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Review on Epidemiology and Economic Significances of Bovine Babesiosis

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Abstract: Bovine Babesiosis is a tick-borne disease of cattle caused by intra-erythrocytic the protozoan parasites of the genus *Babesia*, which affects a wide range of domestic and wild animals and occasionally humans. It is the worldwide most important hemoparasitic disease of cattle that causes significant morbidity and mortalitynext to trypanosomiasis. They are widespread in tropical and subtropical areas including Ethiopia. The one host tick, *Rhipicephalus* (formerly *Boophilus*) species are the principal vector of *Babesia bovis* and *Babesia bigemina*. Availability of host, presence of ticks that act as vector for transmission of infections, presence of parasites within vectors, as well as hosts and environmental conditions are the most important factor for occurrences of bovine babesiosis. Anaemia, fever, depression, anorexia, haemoglobinaemia and haemoglobinuria are the predominant signs. Microscopic examination is the cheapest and fastest methods used to identify *Babesia* parasites, but not reliable for detection of carrier animals. In these cases molecular detection methods, or serological diagnostic procedures are the most important to demonstrate specific antibodies. The most commonly used drugs for the treatment of babesiosis are diminazenediaceturate, imidocarb and amicarbalide. Active prevention and control of Babesiosis is achieved by immunization, chemoprophylaxis and vector control.

Key words: Babesia · Bovine Babesiosis · Epidemiology · Intra-Erythrocyte · Tick

INTRODUCTION

Bovine babesiosis is the most important arthropod borne disease of cattle worldwide that causes significant morbidity and mortality. It is the second most common blood borne parasitic next to trypanosomiasis [1]. Bovine babesiosis is caused by protozoan parasites of the genus *Babesia*, order Piroplasmida, phylum Apicomplexa [2]. Babesiosis, also known as piroplasmosis, tick fever, red water, Texas fever, splenic fever, tristeza, etc [3]. It is a haemolytic disease characterized by fever (40-42°C) which may be sudden in onset, anaemia, icterus, hemoglobinuria, listless, anorexic, jaundice and death. Although some species of *Babesia* such as *B. microti* can affect healthy people, cattle parasites seem to cause disease only in people who are immunocompromised. *Babesia divergens* causes serious disease in humans who have had splenectomise [4].

In 1888, Victor Babes described intra-erythrocytic microorganisms responsible for the death of 50 thousand cattle in Romania and classified them as Bacteria [5]. He named as *Hematococoeusbovis* and later it was changed to *Babesia bovis* [6]. In 1893, Kilborne and Smith described a factor of Texas cattle fever, giving them the rank of genus and name *Babesia* as classifying them as Protozoans [7].

Now a day, it is a disease with a worldwide distribution affecting many species of mammals with a major impact on cattle and man [8]. It is a tick-borne intra-erythrocyticapicomplexan parasites found in a variety of domestic and wild animals and in humans. Mixed infections are responsible for widespread morbidity and mortality in livestock of tropical and subtropical regions of the world. However, the major impact occurs in the cattle industry and the species affecting bovines are the most studied, including *Babesia bovis, B. bigemina*

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and *B. divergens* [9]. Two species are economically important in tropical and subtropical regions of the world, including southern Africa: *B. bovis,* which causes Asiatic red water and *B. bigemina*, which causes African red water. *Babesia divergens* causes an economically important disease in the British Isles and northern Europe [10].

Babesia and its two hosts, the tick vector and vertebrate host, represent a complex system in which the interactions between the three partners are among the longest described [11]. *Babesia bovi*s was probably introduced into southern Africa with the Asian blue tick (*Rhipicephalus* (*Boophilus*) *microplus*) during the latter part of the 19th century. *Babesia bigemina* is principally transmitted by the common, indigenous African blue tick (*Rhipicephalus* (*Boophilus*) *decoloratus*), as well as by *Rhipicephalus* (*Boophilus*) *microplus*. Other tick vectors may also be involved [10]. Babesiosis is fatal for cattle population when no health facilities were provided [12].

The endemic condition of bovine babesiosis in a specific geographic region is related with presence of a vector capable to transmit the infection and the enzootic stability condition depends of the interaction established between tick, parasite and bovine. There is age related immunity to primary infection of cattle. Young calves possess strong innate immunity against infection that lasts for approximately 6months after birth and is abrogated with the removal of the spleen. Infected animals develop a lifelong immunity against reinfection with the same species. There is also evidence of a degree of cross-protection in *B. Bigemina*-immune animals against subsequent *B. bovis* infections [13]. The classical microscopic examination of *Babesia* piroplasms in Giemsa stained thin blood smear is a gold standard test that is relatively cheap and quick method; however, in chronic infection, it has low sensitivity and usually fails to detect carrier animals [14].

Therefore, the objective of this seminar is to review the epidemiology and economic significances of bovine babesiosis.

Literature Review

Etiology and Morphology: Bovine babesiosis is caused by multiple species but three species found most often in cattle are *B. bovis, B. bigemina* and *B. divergens*. Additional species that can infect cattle include *Babesia major*, *Babesia ovata*, *Babesia occultans* and *Babesia jakimovi* [4]. Little has been published about *B. jakimovi*, which was discovered in Russia and does not appear in many descriptions of *Babesia*. To date, more than 100 species have been identified, infecting many mammalian and some avian species [13]. *Babesia* belongs to protozoan parasites of the genus *Babesia*, order Piroplasmida, phylum Apicomplexa and subclass Piroplamsia and are commonly referred to as 'piroplasmas' due to the pear like shaped merozoites which live as small parasites inside RBC of mammals [1] and used the 18s rRNA gene for phylogenetic analysis [15].

On the basis of morphology, babesias are divided into two groups, small babesias (1.0–2.5 µm long) which included *B. bovis, Babesiagibsoni, B. microti, Babesiarodhaini,* etc. and large babesias (2.5–5.0 µm long) which included *B. bigemina*, *Babesiacaballi, Babesiacanis*, etc., The orientation of the parasite in the red blood cells (RBCs) depends on its size because large pyriform parasites meet at their pointed ends at an acute angle to each other and small forms make an obtuse angle to each other [6]. *Babesia* species enter red blood cells (Erythrocytes) at the sporozoite stage then within the red blood cell, the protozoa become cyclical and develop into a trophozoite ring. The trophozoites moult into merozoites, which have a tetrad structure coined a Maltese-cross form. The tetrad morphology, which can be observed under microscope with Giemsa staining of a thin blood smear, is unique to *Babesia*and serves distinguishing feature [16]. Anterior and posterior ends, termed polar rings, delimit the shape of the parasite. Three major organelles (Microtubules, rhoptries and micronemes) concentrate in the anterior polar ring and are collectively known as the apical complex *B.bovis* is smaller than *B. bigemina*, measuring up to 2 µm in length. Under light microscopy, this organism is often found in pairs at an obtuse angle. Conversely, *B. bigemina* can measure 2 to 5 µm in length and extend the full diameter of an erythrocyte. Under light microscopy, *B. bigemina* is also found in pairs but unlike *B. bovis*, the angle is acute. Although both organisms are often found in pairs, single forms of the organism are often found within infected erythrocyte [13].

