per year occur in East, Central and South Africa regions, where losses of US$ 168 million occur in Eastern Africa alone (Tarimo, 2013).

1.9 Status of bovine Theileriosis in Ethiopia

Similar to other countries, there are a considerable number of economically important livestock diseases occurring in Ethiopia. Among others, tick borne haemoparasitic diseases are of the major constraints to the livestock industry of the country (Nejash, 2016). A number of researchers are reporting presence of different theileria species from different area of Ethiopia (table 2). For example Gebrekidan *et al*. (2014) reported a widespread distribution of *Theileria* spp. among domestic ruminants in northern Ethiopia. The circulation of Theileria spp. in Ethiopia is also indicated by Solomon *et al*. (1998), who reported 30.9% seropositivity to *T. mutans* in cattle from the Yabelo district.

In previous studies, conducted by Mekonin *et al*., (1992) *T. orientalis* and *T. velifera* were reported from Gambella region, western Ethiopia. The vectors of *T. mutans*; Rhipicephalus evertsi evertsi and Ambylomma variegatum are also commonly found in different regions of Ethiopia (Feleke *et al*., 2008). Gebrekidan *et al*. (2014) reported the presence of three Theileria species in cattle in Addis Zemen, i.e. *T. velifera*, *T. mutans*, and T. orientalis complex. The average seropositivity of *T. mutans* (54.2%) was reported in Ghibe valley by Feleke *et al*. (2008) indicating that the population is in the state of enzootic instability.

Table 4: Report of Theileria species from different area of Ethiopia

Theileria species	Area	Samples	diagnostic methods test	Reference
T. mutante	southern and eastern Ethiopia	Blood	Microscopic and serology	(Solomon *et al*., 1998; Tomassone *et al*., 2012)
T. annulata	Northern Ethiopia	blood	PCR	(Gebrekidan *et al*., 2014)
T. orientalis	Northern Ethiopia	blood	MT- PCR	(Gebrekidan *et al*., 2016)

In addition some of the past studies confirmed that *T. mutans*, *T. velifera*, and *T. orientalis* infect cattle in western, eastern, and southern Ethiopia (Sileshi, 1996; Sileshi *et al*., 2011; Tomassone *et al*., 2012). But in the recent study by Gebrekidan *et al*. (2014) four species of Theileria in cattle: *T. velifera*, *T. mutans*, *T. orientalis complex* and *T. annulata* were reported from northern Ethiopia **(table 3)** (Addis Zemen, Humera and Sheraro) with infection rates of 66, 8, 4, and 2%, respectively. Furthermore they have reported a new report on the presence *T. annulata*, the cause of Tropical Theileriosis, in Ethiopia, Humera for the first time. There are no clinical or serological reports of the presence of East Coast fever (*T. parva*) and its vector *R. appendiculatus* have not been found in Ethiopia However, there is relatively uncontrolled movement of livestock from southern Sudan, where these diseases and their vectors (the brown ear tick) are found, suggests that there is a considerable risk of the diseases being introduced. If infected ticks become established on the climatically favourable highlands of Ethiopia, close to 100% mortality of improved and indigenous cattle could occur (Sileshi, 1996).

Table 5: Distribution of Theileria spp. infections in cattle in Northern Ethiopia

Study site	No. of anima s	PCR+ n (%)	*T. velifera n* (%)	*T. mutans n* (%)	T. orientalis complex *n* (%)	*T.annulata n* (%)
Addis Zemen	59	48(81.4)	38 (64.4)	6 (10.2)	4 (6.8)	–
Sheraro	21	18(85.7)	17 (81)	1 (4.8)	–	–
Humera	20	14 (70)		1 (5)	–	2 (10)
Total	100	80(8)	66 (66)	8 (8)	4 (4)	2 (2)

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

The most recent study in Ethiopia detected and characterized *T. orientalis* in local cattle using PCR-based tools and found as most pathogenic of *T. orientalis* genotypes is low and dominant is non-pathogenic genotype. Therefore, the low intensity of infection of pathogenic genotypes of *T. orientalis* may explain why clinical oriental theileriosis is uncommon in Ethiopia (Gebrekidan *et al*., 2016).

2. CONCULUSSIONS AND RECOMMENDATIONS

Bovine Theileriosis is the disease caused by tick-borne, haemoprotozoan parasites of the genus Theileria and it is one of the most economically important diseases of bovines, particularly in tropical and sub-tropical regions of the world. The two diseases with the greatest economic impact in cattle are East Coast fever and tropical Theileriosis. Unfortunately presence East Coast fever is not reported till now in Ethiopia but tropical Theileriosis is confirmed from Humera, the border of Ethiopia and Sudan. PCR is the most beneficial molecular tool for diagnosis of infection till date than blood and lymph node smear examination and serological tests. Infection by Theileria parasites limits the movement of cattle between countries and can result in production losses and high mortality in susceptible animals. Because these diseases are most severe in recently introduced animals, they are a constraint on the importation of new breeds or improved stock. Thus controlling is crucial for preventing economic loss associated with this disease. Tick control particularly by using acaricides can limit the incidence of bovine theileriosis but Tick control is not always fully effective and vaccination is the most sustainable option. Even though chemotherapeutic agents such as parvaquone, buparvaquone are available as treatment option; drug resistance is reported from different countries. Therefore based on the above conclusions the following recommendations can be proposed.

- ✓ There is a real threat to the livestock industry in Ethiopia for the possibility of introduction of East Coast fever and its vector R. appendiculatus. Therefore, it is recommended that future Ethiopia policies should focus on developing and implementing surveillance systems and action plans to prevent East Coast fever from becoming established in the country
- ✓ Since drug resistance is reported with currently available drugs for treatment of bovine Theileriosis research should be extended to design new drugs having different modes of action
- ✓ In diseases endemic area farmers should be educated on the importance of ticks not only as external parasites but also as vectors of diseases.
- ✓ In Ethiopia more research should be conducted on bovine tropical theileriosis to design and implement appropriate disease control strategies and prevent disease spreading.
- ✓ In disease endemic area specific attention for tick prevention should be given for imported animals as
- ✓ The currently used control methods against Theileriosis are expensive and have many limitations, so it is recommended to devise and implement cost-effective and integrated control strategies against the infection.

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