



**MEKELLE UNIVERSITY
COLLEGE OF VETERINARY SCIENCES**



**THE OCCURANCE OF CATTLE BABESIOSIS IN MARODIJEH REGION,
SOMALILAND**

By

Mohamed Adam Abdi

November, 2023

Mekelle, Ethiopia

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Mohamed Adam Abdi

**A Thesis Submitted to the College of Veterinary Sciences, Mekelle University, in Partial
Fulfillment of the requirements for the Degree of Master of science in Veterinary Clinical
Laboratory science**

November, 2023

Mekelle, Ethiopia

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a Degree in any other University and that all sources of materials used for this thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as University advisor

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By

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ACKNOWLEDGMENTS

I would like to express my deepest gratitude to Allah, the Most Merciful and Most Compassionate, for providing me with the guidance, strength, and wisdom throughout the course of this research. It is through His blessings and guidance that this work has come to fruition.

I am profoundly indebted to my advisor, Dr. Etsay Kebede, whose unwavering support, expertise, and mentorship have been invaluable. Your guidance and encouragement have played a pivotal role in shaping this research, and for that, I am profoundly grateful.

I extend my heartfelt appreciation to my parents for their unending love, encouragement, and sacrifices that have paved the way for my academic journey. Your unwavering belief in my abilities has been a constant source of inspiration.

I would also like to acknowledge and thank all the staff members of Mekelle University College of Veterinary Medicine for their assistance, resources, and cooperation during this research endeavor. Your contributions have been instrumental in the successful completion of this study.

Additionally, I extend my gratitude to all those known and unknown, whose hands and efforts, directly or indirectly, have contributed to this research. Your support has been a driving force behind this project, and I am truly thankful for your assistance.

In closing, this research would not have been possible without the combined support, encouragement, and assistance of these remarkable individuals and entities. I am humbled and appreciative of their contributions to this work.

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LIST OF ABBREVIATION

CFT:	Complement Fixation Test
CLT:	Central Limit Theorem
CNS:	Central Nervous system
EDTA:	Ethylene-diamine tetra-acetic Acid
ELIZA:	Enzyme Linked Immuno-Sorbent Assay
FAO:	Food Animal Organization
GDP:	Gross Domestic Product
IFA:	Indirect Fluorescent Antibody
IFAT:	Indirect Fluoroscopy Antibody Technique
OIE:	Office des International Epizooties
PCR:	Polymerase Chain Reaction
RBC:	Red Blood Cells
SPSS:	Statistical Package for the Social Sciences
TBDs:	Thick Borne Diseases
USDA:	United States Department of Agriculture.

ABSTRACT

Babesiosis, a tick-borne protozoal disease, poses a significant threat to cattle populations worldwide, including Somaliland. This research aimed to investigate the prevalence of cattle Babesiosis and its associated risk vectors in the Marodijeh region, Somaliland. The major objective of the study was to determine the prevalence of Babesiosis and identify factors associated with its occurrence. A cross-sectional study was conducted, involving a comprehensive examination of cattle populations, tick infestations, and risk factors contributing to the occurrence of Babesiosis. A total of 356 cattle were sampled, and the presence of Babesia species was determined. The Chi-Square (χ^2) test was employed to assess the association between risk factors and the prevalence of Babesiosis. The results of the study indicated that 5.4% of the cattle tested positive for Babesiosis. Among Babesia species, *B. bigemina* had a higher prevalence at 4.8% compared to *B. bovis*, which accounted for 0.6% of the cases. These findings highlight the differential impact of Babesia species in the region. Furthermore, the research explored the influence of factors such as age, sex, body condition, and tick infestation on the prevalence of the disease. Geographic variations, farming systems, and seasonal factors were found to contribute significantly to the differences in Babesiosis prevalence. The Chi-Square analysis confirmed these associations, with p-values supporting the significance of these risk factors. In conclusion, this study underscores the need for region-specific disease control and prevention strategies to effectively manage Babesiosis in the Marodijeh region and similar areas. Such strategies should take into account the differential prevalence of *B. bigemina* and *B. bovis* and address the specific risk factors identified in this study. The results provide valuable insights for local veterinary authorities and policymakers to design targeted interventions for the management of Babesiosis, ultimately improving the health and productivity of cattle populations in the region.

Keywords: Babesia species; Cattle Babesiosis; Marodijeh region; Prevalence; Risk factors; Somaliland; Tick species

1. CHAPTER I: INTRODUCTION

1.1. Background and Justification

Throughout human history, cattle have played a unique role since they were first domesticated in the ancient Fertile Crescent around 10,500 years ago. They are raised for their flesh, dairy products, leather and hides. They are also employed as draught animals in agriculture, pulling ploughs, and in transportation, pulling carts and wagons (FAO, 2012).

The demand for more cattle to produce more meat, milk, and other dairy products increased in tandem with the growth of the human population. This is especially true given the current exponential increase in the human population. More cattle must be kept due to the rise in the consumption of dairy products and meat (beef and veal). The industry that raises cattle must confront the growing problem of balancing the requirement to meet the growing demand for animal products with the need to minimize the negative environmental effects of cattle breeding (FAO, 2012).

There were 987.5 million cattle worldwide in 2020, according to the USDA's global livestock inventory. In 2020, India surpassed Brazil and the United States as the country with the largest global cattle inventory. Brazil, India, and the United States are home to about 65% of the world's cattle. (Water buffalo are included in India's cattle inventory). The United States does not contain more than 90% of the world's cattle. In 2020, the United States possesses the third-largest global cow inventory (beef2live, 2021).

The different traditions and daily lives of Africans revolve around cattle. For the estimated 800 million livestock keepers on the continent, African cattle are priceless assets. In addition to providing much-needed revenue and a vital source of food and nourishment, cattle also produce manure that is rich in nitrogen, which is useful for recharging soils and other purposes. Additionally, they carry out a broad range of sociocultural roles (Dessie & Okeyo Mwai, 2019).

Out of the 14 million households that keep livestock in Ethiopia, the national herd consists of 57 million cattle; over 12 million of these households own at least one cow. Cattle support livelihoods by providing meat, milk, cash, draught power, hauling services, insurance, and social capital (FAO, 2019).

The most important sector in Somaliland is livestock production which is predominately pastoral and agro-pastoral in Somaliland employing over 70% of the population. Livestock production in Somaliland contributes to 60% of GDP and around 85% to foreign export earnings (Mohamed *et al.*, 2018).

Over 65,000 species of single-celled organisms called protozoa are present in both humans and animals. According to Taylor (2000), some species of them are major causes of disease in domesticated cattle. They also cause significant parasitic diseases.

Blood protozoan diseases, which affect 80% of the world's cattle population, include the Theileriosis, Typosomiosis, Babesiosis, and Anaplasmosis. These diseases have an impact on livestock health, productivity, draught power, and food security. These illnesses are significant because they pose a risk to other animals because recovered animals continue to carry the disease for a long time (Senapati *et al.*, 2018).

In Africa, *B. bovis* and/or *B. bigemina* are typically the cause of Babesiosis. *Boophilus microplus* (*B. bovis* & *B. bigemina*), *Boophilus geigy* (*B. bovis* & *B. bigemina*), *Boophilus decoloratus* (*B. bigemina*), and *Rhipicephalus evertsievertsi* (*B. bigemina*) are the tick vectors that their distribution is based on. Transmission rates determine the clinical disease prevalence in a given area (De Vos & Jorgensen, 1992).

Tick-borne diseases (TBDs) cause significant losses among livestock and impact the livelihoods of livestock communities worldwide including Somalia. Infections with Babesia species are endemic in Somalia yet there is a lack of adequate information on their prevalence (Aamir *et al.*, 2020).

Previous studies (Warsame *et al.*, 2020) have reported on the prevalence and impact of Babesiosis in Wajale Somaliland; however, there is still an absence of thorough research on the disease's distribution, epidemiology, and risk factors. The literature that is currently available indicates that Babesiosis may exist in the area, but further research is required to pinpoint its precise prevalence, range, and effects on regional animal populations. Understanding and resolving the issues raised by Babesiosis is crucial, as cattle farming plays a significant role in the livelihoods of many Somaliland residents.

This present study aimed to study the occurrence of Cattle Babesiosis in Marodijeh region Somaliland by detecting parasite using microscope and identifying the different tick species associated with occurrence of this disease. The study aims to address a critical knowledge gap concerning the prevalence, vectors, and risk factors of cattle Babesiosis in the Marodijeh region, Somaliland. By achieving the specified objectives, this research will contribute to more effective disease management and improved cattle health, ultimately benefiting the livelihoods of the local population.

1.2. Objectives

1.2.1. General Objective:

- ❖ The general objective is to study the prevalence of cattle Babesiosis and its vector in Marodijeh region, Somaliland.