Epidemiology: The epidemiology of babesiosis in general depended on several parameters such as availability of host, presence of ticks that act as vector of transmission of infections, presence of parasites within vectors, as well as hosts and environmental condition. These parameters are responsible for spread of infections.

Table 1: Recognized *Babesia* species of bovines

Babesia species	Size and Morphology of organism	Principal Tick vectors	Distribution
B. bovis	2.4 by 1.5 µm (small, more roundedobtuse angle)	*Rhipicephalus microplus, R. annulatus, R. geigyi* and *R. australis*	*The same as Babesia bigemina*
B. bigemina	4.5 by 2.5 µm (Large, round and pryiform, acute angle)	*R. microplus, R. annulatus, R. geigyi, R. decoloratus* and *R. evertsievertsi*	*Africa, Egypt, Asia, America,* Australia and Southern Europe
B. divergen	1.5 by 0.4 µm (small, narrow and obtuse angle)	*Ixodesricinus* and *I. persulcatus*	Northern Europe, Northern Africa
B. ovata	Similar to *B. bigemina*	*Haemaphysalis* sp.	Japan
B. major	2.6 by1.5 µm (large round &pyriform)	*Haemaphysalis* sp.	Europe, North Africa
B. beliceri		*Hyalomma* sp.	Russia
B. occultans	Large	*Hy. marginatum*	Southern Africa
B. jakimovi	Similar to *B. Major*	*Ixodes* sp.	Northern USSAR

Source: [13, 17]

Absence of any one parameter will discontinue the spread of infections. The parasite *Babesia* itself is the weakest point of this system of spread of infection as it needs both vectors and host for its survival and thus dependent on them. The second weakest point is the vector which depends on host and finally the host which support these two parameters to spread the infection but is not dependent on these two parameters. As regarding epidemiology of babesiosis, a state of "endemic stability" where the relationship between host, parasite, vector and environment remained in such a way that clinical disease occurs rarely or not at all [6]. Under instability conditions, some animals become infected with *Babesia* after birth. Therefore, they may develop severe disease if they get the infection later in life [18].

Geographic Distribution: Bovine babesiosis can be found wherever the tick vectors exist, but it is most common in tropical and subtropical areas [4]. Ticks are widely distributed throughout the world particularly in tropical and subtropical countries and 80% of the world cattle are affected with ticks and ticks borne diseases [19]. *Babesia bovis* and *B. bigemina* are present in most areas of the world, with the greatest incidence between the latitudes of 32°N and 30°S, where their *Rhipicephalus* (formerly *Boophilus*species) tick vector commonly occurs [20]. They are particularly important in Asia, Africa, Central and South America, parts of southern Europe and Australia.

Babesia bigemina has the widest distribution but *B. bovis* is generally more pathogenic than *B. bigemina* or *B. divergens* [2]. Although *B. bovis* is usually found in the same general geographic area as *B. bigemina,* slightly different groups of ticks spread these two species and some differences in their distribution can be seen. For example, *B. bigemina* is more widely distributed than *B. bovis* in Africa [21]. *B.bigemina* has been eradicated from the United States of America. In southern Africa, *B. bovis* is restricted to areas where *Rhipicephalus microplus* is exist [18]. Generally both parasites, *B. bovis* and *B. bigemina*, have the same distribution, but in Africa *B. bigemina* is more widespread than *B. bovis* because of the ability of *Rhipicephalus* (*Boophilus*) *decoloratus* and *Rhipicephalus evertsi* to also act as vectors for this species [20]. *Babesia major* can be found in parts of Europe, Northwest Africa and Asia, as well as China. *Babesia ovata* has been described in Japan, China and other parts of eastern Asia. *B. occultans* was reported in Africa and *B. jakimovi* occurs in Siberia. *Babesia divergens* is transmitted almost exclusively by *Ixodesricinus* in northern Europe [22].

Risk Factors

Host Factor: Host factors associated with disease include age, breed and immune status. *Bosindicus* breeds of are more resistance to babesiosisthan *Bostaurus*. This is a result of evolutionary relationship between *Bosindicus* cattle, *Rhipicephalus* (formerly *Boophilus*) species and *Babesia* [13]. Because of natural selection pressure, indigenous populations, having lived for a long time with local ticks and tick-borne diseases have developed either an innate resistance or an innate ability to develop a good immune response to the tick or tick-borne hemoparasitic disease in question [5].

Babesia infection in host depending on age of the host and inverse age resistance like young animals are less and older animals are more susceptible to infection [6]. The severity of the clinical babesiosisincreases with age so, adult are more infected by babesiosis as compared with calves [5]. Resistance to re-infection, acquired as a result of the continuous presence of the parasite, is known as ''*premune immunity'' or premunity* [11]. In endemic area, passive acquired immunity against *Babesia* through colostrum remained until 2 months. Later is followed by innate immunity from 3 to 9 months of age. Therefore, calves exposed to babesiosis during the first 6 to 9 months rarely show clinical symptoms and develop a solid long-lasting immunity. Moreover, it is

estimated that if at least 75% of calves were exposed to *B. bovis* infection by 6 to 9 months of age the disease incidence would be very low and a state of natural endemic stability would exist [23].

Pathogen Factor: Strains vary considerably in pathogenicity; however, *B. bovis* is usually more virulent than *B. bigemina* and *B. divergens* [24]. Many intra-erythrocyte hemoparasites survive the host immune system through rapid antigenic variation which has been demonstrated for *B. bovis* and *B. bigemina* [25].

Environmental Factor: There is a seasonal variation in the prevalence of clinical babesiosis, the greatest incidence occurring soon after the peak of the tick population. Climatic data such as environmental temperature, humidity and rainfall of a particular area are responsible for transmission of bovine babesiosis [6]. From the climatic factors, air and temperature is the most important because of its effect on tick activity; higher temperatures increase its occurrence. Heaviest losses occur in marginal areas where the tick population is highly variable depending on the environmental conditions. Babesiosisinfection in cattle mostly reaches peak in summer [26].

