UNIVERSIDADE FEDERAL DO PARANÁ

AAMIR MUSE OSMAN

SERO-EPIDEMIOLOGICAL SURVEY OF *ANAPLASMA MARGINALE, BABESIA BOVIS,* AND *BABESIA BIGEMINA* IN CATTLE FROM LOWER SHABELLE REGION, SOMALIA

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Rafael F. C. Vieira
Coorientador: Prof. Dr. Abdalla M. Ibrahim

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The Examining Board is designated by the Faculty of the Graduate Program of the Federal University of Parana in CIÊNCIAS VETERINÁRIAS where invited to argue the THESIS of MASTER OF SCIENCES by **AAMIR MUSE OSMAN** , entitled: **Sero-epidemiological survey of *Anaplasma marginale*, *Babesia bovis* and *Babesia bigemina* in cattle from Lower Shabelle Region, Somalia**, under the supervision of Dr. RAFAEL FELIPE DA COSTA VIEIRA, which and after assessment of the candidate and the work, the Examining Board decided for the APPROVAL in the present rite.

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DEDICATION

To those who encouraged me from my birthday till now. To those who made the
impossible, possible. To those who turned
our life into a better life.
MY PARENTS.

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“Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.” – Louis Pasteur

RESUMO

Fundo: A anaplasmose, causada por *Anaplasma marginale*, e a babesiose bovina, causada por *Babesia bovis* e *Babesia bigemina*, são doenças transmitidas por carrapatos que representam importantes ameaças à pecuária. Ambas as doenças são causas importantes de graves perdas econômicas na pecuária em todo o mundo, inclusive na África Subsaariana. Na Somália, o status dessas doenças é desconhecido. Portanto, os objetivos do presente estudo foram i) determinar a ocorrência de anticorpos contra *A. marginale, B. bovis* e *B. bigemina* em bovinos, ii) identificar espécies de carrapatos parasitando os animais, e iii) determinar fatores associados a exposição em fazendas dos distritos de Awdhegle, Wanla Weyn e Afgoye na região inferior de Shabelle, Somália

Métodos: Foram avaliadas 127 amostras de soro e 66 carrapatos de bovinos. Um questionário epidemiológico foi aplicado a cada proprietário da fazenda abordando local de amostragem, idade, sexo, condição corporal e presença de carrapatos. O volume globular (PCV) foi medido pelo método de microhematócrito. Amostras de soro bovino foram rastreadas usando ELISA comercial para a detecção de anti-*A. marginale* (baseado em MSP5), anti-*B. bovis* (BV60 recombinante) e anti-*B. bigemina* (antígenos brutos) e carrapatos foram identificados com base na técnica morfológica e molecular.

Resultados: A soropositividade geral para *A. marginale, B. bovis* e *B. bigemina* foi de 108/127 (85%; IC 95%: 77,6 - 90,8%), 93/127 (73,2%, IC 95%: 64,7 - 80,7%) e 94/127 (74%, IC 95 %: 65,5 – 81,4%), respectivamente. Um total de 15/127 (11,8%, IC 95%: 6,8 - 18,7%), 1/127 (0,08%, IC 95%: 0,02 - 4,3) e 1/127 (0,08%, IC 95%: 0,02 - 4,3%) bovinos foram apenas soropositivos para *A. marginale, B. bovis* e *B. bigemina*, respectivamente. Um total de 93/127 (73,2%; IC 95%: 64,7-80,7%) bovinos foram soropositivos para pelo menos as espécies *Anaplasma* e/ou *Babesia*. Fatores de risco como idade, condição corporal e localização foram analisados, porém apenas a localização dos animais ($p<0,05$)

foi significativamente associada à soropositividade para *Anaplasma marginale, B. bovis* e *B. bigemina* e condição corporal (p< 0,05) foram significativamente associados apenas com *B. bigemina*. Trinta dos 127 (23,6%; IC 95%: 16,54 – 31,9%) bovinos estavam infestados por 66 (37 M, 26 F e três ninfas) carrapatos no momento da amostragem: *Rhipicephalus pulchellus* (43/66; 65,15%, 19M, 24F), *Amblyomma gemma* (12/66; 18,18%, 12M), *Amblyomma lepidum* (6/66; 9,09%, 5M e 1F), *Hyalomma marginatum* (1/66; 1,51%, 1F) e *Hyalmomma rufipes* (1 /66; 1,51%, 1M). Duas ninfas foram identificadas como *R. pulchellus* e *Rhipicephalus pravus*. A concentração média de PCV para bovinos foi de 0,26 L/L, com 54/123 (43,9%, IC 95%:34,9 – 53,1%) bovinos anêmicos. Não foi encontrada associação entre PCV e soropositividade para *A. marginale* (P = 0,10) e *Babesia* spp (P = 0,80).

Conclusão: O presente estudo mostrou uma alta soropositividade para *A. marginale, B. bovis* e *B. bigemina* em bovinos da Somália. Considerando a alta soropositividade, nossos dados destacam a necessidade de novas investigações utilizando técnicas moleculares.

Palavras-chave: Anaplasmose, babesiose, iELISA, Bovino, África Subsaariana.