1.2.2. Specific Objectives:

- ❖ To examine and identify the morphology of cattle Babesia species
- ❖ To examine and identify morphologically the possible hard tick vectors associated with cattle Babesiosis in the study.
- ❖ To identify the risk and exposing factors of cattle Babesiosis.

1.2.3. Research Questions:

- ❖ Are there morphologically known possible Babesia species affecting cattle in the examined sample cattle?
- ❖ Are there possible known ticks that can transmit cattle Babesiosis in the examined cattle samples?
- ❖ Which are the common risky and exposing factors in cattle Babesiosis?

1.3. Limitation of the Study

The current study has the following limitation;

- ❖ Due to financial constraints and time limitations, indirect diagnostic methods were not included in this study.

2. CHAPTER II: LITERATURE REVIEW

2.1. Etiology and Taxonomy

An apicomplexan hemoprotozoan parasite belonging to the family Babesiidae and order Piroplasmida is the cause of bovine Babesiosis (Sharma *et al.*, 2013). Although there are several species that cause it, *B. bovis*, *B. bigemina*, and *B. divergens* are the three that are most frequently found in cattle. *B. major*, *B. ovata*, *B. occultans*, and *B. jakimovi* are additional species that can infect cattle (Spickler *et al.*, 2010).

In tropical and subtropical regions, the productivity and health of cattle are significantly impacted by two species: *B. bigemina* and *B. bovis* (El-Ashker *et al.*, 2015).

2.2. Epidemiology

2.2.1. World Distribution

Bovine Babesiosis can be found wherever the tick vectors exist, but it is most common in tropical and subtropical areas (CFSPH, 2008). Both *B. bovis* and *B. bigemina* are found throughout much of the planet, but their prevalence is highest between 32 °N and 30 °S, which is also where their *Boophilus* tick vector is most frequently seen (Pohl, 2013).

They are especially significant in Australia, areas of southern Europe, Asia, Africa, Central America, and South America. While *B. bovis* and *B. bigemina* are typically found in the same broad geographic area, there are minor variances in the distribution of these two species due to the slight differences in tick groups that disseminate them. For instance, *B. bigemina* is found throughout Africa more extensively than *B. bovis* (Spickler *et al.*, 2010).

In general, the range of *B. bigemina* and *B. bovis* is the same; however, in Africa, *B. bigemina* is more common than *B. bovis* due to the fact that *B. decoloratus* and *R. evertsi* can serve as vectors for this species as well (Pohl, 2013).

In portions of Europe, such as the United Kingdom, Spain, and northern Europe, *B. divergens* is a significant parasite. There is evidence of this species all over Europe, and surveys may have

detected it as far north as North Africa.

I. ricinus, its vector, can endure from northern Scandinavia all the way to the Mediterranean. This tick can only be found in specific microenvironments, such as the underside of forest foliage, rough hill scrub, and damp low-lying ground, since it needs 80% humidity. Parts of Europe, Northwest Africa, Asia, and China are home to *B. major*. There have been descriptions of *B. ovata* in China, Japan, and other eastern Asian countries. Africa has documented cases of *B. occultans*, while Siberia is home to *B. jakimovi* (CFSPH, 2008).

2.2.2. Host Range

Bovines, sheep, goats, horses, pigs, dogs, cats, and occasionally humans are all susceptible to Babesiosis. Eighteen of the more than 100 known Babesia species cause sickness in domestic animals and infect a variety of mammalian hosts (Hamsho *et al.*, 2015).

The primary hosts of *B. bovis* and *B. bigemina* in reservoirs are cattle. Additionally, they have an impact on African buffalo (*Syncerus caffer*) and water buffalo (*Bubalus bubalis*). In Mexico, *B. bigemina* and *B. bovis* have been found in white-tailed deer (*Odocoileus virginianus*). This observation may or may not be significant, although as reservoir hosts, animals other than cattle have typically been regarded as having minimal epidemiological significance (CFSPH, 2008).

Table 1: Distribution of different Babesia species and their vectors and host Parasite

Parasite species	Vertebrate hosts	Pathogenicity	Vectors	Distribution
<i>B. bovis</i>	Cattle, Deer	High	<i>Ixodes</i> , <i>Rhipicephalus</i> (<i>Boophilus</i>)	Australia, Europe, Africa, South & Central

<i>B. bigemina</i>	Cattle,Deer	Moderate	<i>Haemaphysalis</i> , <i>Rhipicephalus</i> (<i>Boophilus</i>)	Europe, Africa, Australia, South & Central America
<i>B. divergens</i>	Cattle	Moderate	<i>Ixodes</i>	Western & Central Europe
<i>B. major</i>	Cattle	Low	<i>Rhipicephalus</i> (<i>Boophilus</i>)	Europe, Russia

Source: (Yadhav et al., 2015)

2.2.3. Life Cycle

All Babesia species have roughly identical life cycles, although there are some minor variations since transovarial transmission occurs in some species (*Babesia* spp. *sensu stricto*) but not in other species (*Babesia microti*) (Saad, et al. 2015). Feeding ticks introduce sporozoites into the erythrocytes of infected cattle, transforming them into trophozoites that divide by binary fission (merogony).

An intra-erythrocytic cycle is caused by the merozoites that are produced when the erythrocyte membrane degrades and they infiltrate new cells. Gametocytes develop in the tick gut after feeding on blood, and they eventually merge to generate diploid zygotes. Before emerging as haploid kinetes, zygotes undergo numerous rounds of multiplication in the digestive cells and most likely in basophilic cells. The ovaries, where additional division takes place, are among the numerous other organs to which the kinetes move. The kinetes travel to the salivary gland following egg hatching, where they change into multi-nucleated stages (sporogony), which eventually generate sporozoites (Simuunza, 2009).

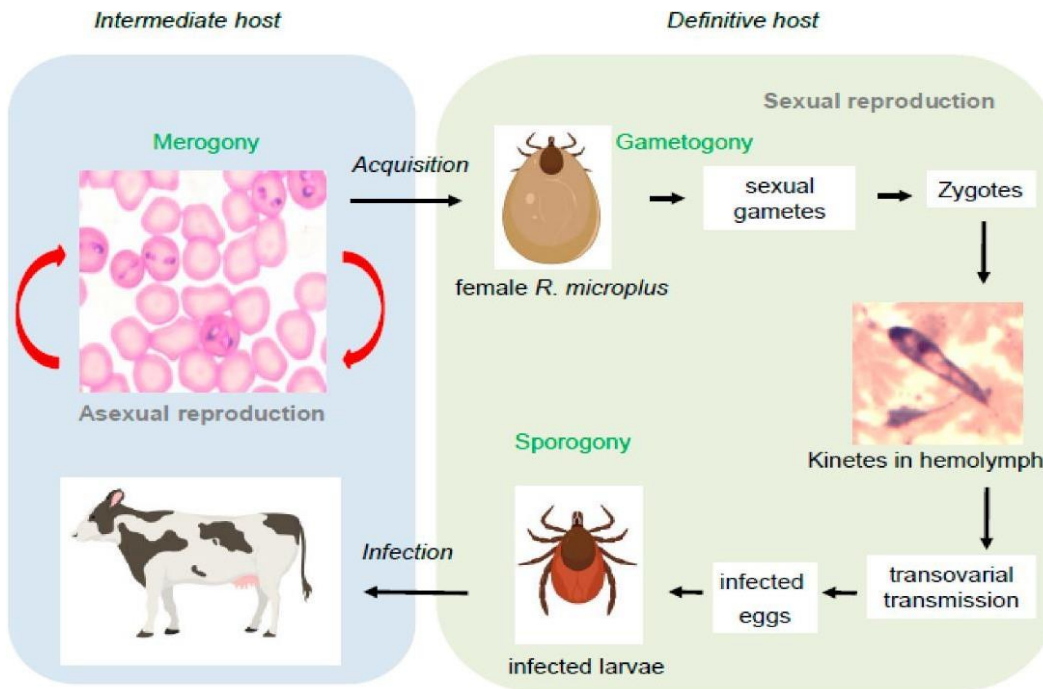


Figure 1: Babesia Species life cycle (Gallego-Lopez et al. 2019)

2.2.4. Risk Factors

2.2.4.1. Host Factors

Age, breed, and immunological condition are host factors linked to disease (Jabbar *et al.*, 2015). Compared to Bos-Taurus, cattle of the Bos-indicus breed are more resistant to Babesiosis. The evolutionary link between babesia, Boophilus species, and Bos-indicus cattle is the cause of this (Radostits *et al.*, 2007).

Indigenous tribes have either established an intrinsic resistance or an innate ability to build an effective immune response to the tick or tick borne hemoparasitic disease in issue because of natural selection pressure, having lived for a long time with local ticks and tick-borne diseases. Compared to goats, sheep were more vulnerable to *B. ovis*. The idea that younger animals are less prone to Babesiosis than older animals due to an inverse age resistance to Babesia infection is commonly expressed; one theory for this could be the passive transfer of maternal antibodies through colostrum (Demessie and Derso, 2015).