Vector: Ticks are effective disease vectors, second only to mosquitoes in transmitting infectious disease. Ticks are widely distributed throughout the world particularly in tropical and subtropical countries and 80% of the world cattle are affected with ticks and ticks borne diseases [19]. Tick vectors of *B. bigemina* and *B. bovis* are *Rhipicephalus microplus* and *Rhipicephalus annulatus*. *Rhipicephalus decoloratus, Rhipicephalus geigyi*and *Rhipicephalus evertsi* are also competent vectors of *B. bigemina*. *Rhipicephalus geigyi* is also a competent vector of *B. bovis* transmitted by feeding of larval stages of one-host *Rhipicephalus* species of ticks [27].

Babesia bigemina and *B. bovis* are transmitted transovarially by boophilid ticks but only tick larvae transmit *B. bovis,* whereas nymphs and adults transmit *B. bigemina* and *B. divergens* [28]. *Ixodesricinus* is the major vector for *B. divergens.* All three of its life stages are thought to be capable of transmitting this organism. *Haemaphysalis longicornis* transmits *B. ovata,* while *B. occultans* is thought to be transmitted by *Hyalomma marginatum, Hy. rufipes* and possibly other members of this genus. The vectors for *B. major* are thought to include *Haemaphysalis punctata* and possibly other members of this genus. *Babesia jakimovi* might be transmitted by a member of the genus *Ixodes* [24].

Fig. 1: Male of *Rhipicephalus* (*Boophilus*) species
Source: [10]

Life Cycle: The life cycle of all *Babesia* species is approximately similar but slight difference exists because in some species transovarialtransmission occur (*Babesia species sensustricto*) while not in other species, *Babesiamicroti,* Zygner*, et al.* [29]. *Babesia* species generally complete their life cycle in 3-stages: gamogony (in the tick gut gametes fusion and formation), sporogony (In salivary glands asexual reproduction occur), merogony in vertebrate asexual reproduction occur. Cattle are infected by feeding ticks which inoculates sporozoitesthat invade erythrocytes where they transform into trophozoitesthat divide by binary fission (merogony) [13].

The erythrocyte membrane breaks down and the released merozoites invade new cells resulting in an intra-erythrocytic cycle. Following a tick blood meal, gametocytesdevelop in the tick gut, which fuse to form diploid zygotes. Zygotes invade the digestive cells and probably basophilic cells where they undergo successive round of multiplication before emerging as haploid kinetes. The kinetes migrate to other organs including the ovaries where further division occurs. After egg hatching, the kinetes migrate to the salivary gland where they transform into multi-nucleated stages (sporogony) which later form *sporozoite*, the infective stage [30]. The ovaries are also invaded which leads to transovarial transmission. The host gets the infection when the larva sucks blood. After one moulting the larva transforms into nymph which also infect as larva. Nymph transforms into adult after moulting and they transmit infection in similar way [31].

Transmission: All species of *Babesia* are naturally transmitted from animal to animal through the bites of ticks and within ticks' transovarian transmission (transmission of infection through eggs from mother ticks) and stage-to-stage transmission (transmission of infection from egg to larvae to nymph to adult) occurs [6].

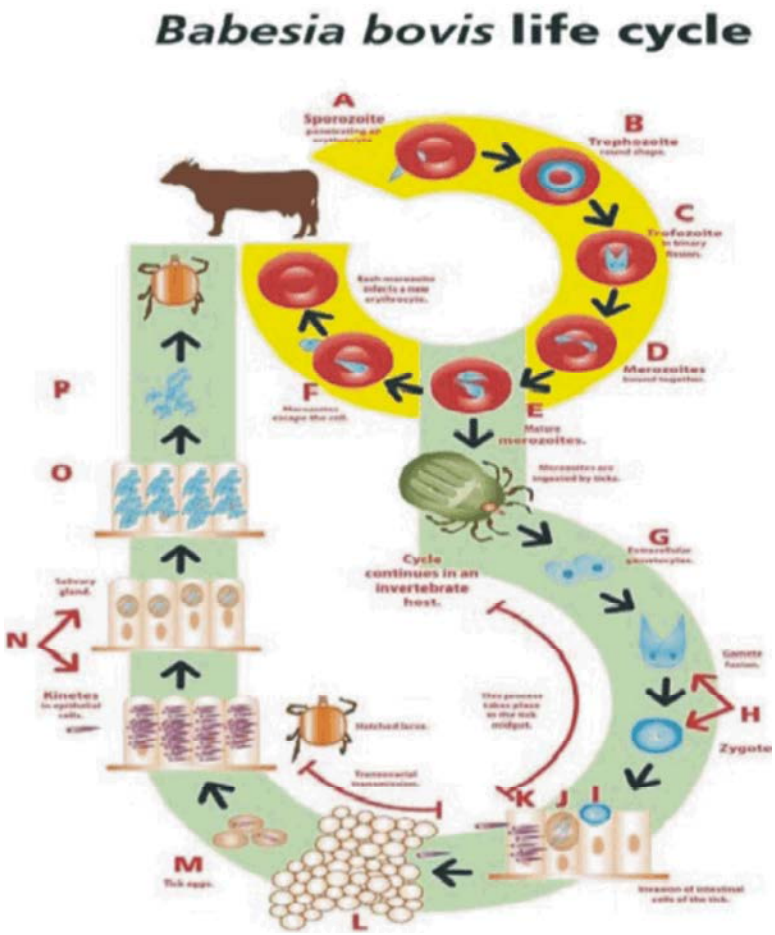

Fig. 2: *Babesia bovis* life cycle. A.B. *bovis* sporozoite invades an erythrocyte and transforms into a trofozoite. B. The trofozoite in a ring shape. C. Two merozoites are generated from each trofozoite by binary fission. D. Merozoites are initially bound together resembling two pears in an acute angle. E. The mature merozoites separate before escaping the erythrocyte. F. Merozoites are liberated from the erythrocyte. Some of them will invade new erythrocytes and develop into trofozoites, while others will be picked up by adult ticks to continue their cycle in the invertebrate host. G. Sexual stages are freed from the red blood cells in the intestinal tick lumen and develop to gametocytes. H. The gametocytes transform into male and female gametes that form a zygote after fusion. I. The zygote develops into an infecting stage and penetrates the tick intestinal cells. J. Fission bodies form and from them motile kinetes develop. K. Kinetes destroy the intestinal cells, escape into the haemolymph and distribute into the different cell types and tissues, including the ovaries. L. In the ovary, embryo cells are infected by kinetes (Transovarial transmission). M. When the female tick lays her eggs, the embryos are already infected. N. Hatched infected larvae attach to a bovine and the kinetes migrate to the salivary glands of the tick, where they form a sporoblast. O. Thousands of sporozoites develop from each sporoblast. P. Tick larvae feed from the bovine blood and the sporozoites are liberated with saliva into the animal's circulatory system [32].