ABSTRACT

Background: Anaplasmosis, caused by *Anaplasma marginale*, and bovine babesiosis, caused by *Babesia bovis* and *Babesia bigemina*, are tick-borne diseases that represent important threats to the livestock industry. Both diseases are important causes of severe economic losses in cattle farming worldwide, including in Sub-Saharan Africa. In Somalia, the status of these diseases is unknown. Therefore, the aims of the present study were i) to determine the occurrence of antibodies against *A. marginale, B. bovis* and *B. bigemina* in cattle, ii) to identify tick species parasitizing the animals, and iii) to determine factors associated with exposure in farms from Awdhegle, Wanla Weyn, and Afgoye districts in the lower Shabelle region, Somalia.

Methods: A total of 127 serum samples and 66 ticks collected from cattle were evaluated. An epidemiological questionnaire was applied to each farm owner addressing sampling location, age, gender, body condition, and presence of ticks. The packed cell volume (PCV) was measured by the microhematocrit method. Cattle serum samples were screened using commercial ELISA for the detection of anti-*A. marginale* (**MSP**5-based), anti-*B. bovis* (recombinant BV60) and anti-*B. bigemina* (crude antigens) antibodies and ticks were identified based on morphological and molecular technique.

Results: The overall seropositivity for *A. marginale, B. bovis* and *B. bigemina* was 108/127 (85%; 95% CI: 77.6 – 90.8%), 93/127 (73.2%, 95% CI: 64.7 – 80.7%) and 94/127 (74%, 95 % CI: 65.5 – 81.4%), respectively. A total of 15/127 (11.8%, 95% CI: 6.8 – 18.7%), 1/127 (0.08%, 95% CI: 0.02 – 4.3), and 1/127 (0.08%, 95% CI: 0.02 – 4.3%) cattle were solely seropositive for *A. marginale, B. bovis*, and *B. bigemina*, respectively. A total of 93/127 (73.2%; 95%CI: 64.7-80.7%) cattle were seropositive for at least *Anaplasma* and/or *Babesia* species. In regardless of associated risk factors like age, body condition, and location were analyzed, only location ($p<0.05$) was found to be significantly associated with seropositive

for *Anaplasma marginale, B. bovis*, and *B. bigemina* and body condition ($p<0.05$) was found to be significantly associated only with *B. bigemina*. Thirty out of 127 (23.6%; 95% CI: 16.54 – 31.9%) cattle were infested by 66 (37 M, 26 F, and three nymphs) ticks at the time of sampling: *Rhipicephalus pulchellus* (43/66; 65.15%, 19M, 24F), *Amblyomma gemma* (12/66; 18.18%, 12M), *Amblyomma lepidum* (6/66; 9.09%, 5M and 1F), *Hyalomma marginatum* (1/66; 1.51%, 1F) and *Hyalmomma rufipes* (1/66; 1.51%, 1M). Two nymphs were identified as *R. pulchellus* and *Rhipicephalus pravus.* The mean PCV concentration for cattle was 0.26 L/L, with 54/123 (43.9%, 95% CI:34.9 – 53.1%) cattle anemic. Association between PCV and seropositivity to *A. marginale* (P = 0.10) and *Babesia* spp (P = 0.80) was not found.

Conclusion: The present study showed a high seropositivity for *A. marginale, B. bovis* and *B. bigemina* in cattle from Somalia. Considering the high seropositivity, our data highlight the need for further investigations using molecular techniques.

Keywords: Anaplasmosis, babesiosis, iELISA, Bovine, Sub-Saharan Africa.

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LIST OF ABREVIATIONS

AU	Abrar University
CI	Confidence Interval
CDC	Centers for Disease Control and Prevention
EDTA	Ethylenediamine tetraacetic acid
FAO	Food and Agriculture Organization
GDP	Gross domestic product
iELISA	indirect enzyme-linked immunosorbent assays
IgG	Immunoglobulin G
mm	Millimeter
MSP5	major surface protein 5
NY	New York
OIE	Office International des Epizooties (now World Organisation for Animal Health)
OR	Odds ratio
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed-Cell Volume
RBC	Red Blood Cell
SPSS	Statistical package for social sciences
UFPR	Universidade Federal do Paraná
USA	United States of America

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1 GENERAL INTRODUCTION

1.1 BACKGROUND:

Somalia is a coastal nation located in Eastern Africa. Livestock is the lifeline of individuals and a source of national wealth, with an estimated cattle population of around 5.1 million heads (Too et al., 2015). The economic importance of cattle for Somalia is due to their role as a food source, currency, and as export earnings. The predominant farming system in Somalia, particularly in the Lower Shabelle region, is the traditional mixed farming system. Several factors limit livestock development in these systems, including tick-borne diseases (TBDs) (Minjauw and McLeod, 2003). Overall poor livestock nutrition and management and a lack of development of approaches and tools for general disease control strategies adapted to the special characteristics of these systems (McDermott et al., 1999).

Tick-borne diseases (TBDs) are among the major causes of economic losses to the livestock industry, and ticks are widely distributed across Somalia (Ocaido *et al.,* 2009; Oura *et al.,* 2011; Osman *et al.,* 2020b). Additionally, the extensive management system being practiced in most of the Somali grazing areas exposes the livestock to frequent and high tick infestation, usually leading to the high prevalence of TBDs with a huge economic impact directly and/or indirectly on the livestock industry. The widespread prevalence of TBDs in cattle in many countries confirms the significant economic impact and has been reported in Ethiopia (Abdela et al., 2018), Kenya (Wesonga, et al., 2017; Adjou et al., 2015), Sudan (Awad et al., 2011), and Nigeria (Kamani et al., 2022).