Adults are more likely than calves to contract Babesiosis due to the severity of the disease increasing with age (El Moghazy *et al.*, 2014).

2.2.4.2. Pathogen Factors

Pathogenicity varies greatly amongst strains; however *B. bovis* is typically more pathogenic than *B. bigemina* or *B. divergens* (CFSPH, 2008). It has been shown that *B. bovis* and *B. bigemina* are among the many intra-erythrocyte hemoparasites that are able to withstand the host immune system through fast antigenic change (Radostits *et al.*, 2007).

2.2.4.3. Environmental Factors

The incidence of clinical Babesiosis varies seasonally, with the highest incidence occurring shortly after the peak tick population. The most significant climatic component is air temperature since it has an impact on tick activity; higher temperatures make tick activity more common. The peripheral areas with highly changing tick populations based on environmental conditions are where the greatest losses happen (Radostits *et al.*, 2007). The summer months account for 33.33% of the peak of bovine Babesiosis infection (El Moghazy *et al.*, 2014).

2.2.5. Morbidity and Mortality

The level of morbidity and death varies widely and is determined by immunization status, prior exposure to a particular species or strain of parasite, and the therapies that are currently being used in the area. Cattle in endemic places contract the disease at an early age and acquire lifelong immunity.

Nevertheless, if tick exposure for young animals is terminated or immunized cattle are introduced, outbreaks may occur in these endemic locations. Disease outbreaks may potentially result from the introduction of Babesia-infected ticks into previously tick-free areas (Yadhav *et al.*, 2015).

Animals typically get infected while they are young, do not get sick, and develop immunity in endemic areas where tick transmission is high year-round. If acaricide therapy, climate change, or other factors reduce tick populations and animals do not contract the disease during the crucial early stage, this endemic stability could be upset and outbreaks could happen. Additionally, outbreaks can occur when vulnerable animals are brought into endemic regions or infected ticks spread to new areas. They can also occur in places where cold seasons temporarily halt tick-

borne transmission (Spickler *et al.*, 2010).

Breed differences exist in naive cattle's sensitivity to illness. *B. cattle* indices and *B. indicus* Compared to *B. Taurus*, *B. Taurus* crosses are more resilient. It has also been found recently that some *Bos-taurus* cattle exhibit varied susceptibility to *B. bovis*: roughly 28% of the adult herd was sensitive to infection but resistant to clinical symptoms. Up to 10% of treated adults and up to 50% of untreated adults may pass away in totally sensitive breeds. The prognosis is uncertain once hemoglobinuria manifests. Compared to infections with *B. bigemina* or *B. divergens*, *B. bovis* infections are typically more lethal, and CNS symptoms indicate a dismal prognosis (CFSPH, 2008).

2.3. Economic Importance

Half a billion cattle worldwide are at risk from bovine Babesiosis, which results in the livestock industry's most significant financial loss (Saad, *et al.*, 2015). Babesiosis, particularly in cattle, is very important economically because, in contrast to many other parasite infections, it affects adults more severely than young cattle, resulting in direct losses from animal deaths and quarantine restrictions that restrict animal mobility. Because the disease kills a lot of genetically superior but extremely sensitive cattle especially dairy cattle imported from Babesia-free areas it also makes it difficult to increase the productivity of local cattle through cross-breeding.

The result is that cattle in endemic areas continue to be of low quality, which hinders the growth of the cattle industry and the welfare of producers and their families (Demessie and Derso, 2015).

2.4. Public Health Importance

Human Babesiosis was first described in 1957 but is now known to have worldwide distribution. Both rising true incidence and greater public knowledge of the illness are probably to blame for the rise in reported cases (Yadhav *et al.*, 2015).

Cattle parasites appear to exclusively infect individuals with impaired immune systems, despite the fact that certain species of Babesia, such as *B. microti*, can also infect healthy individuals. *B. divergens* is a major source of illness in patients with splenectomies. As of 2003, only about

thirty cases of this unusual virus had been reported in Europe. Acute severe hemolysis, hemoglobinuria, jaundice, persistent high fever, chills and sweats, headache, myalgia, lumbar and abdominal pain, and occasionally vomiting and diarrhea are its defining characteristics.

Renal failure and shock are also possible. *B. divergens* infections in people are urgent medical situations. They often advance quickly, and the majority of cases in the past have resulted in death within a week. The case fatality rate is about 40% with supportive care and contemporary antiparasitic medications. Medication therapy alone may be sufficient to cure mild cases (CFSPH, 2008).

Immunocompromised people should exercise caution when visiting areas where Babesiosis is endemic, particularly during the tick season, to avoid contracting *B. divergens*. Wearing suitable clothing (such as long sleeves and long pants) and tick repellents can help prevent exposure to ticks.

After spending time outside, one should check their skin and clothing for ticks and remove any that are discovered. The possibility that *B. divergens* can infect immunocompetent people that is, those who are immunosuppressed but haven't had their spleen removed is not proven. However, out of 190 French blood donors, two had Babesia antibodies. It's unclear if *B. bovis* is zoonotic as well. *B. divergens* most likely caused at least some of the historical instances that were linked to *B. bovis* (Spickler *et al.*, 2010).

2.5. Transmissions

B. bigemina is spread by adult and nymphal stages of *Rhipicephalus spp.* ticks that feed on a single host. *Rhipicephalus microplus* and *Rhipicephalus annulatus* are two tick vectors that carry *B. bovis*. *Rhipicephalus geigy* is another capable vector that transmits *B. bovis* through feeding on the larval stages of one-host *Rhipicephalus spp.* ticks (Yadhav *et al.*, 2015).

Babesia species are easily transmitted to the following generation of ticks in the egg. Babesia zygotes proliferate inside the tick as "*vermicules*," which infect numerous organs within the tick, including the ovaries. Depending on the type of tick and Babesia species, these parasites may occasionally be transmitted transgenerationally over several generations (Spickler *et al.*, 2010).

Even in the absence of cattle, *B. divergens* can persist in tick populations for a minimum of four years. Babesia is induced to go through their final maturity when an infected tick binds to a new host. After larval ticks adhere, *B. bovis* parasites often become infectious two to three days later and can spread through larvae. *B. bovis* does not survive past the larval stage in *R. microplus*. On the other hand, *B. bigemina* is only spread by nymphs and adults, and it takes around 9 days for a larval tick to mature. *B. divergens* can be transmitted by *I. ricinus* in all three stages. Additionally, animals can contract Babesia species through direct blood injection. Although this mode of transmission is regarded as being of minimal consequence, biting flies and fomites contaminated by infected blood may operate as mechanical vectors (CFSPH, 2008).

2.6. Pathogenesis and Clinical Signs

Even though *Boophilus bovis* and *Bos. bigemina* are closely related and spread by the same *Boophilus* ticks, they cause very different diseases in cattle from one another. Both the direct result of the parasite's destruction of red blood cells and an excess of pro-inflammatory cytokines can contribute to the pathophysiology of *B. bovis* infections. Pro-inflammatory cytokines and parasitocidal molecules are produced by macrophages that have been stimulated by the parasite during an acute infection (Simuunza, 2009).

Hemolysis and circulatory disruption are the two main ways that Babesia causes acute illness. Sporozoites are introduced into the host during the tick bite, where they infect red blood cells directly. Babesia sporozoites cause two or occasionally four daughter cells to emerge from the host cell and infect additional erythrocytes when they mature into piroplasms inside the infected erythrocyte. Both intravascular and extravascular hemolysis is caused by its invasion of erythrocytes. The erythrocytes are rapidly destroyed by the fast dividing parasites in the red blood cells, resulting in hemoglobinaemia, hemoglobinuria, and fever.

Anemia may result from this if it is severe enough to kill the patient within a few days and for the packed cell volume to drop below 20%. Depending on the kind of Babesia, the parasitaemia, which is often identified until clinical symptoms manifest, can affect anywhere from 0.2% to 45% of the red blood cells (Demessie and Derso, 2015). The age of the animal as well as the kind and strain of the parasite affect the clinical symptoms. Babesiosis typically affects adults; animals under the age of nine months typically show no symptoms at all. Although the

pathogenicity of strains varies greatly, *B. bovis* is typically more virulent than *B. bigemina* or *B. divergens* (CFSPH, 2008).

Of the bovine Babesia, *B. bovis* is the most pathogenic. Although *B. bigemina* infections in animals are not as severe as *B. bovis* infections, 40% of the red blood cells may be infected by the parasites (Sahinduran, 2012). High fever, ataxia, anorexia, general circulatory shock, and occasionally neurological symptoms due to sequestration of infected erythrocytes in cerebral capillaries are the hallmarks of *B. bovis* infections.