Ticks become infected when they ingest parasites in the blood of infected cattle. In an infected tick, the *Babesia* parasite develops and spreads throughout the tick's organs, eventually invading the salivary glands or eggs. When the infected tick bites cattle, the parasites are injected into the bloodstream where they enter red blood cells [5].

Babesia can be transmitted directly between animals in blood, for instance during transfusions and possibly when smaller amounts of blood are transferred on reused needles or field surgical instruments or by biting flies or fomites, might act as mechanical vectors, although this method of transmission is thought to be of minor importance. Transplacental transmission has been

demonstrated for *B. bovis* and *B. bigemina* in cattle, but seems to be infrequent. Humans are thought to become infected with *B. divergens* in tick bites. Other species of zoonotic Babesia (e.g., *B. microti* of rodents) can be transmitted in blood transfusions and may also infect the fetus in utero on rare occasions. *Babesia divergens* can survive in tick populations for at least 4 years even if cattle are not present. When an infected tick attaches to a new host, Babesia is stimulated to undergo their final maturation. *Babesia bovis* parasites usually become infective within 2-3 days after larval ticks attach and can be transmitted by larvae. In *R. microplus, B. bovis* does not persist after the larval stage. In contrast, *B. bigemina* matures in approximately 9 days after a larval tick attaches and it is only transmitted by nymphs and adults [24].

Incubation Period: In natural infections, incubation periods usually vary from 8 to 15 days [5]. The symptoms of *B. bigemina*and *B. Bovis* infections usually appear 2 to 3 weeks after tick infestation [32]. After inoculation with contaminated blood, the incubation period can be as short as 4-5 days for *B. bigemina* and 10-12 days for *B. bovis* [33].

Morbidity and Mortality: Morbidity and mortality vary greatly and are influenced by prevailing treatments employed in an area, previous exposure to a species/strain of parasite and vaccination status. In endemic areas, cattle become infected at a young age and develop a long-term immunity. However, outbreaks can occur in these endemic areas if exposure to ticks by young animals is interrupted or immuno-naïve cattle are introduced. The introduction of *Babesia* infected ticks into previously tick-free areas may also lead to outbreaks of disease [27].

The endemic stability can be upset and outbreaks can occur if climate changes, acaricide treatment or other factors decrease tick numbers and animals do not become infected during the critical early period. Outbreaks are also seen in areas where cold seasons interrupt tick-borne transmission for a time [21]. In naive cattle, susceptibility to disease varies with the breed. Recently, variable susceptibility to *B. bovis*was also reported in some *B. taurus* cattle: approximately 28% of a population of adult animals was susceptible to infection but resistant to clinical signs. Infections with *B. bovis* are generally more likely to be fatal than infections with *B. bigemina* or *B. divergens* [24]. The overall mortality rate for bovine babesiosis is reported to be 5-10%, even with treatment. *B. bovis* usually causes more severe illnesses than *B. bigemina* or *B. divergens* and mortality can reach 50-100% in untreated animals infected with this organism. The prognosis is guarded once hemoglobinuria develops and CNS signs suggest a poor prognosis [33].

Pathogenesis: The primary mechanism is intra vascular haemolysis (Leading to haemoglobinaemia and haemoglobinuria), resulting in anaemia, hypoxia and secondary inflammatory lesions in various organs, especially liver and kidneys. The secondary mechanism is electrolyte imbalances, complement activation, coagulation disorders and release of pharmacologically active substances resulting in vascular malfunction and hypotensive shock. The main sequelae of the disease are: anaemia due to haemolysis; haemoglobinaemia and haemoglobinuria, Icterus. Pharmacologically active substances such as kinins and catecholamines lead to increased vascular permeability and dilatation of blood vessels resulting in oedema and hypovolaemic shock. Centrilobular liver degeneration and degeneration of kidney tubule epithelium are caused by hypoxia and possibly by immune pathologic reactions. Damage to kidney tubule epithelium impairs ion exchange, resulting in hydrogen ion retention leading to acidosis [23].

Clinical Findings: Clinical signs depend on virulence and pathogenic effects of a particular *Babesia* species and host factors associated with disease include age, breed and immune status [34]. Most cases of babesiosis are seen in adults; animals younger than 9 months usually remain asymptomatic [5].

Acute *B. bovis* often develops in fatal cerebral babesiosis with hyperaesthesia, convulsions and paralysis due to aggregation of red blood cells in the cerebral capillaries and extra vascular, following endothelial damage. Nervous signs are characterized by hyper excitability and the animal may charge moving objects. The vision becomes impaired. Other manifestations include salivation, lachrymation, diarrhoea or constipation, delirium and incoordination of gait [13].

In *B. bigemina* infections, the major signs include fever, haemoglobinuria and anaemia. Intravascular sequestration of infected erythrocytes does not occur with *B. bigemina* infections [5]. Acutely affected cattle are usually not as severely affected as those with *B. bovis* infections. There is no cerebral involvement and recovery in non-fatal cases is usually rapid and complete. However, in some cases the disease can develop very rapidly with sudden and severe anaemia, jaundice and death, which may occur with little warning Animals that recover from *B. bigemina* remain infective for ticks for 4 to 7 weeks and carriers for only a few months [23].

*Babesia. major, B. ovata*and *B. occultans* are mostly thought to cause mild illnesses or asymptomatic infections in cattle, but there are occasional reports of clinical cases. *B. major* has been implicated in anaemia and hemoglobinuria and it is thought to have been responsible for two fatal cases of babesiosis in Hungary, while *B. occultans* appears to have caused babesiosis in a herd of cattle in Italy. *B. ovata* may potentiate the development of anemia in cattle co-infected with *T. orientalis* and it can cause clinical signs in experimentally infected, splenectomized cattle [33]. Recovered animals become carriers, without apparent clinical symptoms, but with possibility to relapse under stress conditions and also remain infective [34].

Post Mortem Lesions: The gross lesions of babesiosis are mainly related to intravascular hemolysis, anemia and jaundice. The mucous membranes are usually pale and may be icteric and the blood can appear thin and watery. Icterus may also be observed in the omentum, abdominal fat and subcutaneous tissues. The spleen is markedly enlarged with a dark, pulpy, friable consistency. The liver may be enlarged and darkened or icteric, with a distended gallbladder containing thick, granular bile. The kidneys are usually dark red or black and the urinary bladder often contains reddish brown urine; however, the appearance of the urine is sometimes normal. The lungs occasionally show signs of pulmonary oedema. Other organs including the heart and brain may have petechiae or ecchymoses or be congested and the surface of the brain can look pink [33]. Acute cases will show haemoglobinuria, but this may be absent in subacute or chronic cases [23].