Anaplasma species are obligate intracellular microorganisms, gram-negative bacteria, living in the blood cells of mammals and cause diseases in animals and humans. The main biological vectors of the *Anaplasma* bacteria include those belonging to the genus *Rhipicephalus* in tropical regions and *Dermacentor* in temperate regions (Kocan et al., 2010) and are mechanically transmitted by biting flies (Aubery and Geale, 2011). The

socioeconomic impact of the disease and the restrictions on trading infected animals internationally led the Office International des Epizooties (OIE) Animal Health Code to categorize Anaplasmosis as a disease that required notification of its presence (OIE, 2018).

Babesia, the causal agent of babesiosis, is tick-borne apicomplexan protozoa (Chauvin et al., 2009). Babesiosis is a zoonotic and hemolytic infection, transmitted by many tick species (Bock et al., 2008), that affects animals and humans, caused by *Babesia* spp. *Babesia bigemina* and *Babesia bovis* are the most economically significant *Babesia* species in the cattle industry of tropical and subtropical countries of the world (Lew and Jorgensen, 2005). The protozoa infect erythrocytes, causing a hemolytic process during their reproduction cycle (Uilenberg 2006). The clinical signs of babesiosis are fever, ataxia, hemoglobinuria, anemia, and weakness, (Lew and Jorgensen, 2005). Additionally, neurological signs have been associated with *B. bovis* infection (Suarez et al., 2012).

Anaplasmosis and babesiosis are widely spread diseases that affect mammals. In cattle, both diseases represent a considerable threat to the cattle industry, associated with direct economic losses such as loss of body weight, decreased milk production and death, and indirect costs such as prevention, control, and treatment. In Somalia, the current status of both diseases is unknown. Prevalence data dating before the Civil War of 1990s, ranged from 0.8% to 72.4% and from 0% to 91.3% for anaplasmosis and babesiosis, respectively, using light microscopy and serological techniques (Heuer et al., 1990a; Osman et al., 2020a,b; Caille, 1987; Schoepf et al., 1984). In this pattern, there is a lack of information on factors associated with both diseases in Somalia. Thus, there is a need to generate information on the seroepidemiological status of *A. marginale, B. bovis,* and *B. bigemina* in cattle from Somalia.

2. LITERATURE REVIEW:

2.1. LIVESTOCK SECTOR IN SOMALIA:

In Somalia, the livestock sector is essential to the social and economic well-being of the country. Livestock is the most important contributor to Somalia's gross domestic product (GDP), livelihoods, and economic growth, with more than half of the population living in rural areas relying on it directly or indirectly (FOA, 2019). The Ministry of Livestock, in conjunction with the Ministry of Planning and National Development, estimated the cattle population at more than 5.1 million heads in 2013. The herd structure comprises 80-87% female herd with 20-30% lactating (Too et al., 2015). Yet, its performance is undermined by several factors, including poor animal nutrition and infectious diseases. Therefore, TBDs are of major socioeconomic importance to the livestock industry, and its current data in Somalia is lacking.

2.2. TICK BORNE DISEASES (TBDs):

Tick-borne diseases (TBDs) are among the major causes of economic losses in animal production (Jirapattharasate et al., 2015). Globally, four main tick-borne diseases (TBDs), namely anaplasmosis, babesiosis, theileriosis, and cowdriosis (heartwater) affect bovines (Jabbar et al., 2015). Anaplasmosis and babesiosis are among the serious illnesses that are more economically significant in tropical and subtropical areas (Filia et al., 2016). Both diseases have been reported in Somalia (Heuer et al., 1990a; Osman et al., 2020a,b; Caille, 1987; Schoepf et al., 1984).

Anaplasmosis and piroplasmosis are widely spread diseases that affects many species of mammals, with a direct effect on cattle (Suarez and Noh, 2011). Globally, both diseases have become gradually known as public health challenges (Schorn et al., 2011; Zanet et al., 2014). The public health challenges are due to the transhumance practiced by

Somali livestock keepers to Ethiopia and Kenya, which facilitates the dissemination of many pathogens. In Africa, TBDs are considered the most important animal disease challenge after trypanosomiasis (Kasaija et al., 2021). In Somalia, the favorable equatorial climatic conditions suitable for livestock production also support large tick populations, which enhances the transmission of TBD agents.

2.2.1 CATTLE ANAPLASMOSIS:

Anaplasmosis is an infectious non-contagious disease, formerly known as gall sickness, caused by obligate intra-erythrocytic bacteria of the genus *Anaplasma* that belongs to the family Anaplasmataceae of the order Rickettsiales (blood (Aubry and Geale, 2011). Globally, cattle anaplasmosis caused by *A. marginale* is of great economic importance (Jongejan and Uilenberg 2004). Because outbreaks are seasonal and infection rates are stable, the significance of anaplasmosis is underestimated in endemic areas (M'ghirbi et al., 2016). Anaplasmosis is transmitted biologically by ticks and mechanically by biting flies or fomites such as needles, syringes, and surgical instruments (Atif, 2015; Jongejan and Uilenberg, 2004; Kocan et al., 2004).

2.2.1.1 GEOGRAPHICAL DISTRIBUTION:

Cattle anaplasmosis occurs in tropical and subtropical areas of the world (Kocan et al., 2010). The geographic distribution of the disease is dependent on the density and distribution of vectors and reservoir hosts (Abdisa, 2019). Kocan et al. (2010) hypothesized that the distribution of anaplasmosis might be expected to continue to change, in part due to global warming, which may influence the movement of the tick hosts.