Haemoglobinuria and anemia may develop later in the illness. The maximal parasitaemia, or the proportion of infected erythrocytes in the bloodstream, is less than 1% in acute instances. In contrast, the parasitaemia in *B. bigemina* infections frequently surpasses 10% and can reach up to 30%. The three main symptoms of *B. bigemina* infections are anaemia, hemoglobinuria, and fever. When *B. bigemina* infections are present, infected erythrocytes do not sequester intravascularly. Infections with *B. divergens* share some similarities with *B. bigemina* infections in terms of parasitaemia and clinical manifestation (OIE, 2010).

Only a small percentage of cases of acute *B. bigemina* in animals result in death. In contrast, animals infected with *B. bovis* frequently experience fatality rates exceeding 50%. Cattle infections are characterized by fever, anorexia, listlessness, dehydration, and increasing hemolysis. Hemoglobinuria and hemoglobinemia, which can lead to jaundice, can also accompany an infection. The clinical indicators listed above are shared by both *B. bigemina* and *B. bovis*; however, their pathophysiology and manifestations differ. Given that *B. bovis* frequently causes a visceral Babesiosis due to thrombus formation, *B. bigemina* can therefore be described as a peripheral Babesiosis with severe anemia (Pohl, 2013).

2.7. Diagnosis

Polymerase chain reaction assays (PCR), serology, transmission trials, and parasite identification in blood or tissues can all be used to diagnose Babesiosis. When cattle exhibit fever, anaemia, jaundice, and hemoglobinuria, Babesiosis should be suspected (CFSPH, 2008).

2.7.1. Direct Microscopic Examination

The quickest and least expensive way to identify Babesia parasites is still by microscopic inspection. For the purpose of direct diagnosis, the various stages of the parasite in the tissues of mammals or arthropod hosts can be identified.

Blood Smears: Thick and Thin the traditional procedure for routine diagnosis has been thought to be blood smear testing, especially in cases that are acute. However, this is not the case with sub-clinical infections; where the parasitemia is typically significantly lower (Demessie and Derso, 2015).

In thin films, species discrimination is good, but in thick films, which are more sensitive, it is poor. In cases where parasitaemias are typically very low, this approach is generally sufficient for the discovery of acute infections, but not for the detection of carriers. Using a fluorescent dye, like acridine orange, in place of Giemsa can improve parasite identification and differentiation (OIE, 2010).

To distinguish Babesia species from one or more animal species that seem identical under stained preparation, blood film evaluation demands a high level of competence (Salih *et al.*, 2015). Films created from freshly drawn blood from capillaries, such as those in the tips of the ears or tails, should be used for samples from live animals whenever possible because capillary blood contains a higher concentration of *B. bovis*.

The parasites *B. bigemina* and *B. divergens* are evenly dispersed throughout the vasculature. Sterile jugular blood should be collected into an anticoagulant such as lithium heparin or ethylene diamine tetra-acetic acid (EDTA) if it is not possible to create new films from capillary blood. Thin blood films, cerebral cortex smears, kidney (freshly dead), spleen (when decomposition is visible), heart muscle, lung, and live animals should all be included in samples from deceased animals (OIE, 2010).

2.7.2. Indirect Diagnostic Methods

Indirect diagnostic techniques, such as serological testing for the detection of antibodies or antigens, are utilized when parasite densities are below the sensitivity of the direct method used or when the life cycle of the parasite prevents direct demonstration in a biological sample. The

enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT), and indirect fluorescent antibody technique (IFAT) are the three most significant serological assays (Salih *et al.*, 2015).

Blood smears are unreliable for identifying carrier animals; in these situations, serological diagnostic techniques that show particular antibodies are needed or molecular detection techniques (Pohl, 2013).

The two most common uses of serology are export certification and surveillance. The most common methods for detecting Babesia antibodies are the enzyme-linked immunosorbent assay (ELISA) and the indirect fluorescent antibody (IFA) test. There have also been reports of agglutination assays (latex and card agglutination tests) and complement fixation. According to Spickler *et al.* (2010), serological cross-reactions can make it more difficult to distinguish between some species in serological testing.

Babesia species can be identified and distinguished using polymerase chain reaction (PCR) assays, which are especially helpful in carriers (CFSPH, 2008). There have also been descriptions of immunoperoxidase and immunofluorescent labelling. These parasites are found inside red blood cells (RBCs), and they can be found in both single and in pairs as well as in all divisional stages, including ring (annular) stages and pear-shaped (pyriform) trophozoites with filamentous or amorphous morphologies.

Amorphous or filamentous forms are typically observed in animals with extremely elevated parasitemia levels. The trophozoites of *B. bovis* are often paired, tiny (1.5–1.5 μm \times 0.5–1.0 μm), and found in the centre of red blood cells. Although *B. divergens* and *B. bovis* seem similar, pairs of them are frequently spotted at the RBC's edge. *B. bigemina* is large and has the ability to fill RBCs (Spickler *et al.*, 2010).

2.8. Prevention and Control

Three main strategies are used to actively prevent and control Babesiosis: vector control, chemoprophylaxis, and vaccination. The three approaches should ideally be used to maximize their respective economic benefits, take advantage of breed resistance, and create and preserve enzootic stability (Demessie and Derso, 2015).

In locations where eliminating ticks is not practical or desired, the removal of the tick vector has allowed for the eradication of bovine Babesiosis; ticks are managed by the use of acaricides and repellents (Beckley, 2013).

Limit the amount of ticks that cattle are exposed to and regularly check the animals and the area. After only one infection with *B. bovis*, *B. divergens*, or *B. bigemina*, cattle acquire a robust, long-lasting immunity; this characteristic has been used in certain nations to immunize cattle against Babesiosis (OIE, 2009). There are several vaccines available that can be used to prevent and control babesia, including live and killed vaccines. As a service to the livestock industries, government-sponsored production facilities generate most live vaccines in calves or in vitro using carefully chosen strains of babesia, primarily *B. bovis* and *B. bigemina* (OIE, 2010).

Some countries employ live, attenuated strains of *B. bovis*, *B. bigemina*, or *B. divergens* for cow vaccinations. The potential for pathogenicity in adult animals, the possibility of contamination with other infections, and hypersensitivity reactions to blood proteins are among the safety concerns associated with these vaccinations.

To reduce the risk of illness, they work best on animals younger than a year old. Older cattle may occasionally need to be vaccinated (e.g., if sensitive cattle are transported into an endemic area). Following vaccination, older animals should be thoroughly observed and treated if any clinical symptoms appear. In the event of an outbreak, animals may receive vaccinations in certain nations. The prevalence of disease can also be reduced by using genetically resistant cattle, such as *B. indicus*. Because it can be impacted by host variables, climate, and management, natural endemic stability as the only control technique is unreliable (Spickler *et al.*, 2010).

2.9. Treatment

The recommended medication for bovine Babesiosis is imidocarb, which can halt clinical infection for up to two months (Saad, *et al.*, 2015). Antiparasitic medication should be administered to sick animals as soon as feasible. Effective cattle babesiocides are amicarbide (Imizol) and midocarb (Imizol), administered at doses of 1-3 mg/kg and 5-10 mg/kg body weight, respectively (Beckley, 2013).

Early diagnosis increases the likelihood of successful treatment; if the animal has already

suffered from anaemia, treatment may not work as intended. Although many medications have been removed because of safety or residual issues, others are said to be helpful against Babesia (CFSPH, 2008).

Trypan blue, a very efficient chemical against *B. bigemina* infections, was the first particular medicine used specifically to treat bovine Babesiosis. However, it did not work against *B. bovis* and had the drawback of discolouring the animal's meat, thus it is rarely used. For commercial considerations, diminazene aceturate which is currently widely used as a Babesiocide in the tropics was removed from Europe (Demessie and Derso, 2015).

Other supportive therapies, such as blood transfusions, might also be required. Imidocarb, when used in chemoprophylaxis, can shield animals against clinical illness and promote the growth of immunity. But this medication isn't available everywhere, and there are worries about residues in meat and milk (CFSPH, 2008).

3. CHAPTER III: MATERIALS AND METHODS

3.1. Study Area

The study area was a purposively selected region in the Somaliland country; which is Marodijeh region which located the western part of Somaliland. The Republic of Somaliland is situated in the Horn of Africa. It is bordered by Djibouti to the west, Ethiopia to the south, and the Puntland region of Somalia to the east. It is bordered by Awdal region to the west, Saaxil region to the north, Togdheer region to the east and Ethiopia to the south (Republic of Somaliland, 2007).

The climate of Marodijeh region is semi-arid. The area often experiences hot summers and mild winters. However, despite being in the tropics, Marodijeh barely experiences extremely hot or extremely cold weather due to its high altitude. In areas with a semi-arid climate, this characteristic is uncommon. The majority of the region's yearly precipitation, at little less than 400 mm, falls between the months of April and September. From 17 C in December and January to 35 C in June, the average monthly temperature ranges (Maroodijeehregional development plan, 2014).