Diagnosis: The diagnosis of bovine babesiosis is an important tool to control and prevent the dissemination of the disease [5]. Diagnosis of babesiosis mainly based on identification of the parasites in blood or tissues, polymerase chain reaction assays (PCR), serology, or transmission experiments. Diagnosis can also depend on clinical signs and babesiosis should be suspected in cattle with fever, anaemia, jaundice and hemoglobinuria [4].

Identification of the Agent

Direct Microscopic Examination: Clinically, babesiosis can be confused with other conditions that cause fever, anaemia, hemolysis, jaundice, or red urine. Therefore, confirmation of diagnosis by microscopic examination of Giemsa stained blood or organ smears is essential [13]. Microscopic examination is still the cheapest and fastest method used to identify Babesia parasites [4]. The sensitivity of thick films is such that it can detect parasitaemias as low as 1 parasite in 106 red blood cells [5]. Thin and thick Blood smear examination has been considered to be the standard technique for routine diagnosis, particularly in acute cases, but not in sub-clinical infections where the parasitemia is usually much lower [35].

Species differentiation is good in thin films but poor in the more sensitive thick films. This technique is usually adequate for detection of acute infections, but not for detection of carriers where the parasitaemias are mostly very low [13]. Parasite identification and differentiation can be improved by using a fluorescent dye, such as acridine orange, instead of Giemsa [36]. Blood film examination requires very much expertise to differentiate between Babesia species from one or more animal species which look similar under stained preparation [37].

Samples from live animals should preferably be films made from fresh blood taken from capillaries, such as those in the tip of the ear or tip of the tail, as *B. bovis* is more common in capillary blood. *Babesia bigemina* and *B. divergens* parasites are uniformly distributed through the vasculature. If it is not possible to make fresh films from capillary blood, sterile jugular blood should be collected into an anticoagulant such as lithium heparin or ethylene diamine tetra-acetic acid (EDTA). Samples from dead animals should consist of thin blood films, as well as smears from cerebral cortex, kidney (freshly dead), spleen (when decomposition is evident), heart muscle, lung and liver [4].

***In vitro* Culture:** In-vitro culture methods used to demonstrate presence of carrier infections of *Babesia* species *B. bovis* has also been cloned in culture. Minimum parasitaemia detectable by this method depends on the facilities available and the skills of the operator but could be as low as 10-10 making it a very sensitive method for the demonstration of infection, with 100% specificity [38].

Animal Inoculation: Confirmation of infection in a suspected carrier animal can also be made by transfusing approximately 500 ml of jugular blood intravenously into a splenectomised calf known to be *Babesia* free and monitoring the calf for the presence of infection. This method is cumbersome and expensive and obviously not suitable for routine diagnostic use. Mongolian gerbils (*Merionesunguiculatus*) have been used to demonstrate the presence of *B. divergens* [5].

Indirect Diagnostic Method

Serological Tests: Among the various serological tests, most important once include complement fixation test (CFT), indirect fluorescent antibody technique (IFAT) and enzyme-linked immunosorbent assay (ELISA). Serology is most often used for surveillance and export certification [4]. Indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) are capable of detecting antibodies of Babesia in sub-clinical infections. And also the complement fixation (CF) test has been described as a method to detect antibodies against *B. bovis* and *B. bigemina* [5] and agglutination assays (latex and card agglutination tests) have been described [4]. IFAT is the most widely used test for the detection of antibodies to *B. bovis* and *B. bigemina* but serological cross reactions make species diagnosis difficult. It is based on the recognition of parasite antigens by serum antibodies in the blood of the tested animal. It is easy to do but requires a good quality antigen which is difficult to obtain [32].

Drawbacks of these tests are the occurrence of false positive and false negative results involving cross-reactive antibodies and/or typical specific immune responses [28]. Antibodies to *B.divergens* can cross-react with other zoonotic members of the *Babesia divergens/ B. odocoilei complex*. There may also be cross-reactivity with organisms such as Plasmodium species or *Toxoplasma gondii* and false positive reactions caused by autoimmune diseases [33]. These serological cross-reactions can complicate the differentiation of some species in serological tests [21].

Molecular Diagnosis: Molecular methods such DNA probes, Polymerase chain reaction, Reverse line blot hybridization and Real time PCR. But the most sensitive and specific methods for detection are molecular [13]. The most common method used to detect Babesia in both the tick and the vertebrate host involves simple or multiplex PCR- amplification of the 18SrRNA gene fragment [39]. An advantage of this method Polymerase Chain Reaction (PCR) is more sensitive and specific technique and it allows identification of the parasite in the early stage of disease which enables early diagnosis, implementation of therapy and avoidance of complications [40].

Polymerase chain reaction (PCR) assays can detect and differentiate *Babesia* species and are particularly useful in carriers [24]. Immunofluorescent and immunoperoxidase labelling have also been described. These parasites are found within RBCs and all divisional stages ring (annular) stages, pear shaped (pyriform) trophozoites either singly or in pairs; and filamentous or amorphous shapes can be found simultaneously.

Biochemical Findings: Babesiosis infected cattle showed significant increase in aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), hypoproteinemia, hypoalbuminemia and decreased albumin to globulin ratio. This may indicate the harmful effect of toxic metabolites of *Babesia* species on liver cells. The significant increase in serum globulins in babesiosis could be attributed to the immune response against *Babesia* [13].

Treatment: The success of the treatment depends on early diagnosis and the prompt administration of effective drugs [5]. If treatment is delayed, however, supportive therapy may be essential if the animal is to survive. Non-specific support includes the use of haematinics, vitamins, intravenous administration of fluids, good nutrition and provision of shade. Blood transfusions may be indicated in cattle with heavy parasitaemias and low PCVs (<0.10); histo-incompatibility seldom occurs at the first transfusion. In acute *B. bovis* infections, use of antioxidants such as vitamin E and high doses of corticosteroids may help to offset the hypotensive and hypercoagulable state of the animal. In cases of cerebral babesiosis, intravenous use of hypertonic solutions of mannitol or glucose may provide temporary relief [10]. Anti-inflammatory drugs, tick removal, iron preparations, dextrose, vitamins (*B. complex*), purgatives and fluid replacements, may be necessary in severe cases of babesiosis [35].

The most commonly used compounds for the treatment of babesiosis are diminazenediaceturate (3-5 mg/kg), imidocarb (1-3 mg/kg) and amicarbalide (5-10 mg/kg); however, the quinuronium and acridinederivatives are also effective. For many years, the babesiacides: quinuroniumsulfate, amicarbalide, diminazeneaceturate and imidocarbdiproprionate were used against bovine babesiosis in most of Europe; however, quinuroniumsulfate and amicarbilide were withdrawn because of manufacturing safety issues and diminazene, which is widely used in the tropics as both a babesiacide and a trypanocide, was withdrawn from Europe for marketing reasons [41]. It is evident that the development of new therapeutics, highly specific against Babesia parasites, combined with a low toxicity profile against the host is highly desirable. Novel chemotherapeutic agents currently in development have recently been reviewed [13].