2.2.1.2 TRANSMISSION:

Anaplasmosis transmissions typically occurs in two forms, biological and mechanical (Abdisa, 2019). In the biological transmission, around 20 tick species have been incriminated as vectors worldwide, including *Rhipicephalus* spp., *Hyalomma* spp., *Demacentor* spp., and *Ixodes* spp. (Jongejan and Uilenberg, 2004; Kocan et al., 2004). Ticks usually feed on cattle during the nymph and adult stages, which is when *Anaplasma* transmission occurs (Palmer et al., 2001). Transovarial transmission between ticks and transplacental in cattle have been reported (Kocan, 2004). Transplacental transmission appears to occur during the second or third trimester of pregnancy (Abdisa, 2019).

In the mechanical transmission, around 12 species of biting flies have been shown to experimentally transmit *A. marginale* to cattle, including (*Stomoxys calcitrans* and *Haematobia irritans*), horse-flies (*Tabanus* spp.), fomites contaminated with blood, and by transplacental route (Aubry and Geale, 2011; Reinbold et al., 2010).

2.2.1.3 PATHOGENESIS AND CLINICAL SIGNS

Pathogenicity of *A. marginale* is related to various factors such as the virulence of the strain, age-related host susceptibility, and breed resistance. Cattle of all ages may be infected with *A. marginale,* but the severity of the disease is age-dependent. Calves are less susceptible to clinical disease. Under 6 months of age, the illness is rare. Animals between 6 months and 1 year of age usually develop mild disease. Animals between 1 and 2 years of age suffer from acute but rarely fatal diseases. The resistance of calves is not due to colostral antibodies from immune dams, but they regenerate red blood cells faster than adults (Coetzee et al., 2015). *Bos taurus* cattle appear to be more likely to develop severe, acute diseases than *Bos indicus* cattle (Smith, 2015). Animals that recover from the disease

may remain carriers for life and become reservoirs for transmission to other susceptible hosts (Abdisa, 2019).

Following transmission, cattle develop rickettsiaemia, accompanied by fever, severe anemia, weight loss, decreased milk production, abortion, and sometimes death during acute infections (Kocan et al., 2003; Urdaz-Rodríguez et al., 2009). During infection, cattle erythrocytes are phagocyted by reticuloendothelial cells, which leads to anemia and icterus without haemoglobinaemia and haemoglobinuria. Moreover, a clinical picture of the disease also includes fever, weight loss, abortions, and lethargy (Rymaszewska and Grenda, 2008).

2.2.2 CATTLE BABESIOSIS:

Globally, cattle babesiosis is caused mainly by the tick-borne apicomplexan parasites *Babesia bovis, B. bigemina,* and *B. divergens* (Suarez and Noh, 2011). In Africa, cattle babesiosis is mainly caused by *B. bovis* and *B. bigemina* due to the presence of its tick vectors. Cattle babesiosis is an acute disease that persists in surviving animals (Goff et al., 2001). Babesia are highly successful intracellular parasites that have evolved extremely developed survival mechanisms over the duration of their long co-evolution with their hosts, allowing them to survive the constant changes and pressures that occur in their environments, including overcoming the host's immune system's activity. (Suarez and Noh, 2011). As a result, these parasites have evolved a complicated life cycle that includes definitive and intermediate hosts in hard ticks and vertebrates. Thus, Babesia infects and multiplies asexually inside red blood cells (RBCs) of their infected vertebrate hosts, then develop sexual forms in the midguts of their invertebrate hosts, where they undertake sexual multiplication (Mehlhorn and Shein, 1984).

The most prevalent clinical signs associated with acute illness are fever, anemia, anorexia, lethargy, hemoglobinuria, tachycardia, and icterus. *Babesia bovis* is renowned for

causing more severe disease, such as cerebral babesiosis, which is marked by convulsions, hyperaesthesia, and paralysis, as well as parasite sequestration in brain capillaries (Suarez and Noh, 2011). The major economic effect of babesiosis is on the cattle industry, particularly over half of the world's 1.2 billion Cattle are at risk of the disease (Bock et al., 2004; Gohil et al., 2013).

2.2.2.1 GEOGRAPHICAL DISTRIBUTION:

The babesias are one of the most global and widespread blood parasites in the world based on numbers and distribution of species in animals, second only to the trypanosomes. *Babesia bovis* and *B. bigemina* are present in many countries between 40°N and 32°S (Bock et al., 2004). Both parasites have a similar distribution, however, *B. bigemina* is more common in Africa than *B. bovis* due to the availability of *Rhipicephalus* (*Boophilus*) *decoloratus* and *Rhipicephalus evertsi* as *B. bigemina* vectors. (Bock et al., 2004). In endemic areas, cattle become infected at a young age and develop long-term immunity. However, outbreaks can occur in these endemic areas if exposure to ticks by young animals is sporadic or immune-naïve cattle are introduced (OIE, 2013).

2.2.2.2 TRANSMISSION:

All babesial parasites described are transmitted by the Ixodid tick (Homer, 2000). All species of babesia are naturally transmitted from animal to animal by tick bites, and within ticks, transovarial (infection transmission through eggs to mother ticks) and transsadial (infection transmission through the egg to larvae to nymph to adult) transmission occurs. (Enbiyale et al., 2018).