The types of animals kept by nomadic families depend on several factors, including the area inhabited and the availability of labour. Cattle are predominant in the western parts of the country, which receive relatively large amounts of rainfall, while goats, sheep, and camels are bred mainly in the drier eastern, central and northern regions of the country (Livestock, n.d, 2015).

The below study area map depicts the geographic location of the Marodijeh region within Somaliland, providing a visual representation of the focal area for our research on bovine Babesiosis. The map aids in visualizing the specific geographic boundaries and landscape features that will be encompassed in our study, helping to pinpoint areas of interest for sample collection, epidemiological assessment, and understanding the disease's distribution in this region.

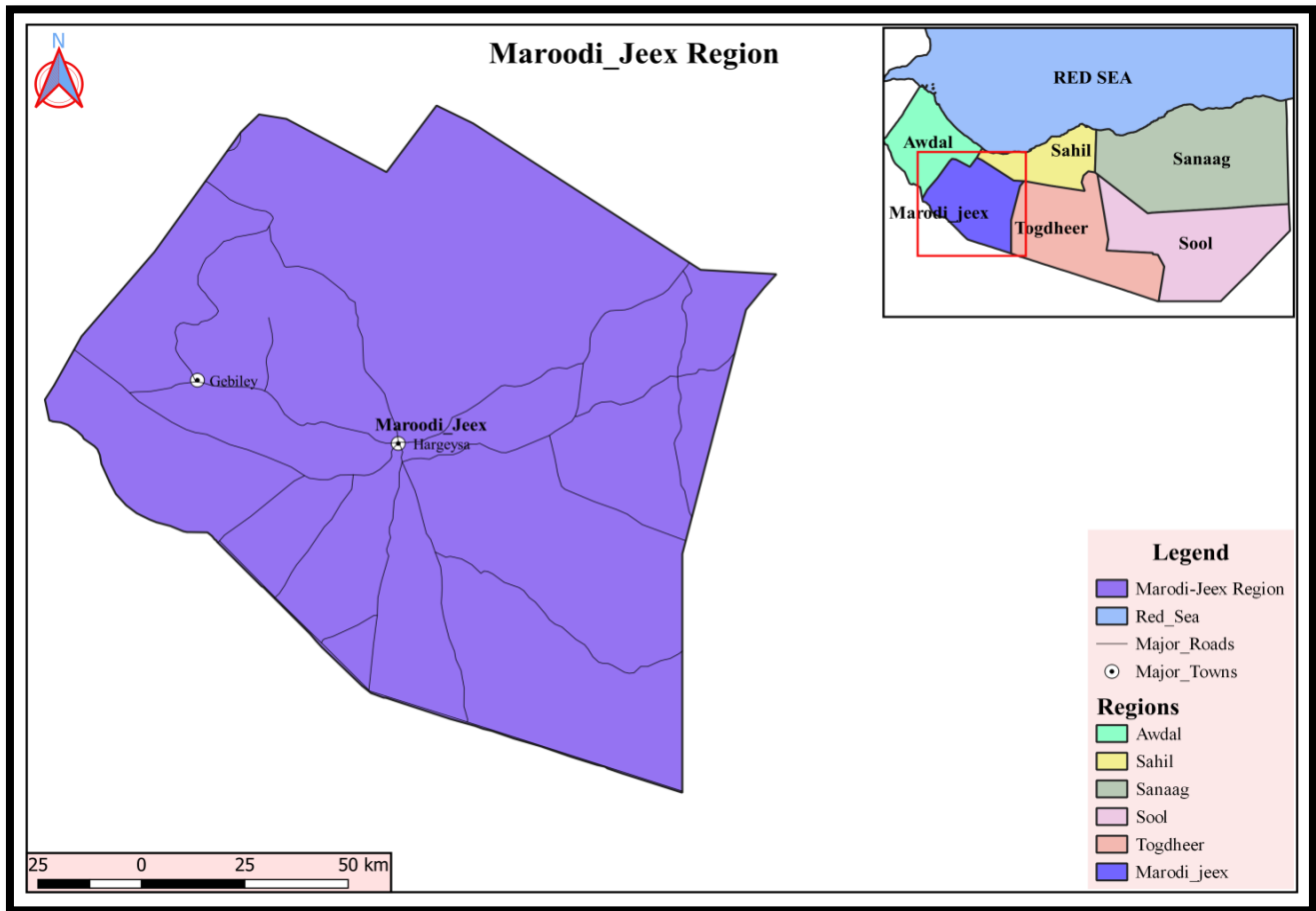


Figure 2: Indicating Location of Mrodijeh region in the Map of Somaliland regions; Source (The World Factbook, 2023).

3.2. Study design.

A cross-sectional study design will be conducted from August 2023 to November 2023.

3.3. Study Population

The study population will be the local cattle of both sexes found in the different districts of Marodijeh region, Somaliland. The two main districts Hargeisa, and Gabiley shown in the map will be collected the samples. The breeds cattle found in Somaliland are predominantly the East African Shorthorn (thoracic humped) Zebu typemanaged under extensive pastoral and agro-pastoral management systems (Somaliland Livestock Policy, 2006). The age of the study animals was determined based on the dentition as described by Pace and Wakeman and classified into 3 groups: <1 year (young), 1 to 3 years (adult), and >3 years (old). The body condition score of cattle was determined according to (Nicholson & Butterworth, 1986).

3.4. Sample Size Determination:

Since there was previous investigation about prevalence of cattle Babesiosis in wajaale district Somaliland, the expected prevalence of cattle Babesiosis in Wajaale was 30% as reported by (Warsame *et al.*, 2022). Thus, during the data collection the sample size of this current designed study will be to estimate the sample size needed with an expected prevalence of 30% using the Thrusfield(2005) formula, by:

- a. Determining the desired confidence level. Let's assume a 95% confidence level.
- b. Determining the desired margin of error. Let's assume a 5% margin of error.
- c. Estimating the expected prevalence of cattle in the population. In this case, it is 30%.
- d. Using the Thrusfield (2005) formula:

$$n = (Z^2 * p * (1-p)) / E^2$$

Where:

- n is the required sample size
 - Z is the Z-score corresponding to the desired confidence level (e.g., for a 95% confidence level, Z = 1.96)
 - p is the estimated proportion of cattle in the population (0.30 in this case)
 - E is the desired margin of error (expressed as a decimal, e.g., 0.05 for a 5% margin of error)
- e. Calculating the sample size.

Using a 95% confidence level, a 5% margin of error, and an expected prevalence of $p = 0.30$:

$$\begin{aligned}n &= (1.96^2 * 0.30 * (1-0.30)) / 0.05^2 \\n &= (3.8416 * 0.30 * 0.70) / 0.0025 \\n &= 0.80832 / 0.0025 \\n &\approx 323.33\end{aligned}$$

However, to increase the absolute precision 10% were added and 356 samples were taken throughout the study period.

3.5. Sample Collection, Transportation and Processing

3.5.1. Blood Collection

All the necessary equipment was gathered, including sterile needles (18–20 gauge), vacutainer tubes or blood collection bags, alcohol swabs or cotton balls, and proper restraining equipment for cattle (Rodriguez-Vivas et al., 2003). After that, cattle were adequately restrained to minimise stress and prevent injury to both the animal and personnel involved in the collection process.

The preferred site for blood collection in cattle is the jugular vein due to its accessibility and size (Uilenberg, 2006). Before needle insertion, the chosen area over the jugular vein will be disinfected using an alcohol swab or cotton ball to reduce the risk of contamination. Then a sterile needle was inserted into the jugular vein at a shallow angle, taking care to avoid injury to the animal and the vein itself. Blood should flow into the vacutainer tube or blood collection bag, with the desired volume typically ranging from 5 to 10 mL (Rodriguez-Vivas et al., 2003).

The collected blood was gently mixed to prevent clot formation and transferred to appropriate containers for storage and transport. Each sample was accurately labelled with the animal's identification information. Detailed records, including the date, time, and relevant clinical information, are needed to ensure traceability and proper documentation (Friedhoff & Tenter, 1996).

3.5.2. Tick Collection

Tick collection is a crucial component of epidemiological studies and research on tick-borne diseases like Babesiosis. To efficiently and effectively collect ticks, specialized instruments were employed. Commonly used instruments for tick collection include fine-tipped forceps, tick removal tools, and vacuum aspirators. Fine-tipped forceps are delicate, precise instruments that allow researchers to gently grasp and remove ticks from the host's skin or fur (Gilot *et al.*, 2017).

The research cattle was examined for ticks on various body parts during tick collection, paying close attention to tick-favored sites such the ears, neck, and udder (Uilenberg, 2006). By making sure that the tick's mouthparts are completely, the ticks were gently and carefully removed from the skin of the cattle (Apanaskevich & Horak, 2008).