Prevention and Control: Active prevention and control of bovine babesiosis is achieved by three main methods: immunization, chemoprophylaxis and vector control. Ideally, the three methods should be integrated to make the most cost effective use of each and also to exploit breed resistance and the development and maintenance of enzootic stability [42].

Vaccination: Cattle develop durable immunity after a single infection with *B. bigemina* and *B. bovis*. This feature has been exploited with the use of live attenuated vaccines to immunize cattle [10]. Caution should be used in their employment as they may be virulent in adult animals, may be contaminated with other disease agents and could lead to hypersensitivity reactions; usually used in younger animals [13]. The *Babesia*strains used in vaccines are of reduced virulence, but are not entirely safe. Reactions to *B. Bigemina* may occur within seven days and to *B. bovis* within 10-14 days after vaccination. A practical recommendation is therefore to limit the use of vaccine to calves aged 3-9 months when non-specific immunity will minimize the risk of reactions. When older animals have to be vaccinated, there is a risk of severe vaccine reactions and they should be observed daily for three weeks after vaccination. Ideally, rectal temperatures of vaccinated cattle should be taken and animals treated when significant fever develops. Because of the risk of abortions, vaccination of pregnant cows is rarely advised.

The immunity lasts for several years in the case of *B. bovis*, but in the absence of natural challenge, it may breakdown in the case of *B. bigemina*. Redwater vaccines can be given at the same time as anaplasmosis and other vaccines, with the exception of heart water vaccine. The incubation period after vaccination against babesiosisand heart water is the same. Treatment is required in animals that react, i.e. showing an increased temperature reaction. At this stage, the owner will not be able to determine against which of the two pathogens the animal is reacting and will therefore not be able to administer appropriate treatment. The incubation period after vaccination against anaplasmosisis longer than in the case of babesiosis and reactions should not overlap; treatment of the animal reacting to the babesiosisvaccine will not influence development of immunity against anaplasmosis. Vaccination against *B. divergens*is not commonly done [10].

Australia is at the forefront in terms of the production and distribution of live attenuated vaccines (Chilled or frozen trivalent live vaccine) worldwide [13]. A formalin-inactivated vaccine has been used with some success in Austria since 1988, while an experimental live vaccine has been successfully used in Ireland [10]. Internationally available strains are attenuated Australian strains of *B. bovis* and *B. bigemina* have been used effectively to immunise cattle in Africa, South America and South East Asia [22]. Tick-transmissible and non-transmissible strains are available. A strain of *B. divergens* with reduced virulence for meriones has also been developed [35].

Chemoprophylaxis: Imidocarb and diminazene are the only babesiacides with useful prophylactic properties for the short term control prevention of babesiosis. Treatment with imidocarb (3mg/kg) will prevent overt *B.bovis* infections for at least four weeks and *B. bigemina* infections for at least eight weeks [10] and *B. divergens* (3–6 weeks protection) [13]. Diminazene (3, 5mg/kg) will protect cattle against the two diseases for one and two weeks, respectively.

Unfortunately, the prophylactic use of imidocarb may interfere with the development of immunity following vaccinations because the residual effect of the drug may eliminate or suppress the infection. The interval between the use of imidocarb and vaccination should be at least eight weeks if immunity to *B. bovis*is required and 16 weeks in the case of *B. bigemina.* If diminazene is used, the intervals for the two parasites should be about four and eight weeks, respectively [10].

Vector Control: Eradication of bovine babesiosis has been accomplished by elimination of tick vector. In areas where eradication of tick is not feasible or desirable; ticks are controlled by repellents and acaricides [13]. Eradication of the tick vectors (the so-called minimum disease situation) is the most desirable, permanent solution to the problem but is rarely considered practical or economical.

The alternative approach, allowing natural endemic stability to develop by practicing limited or no tick control, is similarly unrealistic in areas where *R. Appendiculatus* and *Amblyomma* species are well established. These regions are also endemic for other *Rhipicephalus* (*Boophilus*) species and essential control of other tick species will inevitably affect the epidemiology of redwater. In the long-term, this approach can be achieved by integrating the strategic use of acaricides the application of vaccines in endemically unstable conditions and the use of tick resistant breeds of cattle [10].

Table 2: Prevalence of bovine babesiosis from different areas of Ethiopia

Area	Diagnostic methods	Prevalence	Reference
Central Ethiopia (Bishoftu)	Microscopic examination	0.6%	[47]
Central Ethiopia (Maki and Batu)	Microscopic examination	3.64%	[48]
South Western Ethiopia (Jimma)	Microscopic examination	23%	[46]
Southern Ethiopia (Borena)	Microscopic examination	16.9%	[1]
Western Ethiopia (Ben. Gumuz)	Microscopic examination	1.5%	[45]

Economic Significance: Bovine babesiosis causes most serious economic loss to the livestock industry, endangering half a billion cattle across the world [13]. Babesiosis, especially in cattle has great economic importance, because unlike many other parasitic diseases it affects adults more severely than young cattle, leading to direct losses through death and the restriction of movement of animals by quarantine laws [42]. The economic losses can be considerable, especially when animals with no immunity are moved into an endemic area [33]. The disease is also a barrier to improving productivity of local cattle by cross-breeding due to the high mortality of genetically superior but highly susceptible cattle, especially dairy cattle, imported from *Babesia* free 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 [42].

The impact of this disease varies from fever, anorexia, anaemia, threatened abortion and death in the acute form of infections. They also impose a great economic burdenon the tropical and subtropical developing countries [13]. The cattle babesiosis in respect of economic impact then we could see losses occurs due to mortality, decreased milk or meat production, abortions, reduction of draft power, cost under the head of control measures, including increased cost of management to maintain ill animals. An annual loss of 16.9, 5.1, 5.4, 6.8, 21.6, 19.4, 57.2, 3.1 and 0.6 million US dollars in Australia, Kenya, Zimbabwe, Tanzania, South Africa, China, India, Indonesia and Philippines, respectively, have been estimated due to babesiosis and anaplasmosis [6].

Public Health Importance: Human babesiosis was first described in 1957 but is now known to have worldwide distribution. The increase in reported cases is likely due to increases in actual incidence as well as increased awareness of the disease [27]. The four identified babesia species definitively confirmed that infect humans so far are *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum* [43], but major one is *Babesiamicroti* particularly in North America [6].