Babesia bigemina are transmitted by *Rhipicephalus (Boophilus) microplus*, *Rhipicephalus annulatus, R. (Boophilus) decoloratus, R. geigyi* and *R. evertsi* are also

competent vectors. Tick vectors of *B. bovis* are *R. (Boophilus) microplus, R. annulatu,* and *R. geigyis*, a competent vector (Sharaf *et al.,* 2017).

Rhipicephalus ticks transmit *B. bigemina* and *B. bovis* transovarially, although only tick larvae transmit *B. bovis,* whereas nymphs and adults transmit *B. bigemina* and *B. divergens* (Esmaeil et al., 2015). As a result, transmission rates in this species are higher than in *B. bovis*, and in areas where both species are present, endemic stability is more likely to occur for *B. bigemina* than for *B. bovis*. (Bock et al., 2004).

2.2.2.3 PATHOGENESIS AND CLINICAL SIGN

After penetration of the host cell, the parasite multiplies via repeated binary fission within the erythrocyte, resulting in up to 16 merozoites. Multiplication of the parasites damages the erythrocyte cell membrane, causing increased osmotic fragility and subsequent intravascular and extravascular haemolysis (Beugnet and Moreau 2015). Indirect pathways of cell destruction are also important contributors to the pathogenicity of *Babesia*-induced anaemia, which is the predominant clinical syndrome. Immune-mediated haemolytic anaemia is assumed to occur with all *Babesia* spp. following the production of anti-erythrocyte membrane antibodies (Hunfeld et al., 2008). *Babesia* activates antibody-mediated cytotoxic destruction of erythrocytes, leading to anaemia, haemoglobinaemia, haemoglobinuria, thrombocytopenia. In cases of massive infection, tissue hypoxia is severe Babesiosis in both dogs and ruminants, particularly affecting the central nervous system, kidneys, and muscles, leading to death caused by multiple organ dysfunction syndromes (Beugnet and Moreau 2015; Suarez et al., 2019). *B. bovis* is more virulent than *B. bigemina*, causing more dramatic clinical signs and symptoms including vascular sequestration and vaso-occlusion, as well as greater mortality in the vertebrate host (Suarez et al., 2019).

2.2.3 LABORATORY DIAGNOSIS OF TICK-BORNE DISEASES

A variety of diagnostic tests are available and researchers are still working to improve existing tests and develop new ones (OIE Manual, 2018). Diagnosis of babesiosis and anaplasmosis in animals can be achieved by parasitological, serological, and molecular methods. The choice of a particular test will be guided by economic principles and the availability of expertise in the laboratory (Wilkowsky, 2018). Reliable interpretation of results from diagnostic tests will depend on test validity as well as on proper sample selection/collection, the sample size, and the way the diagnostic tests are conducted (OIE Manual, 2018).

The traditional method of identifying the agent in infected animals is by microscopic examination of thick and thin films stained with Giemsa or Romanowsky type stain. This method is still used today in most diagnostic laboratories (Sharaf et al., 2017). Blood is usually collected, combined with an anticoagulant, and smeared on a glass slide, air-dried, fixed with methanol, and stained with Giemsa or a similar stain for several minutes. The slide is then washed thoroughly and dried. Intraerythrocytic parasites are observed under a microscope using a 100X objective with oil immersion (Sharaf et al., 2017; Mosqueda et al., 2012).

Serological methods such as the Indirect Fluorescent Antibodies Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA), and the Complement Fixation Test (CFT) are used routinely (Alvarez and Figueroa 2019). For epidemiological surveys, the favored tests are serological. The IFAT is the most popular serological technique used to distinguish between *Babesia*, *Anaplasma* spp. and to demonstrate the presence of antibodies in a population. The advantage of ELISA over IFAT is that interpretation of results is less subjective and is easily automated for large samples (Zintl et al., 2003; Sharaf et al., 2017).

When a large number of serum samples is processed, the IFAT test becomes time-consuming and not very effective; this is mainly because each sample must be analyzed at one time by the diagnostician, and reading each sample may take several minutes (Bose and Peymann, 1994). Other methods based on automation, like ELISA, are very useful (Mosqueda et al., 2012). ELISA has the advantage of non-subjectivity, the capacity to read a large number of samples easily, and presents higher specificity than the IFAT (Mosqueda et al., 2012). Fortunately, IFAT and ELISA are equally effective in detecting positive samples (Zintl et al., 2003).

Tick-borne pathogens are difficult to detect because of the low number of parasites in peripheral blood. Therefore, DNA-based molecular methods have been developed with great advantages, such as high analytical sensitivity and specificity rates (Alvarez et al., 2019).

Nested PCR is a suitable assay for international trade, especially for *B. bovis*, which usually shows low parasitaemia or carrier status (OIE, 2018). A nested PCR assay also has been used for the detection of tick-borne pathogens not only in cattle but also in water buffaloes (*Bubalus bubalis*). Both types of animals have been detected as asymptomatic carriers (Romero et al., 2016). It is also possible to perform DNA extraction from already stained blood smears, enabling an easier way to detect asymptomatic carriers in laboratory collection samples (Shayan and Rahbari, 2005).

2.2.4 CONTROL OF TICK-BORNE DISEASES

Active prevention and control of TBDs are achieved by three main methods: vaccination, chemoprophylaxis, and vector control.