The ticks were placed into vials or containers filled with 70% ethanol and labeled with patient details, like the host animal's identification, the collecting date, and the location. To maintain the integrity of the collected ticks until further examination, it was stored in a cool, dry place (Uilenberg, 2006).

3.5.3. Sample Transportation.

To maintain sample integrity and permit correct analysis, it was essential to ensure the safe transportation and storage of blood and tick samples acquired for the study of Babesiosis parasites (Smith et al., 2021). Blood samples were properly transferred into the proper containers after sample collection, and ticks will be stored in vials filled with 70% ethanol. The host animal's identification, the collection date, and the location were all written on the labels of every container (Apanaskevich & Horak, 2008).

To avoid deterioration and preserve the integrity of the specimens until further investigation, the collected samples were kept in a cool, dry location (Uilenberg, 2006).

3.5.4. Sample Processing

During sample processing the collected samples was processed in Hargiesa veterinary laboratory center.

3.5.4.1. Blood Sample Processing

The acquired blood was initially screened using the thick smear method to determine whether parasites are present and to discriminate between positive and negative samples. In order to make thick films, a small drop of blood was applied on a clean glass slide, and it was then disseminated over a relatively limited area using the corner of another slide. This droplet; it is just air dried and stained with Giemsa. This method is more sensitive for identifying Babesia species because RBCs are lysed and parasites are concentrated, but it is more difficult to distinguish between different species (Choramo, A. & Ibrahim, 2017).

A drop of the gathered blood was then applied to a microscope slide to create a thin blood smear. The smear was applied uniformly, and then let to air dry. The blood smear was stained with a particular stain, like Giemsa to improve visibility (Ibrahim et al., 2013). A light microscope was used to analyze the stained blood smear. Small, round or oval-shaped creatures called Babesia

parasites found inside red blood cells. Depending on the Babesia species, they can be seen as rings, tetrads, or piriform structures (Homer et al., 2000).

The Babesia species was determined by the size, shape, and distribution of the parasites within red blood cells. *B. bigemina* or *B. bovis* infection was indicated by morphological traits such the presence of paired organisms (Homer et al., 2000).

3.5.4.2. Tick Sample processing

The collected ticks were processed using a stereomicroscope. Morphological analysis which was the main technique for identifying ticks were performed by seeing the examinable details include shape, size, coloring, the existence of particular marks, and mouthparts. For differentiating between tick species identification manuals and taxonomic keys, which are frequently devoted to certain geographical areas, tick species, or genera were used (Apanaskevich & Horak, 2008).

3.6. Questionnaire Survey

In order to clarify potential risk factors linked to the tick-borne disease cow Babesiosis, questionnaire survey data collecting were conducted. A structured questionnaire survey for gathering important information from cattle owners were developed in order to thoroughly examine these risk variables. With an emphasis on the availability of ticks, which serve as vectors for the Babesia parasites, the survey intends to look at several aspects that may contribute to the prevalence and transmission of bovine Babesiosis.

The survey asked about the practices employed to manage cattle, the methods used to control ticks, the area in which the cattle are kept, and the patterns of tick infestation, all of which are known to affect the risk that animals may get Babesiosis. This survey aims to give a thorough understanding of the risk factors associated with cattle Babesiosis by combining the responses from a varied set of participants (Smith et al., 2021; Johnson et al., 2019).

Collected data on the risk factors of Babesiosis disease was a critical component of epidemiological research, enabling a better understanding of the disease's prevalence and its impact on animal populations. To ensure a comprehensive investigation, 60 survey questionnaires was distributed, that aligning with the principles of the Central Limit Theorem (CLT) (Zarzycki, 2011).

3.7. Data Analysis

The gathered data were cleaned up, properly arranged, coded, and entered into Microsoft Excel before being analyzed with SPSS statistical software version 20. To summarize the data, descriptive statistics were used, and the results will be expressed as cross tabulation. Chi-square was used to compare the relationship between the variables. The cutoff for significant differences in all analyses was 95% confidence interval and P-value 0.05.

4. CHAPTER IV: RESULTS

4.1. Prevalence of Bovine Babesiosis

In the current study, based on the prevalence of bovine Babesiosis, 19 (5.4%) out of 356 samples obtained from Hargeisa and Gabiley districts were examined positive for Babesiosis. In Hargeisa, 7 (36.8%) of the animals were examined positive, while 179 (53.1%) tested negative. 12 animals (63.2%) were positive in Gabiley, whereas 158 animals (46.9%) tested negative. The disease is clearly prevalent in both districts, with Gabiley (63.2%) having a higher frequency than Hargeisa (36.8%).

Table 2: The prevalence of bovine Babesiosis in Hargeisa and Gabiley districts

District	Tested Animal				Chi-square	df	Sig.
	Positive		Negative				
	No	Prevalence %	No	Prevalence %			
Hargeisa	7	36.8%	179	53.1%	1.90915	1	0.167
Gabiley	12	63.2%	158	46.9%			
Total	19	100.0%	337	100.0%			

As shown in the above table, the chi-square statistic was 1.909, with 1 degree of freedom. The p-value associated with the chi-square statistic was 0.167. The results did not reveal a statistically significant difference ($\chi^2 (1) = 1.909$, $p = 0.167$). Therefore, based on this analysis, there is no strong evidence to suggest that the prevalence of Babesiosis differs between these two districts in Somalia.

Table 3: Prevalence of bovine Babesiosis on the basis of Babesia species identified.

Total of Animals Tested	Babesia Spp Identified	Positive	Prevalence (%)
356	<i>B. bovis</i>	2	0.6%
	<i>B. bigemina</i>	17	4.8%
	Total	19	5.4%

The descriptive analysis shown in the above table provides an overview of the prevalence of *B. bovis* and *B. bigemina* in the sample of 356 animals tested. It is evident that *B. bigemina* has a higher prevalence (4.8%) compared to *B. bovis* (0.6%). When considering both species together, the overall prevalence of Babesia species in the sample was 5.4%.

4.2. Prevalence of Risk Factors Associated To Bovine Babesiosis

Table 4: Prevalence of bovine Babesiosis on the basis of risk factors

Animal Related Risk Factors		Tested Animal						
		Positive		Negative		Chi-square	df	Sig.
		No	Prevalence %	No	Prevalence %			
Age	< 1 year	2	10.5%	69	20.5%	2.216194	2	0.330187
	1 - 3 years	9	47.4%	109	32.3%			
	> 3 years	8	42.1%	159	47.2%			
Sex	Male	9	47.4%	138	40.9%	0.305694	1	0.580335
	Female	10	52.6%	199	59.1%			
Body condition score	Good	0	0.0%	111	32.9%	61.13074	2	.000*
	Moderate	4	21.1%	185	54.9%			
	Poor	15	78.9%	41	12.2%			
Tick infestation	No tick	2	10.5%	275	81.6%	159.121	3	.000*
	<i>Rhipicephalus</i>	12	63.2%	5	1.5%			
	Mixed	5	26.3%	39	11.6%			
	Others	0	0.0%	18	5.3%			

Note: (Mixed=*Rhipicephalus* + Others, Others = *Hyaloma* spp and *Ambyloma* spp.)

The above table of the chi-square analysis aimed to determine whether age, sex, body condition score, and tick infestation were significantly associated with the prevalence of the cattle Babesiosis in the tested animals shown that the analysis did not reveal a significant association between age and the disease prevalence ($p = 0.330$). Similarly, there was no significant association between sex and the disease prevalence ($p = 0.580$).

Different to the previous results, there was a significant association between body condition score and the disease prevalence ($p < 0.001$). Animals with poor body condition had a notably

higher prevalence. Similarly there was a significant association between tick infestation and the disease prevalence ($p < 0.001$). Animals with *Rhipicephalus* tick infestation had a higher prevalence.