Parasitaemias may range between 1 and 80% causing severe intravascular haemolysis with haemoglobinuria. The subsequent nonspecific clinical presentation can be easily confused with malaria; jaundice due to severe hemolysis is accompanied by persistent non periodic high fever (40 to 41°C), shaking chills, intense sweats, headaches and myalgia as well as lumbar and abdominal pain, vomiting and diarrhea may be present. Total hemoglobin levels may fall to 70 to 80g/ litre, in the most severe cases; patients develop shock like symptoms, with renal failure induced by intravascular hemolysis and pulmonary oedema. Unless treated rapidly, the infection is usually fatal. During the incubation period of 1 to 3 weeks, patients frequently complain of general weakness and discomfort. Acute illness appears suddenly, generally with hemoglobinuria as the presenting symptom [13].

Status of Bovine Babesiosis in Ethiopia: Tick-borne diseases and their vectors are wide spread in Ethiopia and they cause considerable losses to the livestock economy, ranking third among the major parasitic disasters after trypanosomes and endoparasitism [44]. They affect the production in various ways, such as growth rate, milk production, fertility, the value of hides and mortality. Bovine babesiosis is one of the most important diseases in Ethiopia because, it occurs sometimes in acute forms with serious recognized clinical manifestations yet lowering the productive performance of the affected animals [5]. Different researchers have reported the prevalence of bovine babesiosis from different areas of Ethiopia (Table 2). The study from BenishangulGumuz Regional State, Western Ethiopia, was reported an overall prevalence of 1.5% from which *B. bovis* was found to be 1.24% and *B. bigemina* was 0.248% [45]. Another study in and around Jimma town, southwest Ethiopia by Lemma *et al*. [46] was reported overall prevalence rate of bovine babesiosis as 23%. A study from Southern Ethiopia in Teltele District, Borena Zone, indicated the overall prevalence of 16.9% with a relatively similar prevalence of both *B. bovis* and *B. bigemina* [1]. A study from Bishoftu, central Ethiopia, was reported a prevalence of 0.6% with an equal prevalence of both *B. bovis* and *B. Bigemina* [46].

CONCLUSION AND RECOMMENDATIONS

Bovine babesiosis is the most important tick-borne disease of cattle worldwide that causes significant morbidity and mortality. It is caused by an apicomplexanhaemo protozoan parasite of the genus *Babesia*. The most prevalent species are *B. bovis* and *B. bigemina* found throughout most tropical and subtropical regions including Ethiopia. The principal vectors of *B. bovis* and *B. bigemina* are *Rhipicephalus* (formerly *Bophillus*) species of ticks and these are widespread in tropical and subtropical countries. The disease is a barrier to improving productivity of local cattle by cross-breeding due to the high mortality of genetically superior cattle. Active prevention and control of Bovine Babesiosis is achieved by immunization, chemoprophylaxis and vector control. Therefore, based on the above conclusion the following recommendations were forwarded:

- Awareness should be created on mode of transmission, control and prevention methods of *Babesia* to livestock owners.
- Since chemical control can result in resistance and environmental contamination, environmentally friend control mechanisms like vaccination and biological methods should be further developed.
- Ethiopia should develop and implement surveillance systems and action plans to prevent bovine babesiosis from spreading.
- The veterinarians and other respected organizations should give attention to control and eradicate of the disease.

REFERENCES

1. Hamsho, A., G. Tesfamarym, G. Megersa and M. Megersa, 2015. A Cross-Sectional Study of Bovine Babesiosis in Teltele District, Borena Zone, Southern Ethiopia Journal of Veterinary Science and Technology, 6(3): 230.
2. OIE, 2018. Bovine babesiosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Paris, France.
3. Ristic, M., 2018. Babesiosis of Domestic Animals and Man: 0. CRC Press, pp: 15.
4. Abdela, N. and K. Jilo, 2016. Bovine Babesiosis and its Current Status in Ethiopia: A Systemic Review. Advances in Biological Research, 10(3): 138-146.
5. Wodaje, A., B. Adudna and M. Hamid, 2019. A Review on bovine babesiosis.International Journal of Advanced Research in Biological Sciences, 6(1): 63-70.
6. Laha, R., M. Das and A. Sen, 2015. Morphology, epidemiology and phylogeny of Babesia: An overview. Tropical Parasitology, 5(2): 94.
7. Kjemtrup, A.M. and P.A. Conrad, 2000. Human babesiosis: an emerging tick-borne disease. International Journal for Parasitology, 30: 12-13, pp: 1323-1337.
8. Schorn, S., K. Pfister, H. Reulen, M. Mahling and C. Silaghi, 2011. Occurrence of Babesia spp., *Rickettsia* spp. and *Bartonella* spp. in Ixodesricinus in Bavarian public parks, Germany. Parasites and Vectors, 4(1): 135.
9. Bock, R., L. Jackson, A. De Vos and W. Jorgensen, 2004. Babesiosis of cattle. Parasitology, 129(7): 247-269.
10. Penzhorn, B., 2015. Bovine babesiosis, Livestock Health and Management Production, High Impact Disease› Vector-borne Disease, pp: 1-16.
11. Chauvin, A., E. Moreau, S. Bonnet, O. Plantard and L. Malandrin, 2009. Babesia and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. Veterinary Research, 40(2): 1-18.
12. Saad, F., K. Khan, S. Ali and N. Akbar, 2015. Zoonotic significance and Prophylactic Measure against babesiosis. International Journal Current Microbiol Applied Science, 4(7): 938-953.
13. Demeke, D., A. Endris, E. Birhanu and A. Samuel, 2018. Review on bovine Babesiosis. Acta. Parasitol. Globalis, 9(1): 15-26.
14. Bal, M.S., V. Mahajan, G. Filia, P. Kaur and A. Singh, 2016. Diagnosis and management of bovine babesiosis outbreaks in cattle in Punjab state. Veterinary World, 9(12): 1370.
15. Mohammed, E.S. and I. Elshahawy, 2017. The Current Prevalence of Bovine Babesiosis and Theileriosis Infection in Egypt. Clinical and Medical Images International Journal, 1(1): 00004.
16. Herwaldt, B.L., S. Cacciò and F. Gherlinzoni, 2003. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe, Emerging Infectious Disease, 9(8): 942-48.
17. Ganzinelli, S., A. Rodriguez, L. Schnittger and M. Florin-Christensen, 2018. Babesia in Domestic Ruminants. In Parasitic Protozoa of Farm Animals and Pets, pp: 215-239.