The most widely used method for controlling ticks is the direct application of acaricides to host animals. In most of cases, the application of acaricides is repeated after

21–30 days (Ghosh et al., 2006). However, the use of acaricides has had limited ability to reduce tick infestations, and it is often accompanied by serious drawbacks, including the selection of acaricide-resistant ticks, environmental contamination, and contamination of milk and meat products with drug residues (Merino et al., 2013).

Several groups of compounds have been used in the chemical control of TBDs. Such as imidocarb dipropionate, diminazeneaceturate, and tetracycline antibiotics remain freely available in most endemic countries (OIE, 2010).

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3 RATIONALE AND OBJECTIVES

3.1 RATIONALE:

Tick-borne diseases (TBDs) constitute a serious animal disease threat in Africa (Young et al., 1988), particularly among smallholder farmers in the east, central and southern parts of Africa (Jongejan and Uilenberg, 2004). In Somalia, the tropical climate is ideal for animal production, but it also supports enormous tick populations, which increases TBD transmission. Thus, considering cattle industry in Somalia is growing, in addition, there is a lack of data on ticks infesting cattle and prevalence data on *A. marginale*, *B. bovis* and *B. bigemina* dates before the Civil War with low sensitive and low specificity methods, Therefore, there is a need to generate information about the epidemiological situation of this diseases.

Until a decade ago, ticks and TBDs received little attention since most studies were focused on bovines, probably due to their higher economic growth (Ahmed et al., 2006). However, as the socioeconomic importance of livestock in food security and poverty reduction in response-poor farming communities around the world has grown, more focus is now being paid to a better understanding of ticks and TBDs in livestock (Ghafar et al., 2020).

On the other hand, one of the major risks to public health are transboundary diseases due to the transhumance practiced by Somali livestock keepers to Ethiopia and Kenya, which facilitates the dissemination of many pathogens. Also, animals from different groups circulating in common areas, such as watering points, markets, grazing spaces, and along trading routes, are very common in Somalia and maintain a high disease transmission index (FAO, 2018).

3.2 HYPOTHESIS:

- *Anaplasma* marginale, *Babesia bovis,* and *Babesia bigemina* are highly prevalent in cattle from Somalia.

3.3 OBJECTIVES:

3.3.1 OVERALL OBJECTIVE:

- To screen cattle antibodies against *A. marginale*, *B. bovis,* and *B. bigemina* and to determine factors associated with exposure to these pathogens in the Lower Shabelle region, Somalia.

3.3.2 SPECIFIC OBJECTIVES

- To determine the seroprevalence of *A. marginale*, *B. bovis,* and *B. bigemina* in cattle from the Lower Shabelle region, Somalia.
- To evaluate the hematologic status of animals.
- To collect and identify tick species parasitizing animals.
- To determine factors associated with exposure to *A. marginale*, *B. bovis,* and *B. bigemina*.

4 MANUSCRIPT: SERO-EPIDEMIOLOGICAL SURVEY OF *ANAPLASMA MARGINALE*, *BABESIA BOVIS,* AND *BABESIA BIGEMINA* IN CATTLE FROM LOWER SHABELLE REGION, SOMALIA

4.1 ABSTRACT

Background: Bovine babesiosis and Anaplasmosis, an important threat to the livestock industry, is a tick-borne disease mainly caused by *Anaplasma marginale*, *Babesia bovis*, and *Babesia bigemina*. In Somalia, the current distribution of these diseases is unknown. Therefore, this study aimed to determine the seropositivity for *A. marginale, B. bovis,* and *B. bigemina* in cattle from Awdhegle, Wanla Weyn, and Afgoye districts in the Lower Shabelle region, Somalia.

Methods: A total of 127 serum samples and 66 ticks from cattle were evaluated. An epidemiological questionnaire was applied to each farm owner addressing sampling location, age, gender, body condition, and presence of ticks. The packed cell volume (PCV) was measured by the microhematocrit method. Cattle serum samples were screened using commercial ELISA for the detection of anti-*A. marginale* (MSP5-based), anti-*B. bovis* (recombinant BV60) and anti-*B. bigemina* (crude antigens) antibodies, and ticks were identified based on morphological and molecular technique.

Results: The overall seropositivity for *A. marginale, B. bovis* and *B. bigemina* was 108/127 (85 %; 95 % CI: 77.6 – 90.8 %), 93/127 (73.2%, 95 % CI: 64.7 – 80.7%) and 94/127 (74%, 95 % CI: 65.5 – 81.4%) respectively. IgG antibodies to only *A. marginale, B. bigemina,* and *B. bovis* were detected in 15/127 (11.8%, 95% CI: 6.8 – 18.7), 1/127 (0.01%, 95% CI: 0.01 – 4.3), and 1/127 (0.01%, 95% CI: 0.02 – 4.3) cattle, respectively. A total of 93/127 (73.2%; 95%CI: 64.7-80.7%) cattle were shown coinfection with at least two *Anplasma* and/or *Babesia* species. Regardless of associated risk factors like age, body condition, and location were analyzed, only location ($p<0.05$) was found to be significantly associated with seropositive for *Anaplasma marginale, B. bovis*, and *B. bigemina* and body condition

(p<0.05) was found to be significantly associated only with *B. bigemina*. 30/127 (23.6%; 95% CI: 16.54-31.9%) cattle were infested by ticks: *Rhipicephalus pulchellus* (68.18%), *Amblyomma gemma* (18.18%), *Amblyomma lepidum* (9.09%), *Hyalomma marginatum* (1.51%), *Hyalmomma rufipes* (1.51%) and *Rhipicephalus pravus* (1.51%).