Table 5: Prevalence of bovine Babesiosis on the basis of environmental and management risk factors

Environmental and Management risk factors		No	Prevalence %
1. Gender of the respondent	Male	31	51.70%
	Female	29	48.30%
2. Residence area of the respondent	Hargeisa	29	48.30%
	Gabiley	31	51.70%
3. Type of cattle owned breeds?	Local	60	100.00%
	Exotic	0	0.00%
4. What type of farming system do you use?	Intensive system	2	3.30%
	Semi-intensive system	13	21.70%
	Extensive system	45	75.00%
5. Are ticks available in your area?	Yes	60	100.00%
	No	0	0.00%
6. If yes, do they cause your animal any problems?	Yes	60	100.00%
	No	0	0.00%
7. How do you manage the health problems of your cow?	By myself	13	21.70%
	By animal health professionals	47	78.30%
8. When your animal was lastly treated?	< 3 Months ago	6	10.00%
	Six Months ago	24	40.00%
	< A year ago	21	35.00%
	More than year ago	9	15.00%
9. How do you treat your animal when ticks are infested?	Dipping	13	21.70%
	Using anti-parasitic drugs	43	71.70%
	Traditional treatment	4	6.70%
	Others	0	0.00%
10. How frequent your animals where treated?	Once	17	28.30%
	Two times	13	21.70%
	Three times	30	50.00%
	More than Three times	0	0.00%
11. Is there a seasonal fluctuation of ticks in your area?	Yes	60	100.00%
	No	0	0.00%
12. If yes, which seasons are they have high burden?	Spring	0	0.00%
	Summer	6	10.00%
	Autumn	27	45.00%

13. Select the clinical signs that you see your animals when ticks infested?	Winter		27	45.00%
	Responses			Percent of Cases
	N	Percent		
Fever	26	14.4%		43.3%
Anemia	47	26.1%		78.3%
Itching	42	23.3%		70.0%
Restlessness	25	13.9%		41.7%
Reduced Feed Intake	34	18.9%		56.7%
Hematuria	6	3.3%		10.0%
Total	180	100.0%		300.0%

Among 60 livestock owners whose animals collected the blood samples were interviewed to assess various environmental and management risk factors related to bovine Babesiosis. The investigation focused on factors such as the gender of the respondent, residence area, cattle breeds, farming systems, tick presence and management, animal health care practices, seasonal tick variations, and clinical signs.

The findings, as seen in the above table, are saying that there was almost an equal distribution of male (51.70%) and female (48.30%) respondents. The majority of respondents came from Gabiley (51.70%), compared to Hargeisa (48.30%). All respondents owned local cattle breeds (100.00%), with no respondents having exotic breeds. The extensive farming system was the most common (75.00%), followed by the semi-intensive system (21.70%) and the intensive system (3.30%).

All respondents reported the presence of ticks (100.00%) in their area, with all ticks causing problems for the animals (100.00%). There was also a significant majority seeking the help of animal health professionals for health management (78.30%). Most animals were last treated within the past year, with a substantial portion treated between six months and a year ago (35.00%). Anti-parasitic drugs (71.70%) were the most commonly used treatment for tick infestations, followed by dipping (21.70%). Three times a year (50.00%) was the most frequent treatment schedule. Respondents reported seasonal tick fluctuations, with ticks having a high burden in both autumn and winter (45.00% each), while summer had a lower burden (10.00%).

In the clinical signs the respondents were asked to select one or more clinical signs that were present in their animals when tick infested and then the signs were dichotomously categorized,

with a value of 1 indicating their presence. The findings show that fever was reported in 26 answers, representing 14.4% of the total cases. Anaemia was reported in 47 answers, accounting for 26.1% of the total cases. Itching was present in 42 answers, with a prevalence of 23.3%. Restlessness was observed in 25 cases, making up 13.9% of the total cases. Reduced feed intake was reported in 34 answers, constituting 18.9% of the answers, and hematuria, the presence of blood in the urine, was noted in 6 answers, with a prevalence of 3.3%. Anemia was the most frequently reported clinical sign, reported 26.1% of the answers, while hematuria had the lowest prevalence at 3.3%.

5. CHAPTER: DISCUSSION

The current study reported from Hargeisa and Gabiley, Somaliland, found a relatively lower prevalence of bovine Babesiosis (5.4%) compared to some of the other studies, such as Warsame et al., (2022) (21%), and Hamsho et al., (2015) (16.9%), from Somaliland and Ethiopia, respectively. This suggests that the prevalence of Babesiosis can vary significantly between different regions or research contexts. The results from Ethiopia by Choramo and Nuraddis, 2017 (12.8%) and Chernet B and Feyera G, (2017) (6.8%) fall within the range of prevalence found in your study. This indicates that Babesiosis prevalence can vary within the same country, depending on factors such as climate, cattle management practices, and the presence of the disease vectors. The relatively consistent use of blood smear as the examination method suggests that methodological differences are unlikely to be the primary cause of variation.

It's essential to consider the geographic and environmental factors that may influence the prevalence of Babesiosis. For example, the prevalence in urban and peri-urban areas Choramo and Nuraddis (2017) may differ from that in rural areas Hamsho et al., (2015). The variations in bovine Babesiosis prevalence rates among these studies are likely the result of a combination of geographic, ecological, and local factors. These differences emphasise the importance of tailored disease control and prevention strategies in different regions to effectively reduce the impact of Babesiosis on cattle populations.

According to the prevalence of Babesia species, the current study shows that it is evident that *B. bigemina* has a higher prevalence (4.8%) compared to *B. bovis* (0.6%). When considering both species together, the overall prevalence of Babesia species in the sample was 5.4%. Similarly, the prevalence of *B. bigemina* (9.8%) was higher than *B. bovis* (2.2%) in Abdela N, et al., (2018). However, the prevalence of Babesia species in Chernet B and Feyera G, 2017 study was different, with *B. bovis* at 6.5% and *B. bigemina* at 0.3%. This suggests that in Chernet B and Feyera G, study, *B. bovis* was more prevalent than *B. bigemina*.

Similarly, in Hamsho et al.'s 2015 study, the prevalence rates were notably different, with *B. bovis* at 9.9% and *B. bigemina* at 7%. These findings indicate that the prevalence of *B. bovis* was higher than that of *B. bigemina* in their study. In Wodajnew et al.'s (2015) study, the prevalence of *B. bovis* was 1.243%, while that of *B. bigemina* was 0.248%, with *B. bovis* again being more

prevalent. These variations in prevalence could be attributed to differences in geographic location, climate, tick vector distribution, and cattle management practices.

In terms of the prevalence of associated risk factors, the current study found no significant association between age and disease prevalence ($p = 0.330$) or sex and disease prevalence ($p = 0.580$). Choram and Nuraddis (2017) also observed a non-significant difference in prevalence between sexes. In contrast, Wodajnew et al. (2015) reported that age was significantly associated with disease occurrence, with older cattle being more affected. These variations could be due to differences in the age structure of the cattle populations and the presence of other confounding factors in the study areas.

Differences were noted in the association between body condition and disease prevalence. The current study found a significant association ($p < 0.001$) between body condition and prevalence. Hamsho et al. (2015) also reported a significant difference, with poor body condition animals having the highest prevalence. In contrast, Chernet B and Feyera G (2017) found no significant difference based on body condition. These variations may be influenced by the local nutrition, management practices, and overall health of the cattle.

Tick infestation showed significant associations with disease prevalence across studies. The current study and Chernet B and Feyera G (2017) reported a significant association between tick infestation and disease prevalence. Choram and Nuraddis (2017) also indicated a strong association between Babesia infection and tick species, particularly *Rhipicephalus*. The presence of specific tick vectors in different regions may explain these variations in prevalence.

Regarding the other associated risk factors related to the environment and management, in the current study, all respondents exclusively owned local cattle breeds. Extensive farming systems were the most prevalent (75%), followed by semi-intensive (21.70%) and intensive systems (3.30%). In all cases, respondents reported the presence of ticks in their areas, with ticks causing problems for their animals. This aligns with the findings of Abdela N. et al. (2018), who noted a high prevalence of Babesiosis in extensively managed cattle (12.6%). Similarly, Choram and Nuraddis (2017) observed a Babesiosis prevalence of 10.3% in extensively managed cattle, and Wodajnew et al. (2015) identified autumn as the season with the highest infection rate (2.99%).

The variation in Babesiosis prevalence based on farming systems may be attributed to differences in tick exposure and management practices. Extensively managed cattle often have greater exposure to tick vectors due to their environment, potentially leading to higher disease prevalence. Conversely, cattle under intensive management systems may benefit from enhanced tick control measures, reducing the risk of Babesiosis.

In the studies conducted by Choramo and Nuraddis (2017) and Namomsa I. et al. (2023), local cattle breeds were predominant, and extensive management systems were commonly practiced. However, the prevalence of Babesiosis in these systems varied. Choramo and Nuraddis (2017) reported a prevalence of 10.3% in extensively managed cattle, while Namomsa I. et al. (2023) did not provide specific prevalence rates based on management systems.

Despite the similar predominance of local breeds and extensive management systems, variations in the prevalence of Babesiosis could be attributed to specific geographic locations, tick populations, and local climate conditions. These factors play a vital role in the transmission of Babesia parasites and influence disease prevalence.

Wodajnew et al. (2015) highlighted seasonal variations in Babesiosis prevalence, with autumn exhibiting the highest infection rate. This is consistent with the findings of the current study, which reported that ticks had a high burden during both autumn and winter (45% each). The influence of seasonal variations on tick populations and disease transmission underscores the importance of considering the timing of disease control measures.