18. Naser, M.A.E., 2017. Prevalence and Risk Factor of Bovine Babesiosis in South Darfur State, Sudan (Doctoral dissertation), Sudan University of Science and Technology, pp: 52.
19. Ghosh, S., G.C. Bansal, S.C. Gupta, D. Ray, M.Q. Khan, H. Irshad and J.S. Ahmed, 2007. Status of tick distribution in Bangladesh, India and Pakistan. Parasitology Research, 101(2): 207-216.
20. Pohl, A.E., 2013. Epidemiology study of tick-borne diseases in cattle in Minas Gerais, Brazil. Doctoral Dissertation, Lmu, pp: 115.
21. Spickler, A., J. Roth and G. Dvorak, 2010. Emerging and exotic diseases of animals, 4th ed. CFSPH Iowa State University, lowa USA, p: 102-105.
22. Bock, R., L. Jackson, A. De Vos and W. Jorgensen, 2008. Babesiosis of cattle. In. ticks Biology, Disease and Control, Bowman, A.S. and Nuttall, P.A., eds. Cambridge University Press, Cambridge, UK, pp: 281-307.
23. Laha, R., M. Das, A. Goswami and P.A. Singh, 2012. Clinical case of babesiosis in a cross bred cow of Meghalaya. Indian Journal of Animal Research, 46(3): 302-305.
24. CFSPH, 2008. Bovine babesiosis, Iowa state university, Ames, Iowa.
25. Radostits, O.M., G.C. Gay, K.W. Hinchiff and P.O. Constable, 2007. A text book of the disease of cattle, sheep, goat, pigs and horses 10th ed. London: Saunders Elsevier, pp: 1110-1489, 1527-1530.
26. El-Moghazy, H., M. Ebied, M. Abdelwahab and A. ElSayed, 2014. Epidemiological studies on bovine Babesiosis and Theileriosis in Qalubia governorate. Benha Veterinary Medical Journal, 27(1): 36-48.
27. Yadhav, C., M. Chandana, Y. Sailalithkumar, N. Sujitha, M. Lavanya and C. Madhavilatha, 2015. An overview of Babesiosis. International Journal in Research Pharmacetical Science, 3(1): 287-295.
28. Esmaeilnejad, B., M. Tavassoli, S. Asri-Rezaei, B. Dalir-Naghadeh, K. Mardani, M. Golabi, J. Arjmand, A. Kazemnia and G. Jalilzadeh, 2015. Determination of prevalence and risk factors of infection with Babesiaovis in small ruminants from West Azerbaijan province, Iran by polymerase chain reaction. Journal of Arthropod-borne Diseases, 9(2): 246.
29. Zygner, W., S. Jaros and H. Wêdrychowicz, 2008. Prevalence of Babesiacanis, Borreliaafzelii, and Anaplasmaphagocytophilum infection in hard ticks removed from dogs in Warsaw (central Poland). Veterinary Parasitology, 153(1-2): 139-142.
30. Simuunza, M.C., 2009. Differential diagnosis of tick-borne diseases and population genetic analysis of *Babesia bovis* and *Babesia bigemina* (Doctoral dissertation, University of Glasgow).
31. Mandal, S., 2012. Veterinary Parasitolgy 2nd edition. India: Panacea Computer, pp: 355-365.
32. Mosqueda, J., A. Olvera-Ramirez, G. Aguilar-Tipacamu and G.J. Canto, 2012. Current advances in detection and treatment of babesiosis. Current Medicinal Chemistry, 19(10): 1504-1518.
33. CFSPH, 2018. Bovine babesiosis, Iowa state university, Ames, Iowa.
34. Figueroa, J.V., J.M. Hostis and E. Camus, 2010. Bovine babesiosis. In: Lefevre, P.C., Blancou, J. Chermette, R., Uilenberg, G, editors. Infectious and Parasitic Diseases of Livestock: bacterial diseases, fungal diseases, parasitic diseases. Paris, France: Lavoisier, pp: 1819-38.
35. Zintl, A., G. Mulcahy, H.E. Skerrett, S.M. Taylor and J.S. Gray, 2003. *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clinical Microbiology Reviews, 16(4): 622-636.
36. OIE, 2010. Bovine Babesiosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Paris, France.
37. Salih, D.A., A.M. El-Hussein and L.D. Singla, 2015. Diagnostic approaches for tick-borne haemoparasiticdiseases in livestock. Journal of Veterinary Medicine and Animal Health, 7(2): 45-56.
38. Kahn, C., 2005. The Merck Veterinary Manual 9th edition, USA, Merck and Company Incorporated, pp: 18-32
39. Alhassan, A., W. Pumidonming, M. Okamura, H. Hirata, B. Battsetseg, K. Fujisaki, N. Yokoyama and I. Igarashi, 2005. Development of a single-round and multiplex PCR method for the simultaneous detection of Babesiacaballi and Babesiaequi in horse blood. Veterinary Parasitology, 129(1-2): 43-49.
40. Skotarczak, B., 2008. Babesiosis as a disease of people and dogs. Molecular diagnostics: a review. Veterinarni Medicina, 53(5): 229-235.
41. Vial, H. and A. Gorenflot, 2006. Chemotherapy against babesiosis. Veterinary Parasitology, 138(1-2): 147-160.
42. Demessie, Y. and S. Derso, 2015. Tick Borne Hemo parasitic Diseases of Ruminants: A Review, Advance in Biological Research, 9(4): 210-224.

43. Abraham, A., J. Thekkiniath, N. Kilian, L. Lawres, R. Gao, K. De Bus, L. He, X. Yu, G. Zhu, M.M. Graham and X. Liu, 2018. Establishment of a continuous in vitro culture of Babesiaduncani in human erythrocytes reveals unusually high tolerance to recommended therapies. Journal of Biological Chemistry, 293(52): 19974-19981.
44. Desalegn, T., A. Fikru and S. Kasaye, 2015. Survey of Tick Infestation in Domestic Ruminants of Haramaya District, Eastern Hararghe of Ethiopia. Journal of Bacteriology and Parasitology, 6(5): 1.
45. Wodajnew, B., H. Disassa, T. Kabeta, T. Zenebe and G. Kebede, 2015. Study on the Prevalence of Bovine Babesiosis and Its Associated Risk Factors in and Around Assosa Woreda, Benishangul Gumuz Regional State, Western Ethiopia. Research, 7(8): 33-39.
46. Lemma, F., A. Girma and D. Demam, 2015. Prevalence of Bovine Babesiosis in and Around Jimma town South Western Ethiopia. Advances in Biological Research, 9(5): 338-343.
47. Sitotaw, T., F. Regassa, F. Zeru and A.G. Kahsay, 2014. Epidemiological significance of major hemoparasites of ruminants in and around Debre-Zeit, Central Ethiopia. Journal of Parasitology and Vector Biology, 6(2): 16-22.
48. Bariso, M. and Y. Worku, 2018. Cattle Ticks and Tick Borne Haemoparasite Species Identification and Associated Risk Factors in Two Districts of West Arsi Zone, Ethiopia. J. Vet. Sci.Ani. Husb., 6(5): 501.