Conclusion: The present study showed a high seropositivity for *A. marginale, B. bovis,* and *B. bigemina* in cattle from Somalia. Considering the high seropositivity our data highlight the need for further investigations using molecular techniques.

Keywords: *Anaplasma marginale, Babesia bovis*; *Babesia bigemina;* iELISA; Bovine; Sub-Saharan Africa

4.2 INTRODUCTION:

Tick-borne diseases (TBDs) are a major constraint of the growth of the livestock industry around the world (Suarez et al., 2011). It has a significant economic impact, which is growing every year. TBDs affect 80% of the world's cattle population, with an estimated cost of between USD 13.9 billion and USD 18.7 billion (Rochlin and Toledo, 2020). TBDs constitute the most serious animal disease threat in Africa (Young et al., 1988), particularly among smallholder farmers in the east, central and southern parts of Africa (Jongejan and Uilenberg, 2004). In Somalia, the tropical climate is ideal for animal production, but it also supports enormous tick populations, which may increase TBD transmission.

Anaplasma species belong to obligate intracellular microorganisms, gram-negative bacteria, living in the blood cells of mammals and cause diseases in animals and humans. The main biological vectors of the *Anaplasma* bacteria are Ixodid ticks (Rymaszewska and Grenda 2008). The socioeconomic impact of the disease and the restrictions on trading infected animals internationally led the Office International des Epizooties (OIE) Animal Health Code to categorize Anaplasmosis as a disease that required notification of its presence (OIE, 2018).

Babesia, the causal agent of babesiosis, is tick-borne apicomplexan protozoa (Chauvin et al., 2009). Babesiosis is a zoonotic and hemolytic infection, transmitted by many tick species (Bock et al., 2008), that affects animals and humans, caused by *Babesia* spp. *Babesia bigemina* and *Babesia bovis* are the most economically significant *Babesia* species in the cattle industry of tropical and subtropical countries of the world (Lew and Jorgensen, 2005). The protozoa infect erythrocytes, causing a hemolytic process during their reproduction cycle (Uilenberg 2006). The clinical signs of babesiosis are characterized by fever, ataxia, hemoglobinuria, anemia, and weakness (Lew and Jorgensen, 2005). Additionally, neurological signs have been associated with *B. bovis* infection (Suarez et al., 2012).

Anaplasmosis and babesiosis are widely spread diseases that affect mammals. Both diseases are a huge threat to the cattle industry as they are associated with direct economic losses like loss of body weight, decreased milk production and death of animals, and indirect costs of prevention and treatment. In Somalia, there is a lack of data on both diseases, with the available prevalence rates dating before the Civil War 1990s of babesiosis ranging from 0% to 91.3%, and anaplasmosis from 0.8% to 72.4% using microscopic and serological technique (Heuer et al., 1990a; Osman et al., 2020a, b; Caille, 1987; Schoepf et al., 1984). However, all of the studies lacked adequate information to assess the factors associated with disease occurrence despite its critical for the control and prevention of these diseases.

The livestock sector is of significant social and economic importance in Somalia. More than half of the population living in rural areas depend directly or indirectly on livestock. Moreover, the sector contributes about 40 percent in the Gross Domestic Products (GDP) of the country and more than 50% of export earnings (CIA, 2020). Considering the significant livelihood benefits derived from cattle in Somalia, epidemiological studies are crucial in studying the distribution and associated factors of tick-borne infections in specified livestock

populations. The aim of this study was to screen cattle antibodies against *A. marginale*, *B. bovis,* and *B. bigemina*, and to determine factors associated with exposure to these pathogens in the Lower Shabelle region, Somalia

4.3 MATERIALS AND METHODS

4.3.1 STUDY AREA AND SAMPLING

A cross-sectional study was carried out between November 2019 to January 2020, which is the dry season in Somalia, on Wanle Weyn (2^{O}08´17.16” N 45^{O}07´16.32” E), Awdhegle (1.9805^{O}N 44.8330^{O}E) and Afgoye (2°08′47.67″N 45°07′ 08.11″E) districts, Lower Shabelle Region. Districts were selected due to security and infrastructure reasons. This region presents a Mid-Latitude Steppe and Desert Climate (Köppen: Bsh) and is characterized by higher land productivity and higher rainfall compared to the rest of Somalia (FAO, 2018). Herds studied herein were selected based on the accessibility and owner’s willingness to cooperate with this study.

A non-probabilistic convenience sampling was performed. A total of 127 cattle blood samples were collected by jugular venipuncture in Awdhegle (n=63), Wanla Weyn (n=46), and Afgoye (n=18) districts. Animals were physically restrained, and blood samples (10 mL) were collected by venipuncture of the jugular vein using vacuum tubes containing EDTA (BD Vacutainer®, Franklin Lakes, NJ, EUA) for packed cell volume determination. Five milliliters were placed into tubes containing a serum separator gel (BD Vacutainer®) and kept at room temperature (25 ◦C) until visible clot formation. The samples were then centrifuged at 1500 × g for 5 min, serum separated, and kept at − 20 ◦C for serological testing.