6. CHAPTER VI: CONCLUSION AND RECOMMENDATIONS

The study has provided valuable insights into the prevalence of bovine Babesiosis in the Marodijeh region. The study findings showed that the prevalence of bovine Babesiosis is 19 (5.4%) out of 356 samples obtained from Hargeisa and Gabiley districts were examined positive for Babesiosis. The study also showed that It is evident that *B.bigemina* has a higher prevalence (4.8%) compared to *B. bovis* (0.6%).

Furthermore, the analysis of risk factors demonstrated that the analysis did not reveal a significant association between age and the disease prevalence ($p = 0.330$). Similarly, there was no significant association between sex and the disease prevalence ($p = 0.580$). Different to the previous results, there was a significant association between body condition score and the disease prevalence ($p < 0.001$). Animals with poor body condition had a notably higher prevalence. Similarly there was a significant association between tick infestation and the disease prevalence ($p < 0.001$).

It is evident from the findings that Babesiosis is a significant concern for the local cattle population. The prevalence rates reported in this study, as well as the comparison with related research, highlight the complex nature of this disease and its associations with various risk factors. Geographic variations, such as tick vector distribution and local climate conditions, play a substantial role in disease prevalence. Additionally, factors like farming systems, cattle breeds, and seasonal variations further influence the occurrence of Babesiosis. Understanding these intricacies is paramount for the development of region-specific disease control and prevention strategies that can effectively reduce the impact of Babesiosis on cattle populations in the Marodijeh region and similar regions.

Based on the findings and discussions, several recommendations can be made to address the occurrence of cattle Babesiosis in Marodijeh Region, Somaliland:

- ✓ Given the significant role of tick infestations in the transmission of Babesiosis, it is crucial to implement and promote tick control measures.
- ✓ Recognizing the geographic and ecological variations in disease prevalence, it is recommended to develop region-specific disease management strategies.

- ✓ Regular health assessments, including body condition scoring, should be conducted to identify cattle at higher risk.
- ✓ Given the seasonal variations in disease prevalence, it is important to implement disease control measures during periods of high risk.
- ✓ Encouraging cattle owners to seek the help of animal health professionals is vital for early disease detection and effective treatment.
- ✓ Continued research is essential to monitor disease trends, understand local factors influencing Babesiosis, and assess the efficacy of disease control interventions.

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8. ANNEXES

Table 6: Description of body condition score

Annex I: Body condition score		
Score	Condition	Features
1	P-	Marked emaciation (animal could condemned at ant mortem examination).
2	P	Transverse process project prominently, neural spines appear sharply.
3	P+	Individual dorsal spines are pointed to the touch, hips, pins, tail head and ribs are prominent. Transverse process visible, usually individually.
4	M-	Ribs, hips and spines clearly visible muscle mass between hook of spines slightly more flesh above the transverse process.
5	M	Ribs usually visible little fat cover dorsal spine barely visible.
6	M+	All smooth well covered, dorsal spines cannot be seen, but are easily felt.
7	G-	All smooth well covered, but fat deposits are not marked, dorsal spine can be felt with firm pressure but rounded rather than sharp.
8	G	Fat cover in critical areas can be easily seen and felt, transverse process cannot be seen.
9	G+	Heavy deposit of fat clearly visible on tail, head, brisket and cad, dorsal spine, ribs, hook and spines fully covered and cannot be felt even with firm pressure.
<p>Note: Body condition scores are as following 1, 2 and 3 are poor body condition, 4, 5 and 6 are medium body condition, and 7, 8 and 9 are good body condition.</p>		

Source: Nicholson and Butterworth (1986).

Annex II: Structured questioner

1. Gender of the respondent.
 - a. Male
 - b. Female
2. Residence area of the respondent
 - a. Hargeisa

- b. Gabiley
- 3. Type of cattle owned breeds?
 - a. Local
 - b. Exotic
- 4. What type of farming system do you use?
 - a. Intensive system
 - b. Semi-intensive system
 - c. Extensive system
- 5. Are ticks available in your area?
 - a. Yes
 - b. No
- 6. If yes, do they cause your animal any problems?
 - a. Yes
 - b. No
- 7. How do you manage the health problems of your cow?
 - a. By myself
 - b. by animal health professionals
- 8. When your animal was lastly treated?
 - a. < 3 Months ago
 - b. Six Months ago
 - c. < A year ago
 - d. More than year ago
- 9. How do you treat your animal when ticks are infested?
 - a. Dipping
 - b. Using anti-parasitic drugs
 - c. Traditional treatment
 - d. Others
- 10. How frequent your animals where treated?
 - a. Once
 - b. Two times
 - c. Three times

- d. More than Three times
11. Is there a seasonal fluctuation of ticks in your area?
- a. Yes
 - b. No
12. If yes, which seasons are they have high burden?
- a. Spring
 - b. Summer
 - c. Autumn
 - d. Winter
13. Select the clinical sings that you see your animals when ticks infested?
- Fever
 - Anemia
 - Itching
 - Restlessness
 - Reduced Feed Intake
 - Hematuria
 - Others
14. If others please specify?
-

Table 7: Animal data recording format

Annex III: Data recording format				
ID	Sex	Age	Body Condition	Tick Infestation
1				
2				
3				
4				
5				

Annex IV: Figures showing research activity.



Figure 3: Preparation of the sampling materials.



Figure 4: Blood Sample collection.



Figure 5: Survey Questioner collection



Figure 6: Blood Sample processing



Figure 7: Blood sample examination



Figure 8: Tick Sample examination

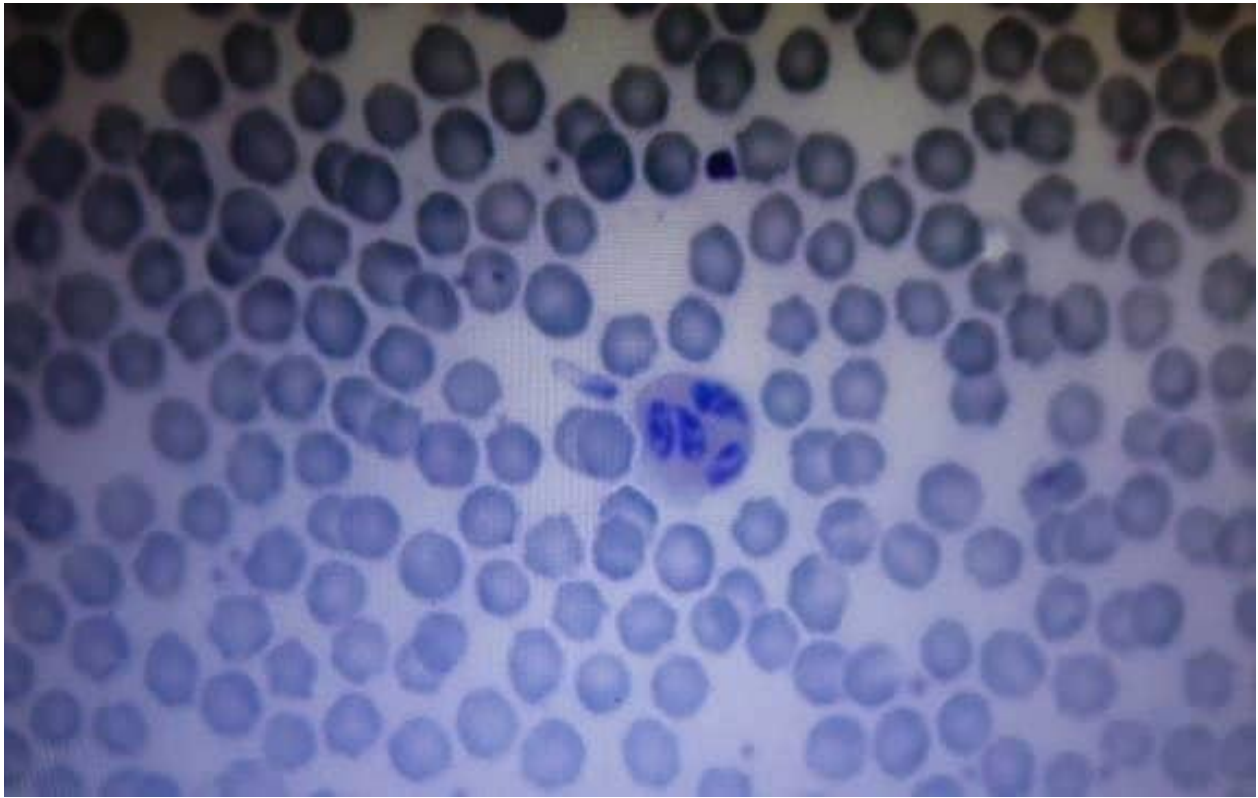


Figure 9: Babesia positive result
(16-10-2023)

Statutory Declaration

I declare that this thesis presents the work carried out by myself and does not incorporate without the acknowledgement of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged.

Name of the Candidate: _____ Signature: _____