4.3.2 DNA EXTRACTION AND PCR ASSAYS

DNA was extracted from the ticks' right fourth leg using a commercial kit (MagaZorb® DNA Mini-Prep Kit, Promega, Madison, Wisconsin), according to the manufacturer's instructions. All samples were submitted to conventional PCR for the mitochondrial 16S rRNA gene of ticks (Fukunaga et al., 2001) to monitor the DNA extraction.

Amplicons obtained from eight ticks were sequenced in both directions by Sanger method. Partial nucleotide sequences of the mitochondrial 16S rRNA gene of ticks were deposited in GenBank® database (accession nos. ON532090, ON532091, ON532092, ON532093, ON532094, ON532095, ON532096, ON532097).

Phylogenetic analysis of 16S rRNA partial sequences of ticks obtained from cattle, Somalia. Sequences were aligned using MAFFT 7.110 (https://mafft.cbrc.jp/alignment/server). Phylogenetic analyses were based on Bayesian inference using Beast version 1.8.4 (https://beast.community/index.html). Three independent runs of 100 million generations of Monte Carlo Markov chain with 1 sampling/10,000 generations and a 10% burn-in. GTR plus gamma was estimated as substitution models based on Akaike information criterion, using jModeltest version 2.1.10 (https://github.com/ddarriba/jmodeltest2/releases/tag/v2.1.10r20160303). The tree was rooted with *Ixodes ricinus* (GenBank accession no. L34292) (Fig. 1).

4.3.3 PACKED CELL VOLUME:

Packed cell volume (PCV) was determined using the microhematocrit centrifugation technique (Brar et al. 2011). A PCV of 0.26 L/L or less was used as an indicator of anemia (Marcotty et al., 2008).

4.3.4 DETECTION OF ANTIBODIES AGAINST *A. marginale, B. bovis,* AND *B. bigemina.*

Cattle serum samples were screened for anti-*A. marginale* antibodies by a commercial MSP5-based on indirect ELISA (Imunodot Diagnostics, Jaboticabal, BR). Anti-

B. bovis (recombinant BV60) and anti-*B. bigemina* (Crude antigen) antibodies were detected by iELISA (Imunodot Diagnostics) following the protocol described by (Machado et al., 1997). The plates (Maxisorp®; Nunc, Thermo Scientific, Brazil) were incubated in a moist chamber at 37 ºC for 90 minutes. After three washings with PBS-Tween 20 buffer, the positive and negative reference serum and tested serum samples, previously diluted with PBS-Tween 20 solution plus 5% normal Rabbit serum at the following dilutions 1:400 *A. marginale, B. bovis* and *B. bigemina,* were added to the ELISA plates. The plates were incubated again at 37 ºC for 90 min. After three washes with PBS-tween 20 buffer, the bovine conjugated IgG (Sigma®, St. Louis, USA) was added to the ELISA plate in the 1:30000 dilution in PBS-tween 20 plus 5% of normal rabbit serum, with subsequent incubation and washing. Finally, the substrate of the alkaline phosphatase enzyme, P-nitrophenyl phosphate (Sigma®, St. Louis, MO, USA) diluted at 1 mg/mL in diethanolamine buffer pH 9.8 (Sigma®, St. Louis, USA) was added. The ELISA plates were sealed with aluminum foil and incubated at room temperature for 30 min.. The reading was performed in an ELISA reader (B.T.-100; Embrabio, São Paulo, Brazil), with a 405 nm filter. The cutoff values, calculated as 2.5 times the mean absorbance of the negative control sera (Machado et al., 1997), were as follows 0.248 for *B. bovis*; 0.256 for *B. bigemina*; and 0.190 for *A. Marginale*.

4.3.5 DATA MANAGEMENT AND ANALYSIS

A non-parametric Mann–Whitney test was used to compare the PCV concentration between infected and non-infected cattle. Data analyses were performed with SPSS Statistics software® (IBM Corp, Armonk, NY, USA, version 26). The chi-square test was used to evaluate significant differences in infection rate in animals of different ages, body conditions, tick presence location, and PCV. Odds ratio (OR), 95% confidence intervals (95% CI), and P-values were calculated separately for each variable, and results were

considered significant when P ≤ 0.05. Data were compiled and analyzed in Epi Info™ software, version 7.2.3.1 (Centers for Disease Control and Prevention, CDC, USA).

4.4 RESULTS:

A total of 66 (37 M, 26 F, and three nymphs) ticks were collected from 30/127 (23.6%; 95% CI: 16.54-31.9%) cattle with a mean of 2.2 ticks per animal. Adult ticks were identified as *Rhipicephalus pulchellus* (43/66; 65.15%, 19M, 24F), *Amblyomma gemma* (12/66; 18.18%, 12M), *Amblyomma lepidum* (6/66; 9.09%, 5M and 1F), *Hyalomma marginatum* (1/66; 1.51%, 1F) and *Hyalmomma rufipes* (1/66; 1.51%, 1M). Two nymphs were identified as *R. pulchellus* and one *Rhipicephalus pravus* tick.

The PCV data were not normally distributed (Shapiro– Wilk normality test, W = 0.98, P = 0.025). The mean PCV concentration for cattle was 0.26 L/L. A total of 54/123 (43.9%, 95% CI:34.9 – 53.1%) cattle were anemic. Statistical difference (U = 657.5, Z = − 2.01, P = 0.044) was found in mean PCV between *Anaplasma*-seropositive D(0.26 L/L) and *Anaplasma*-seronegative cattle (0.28 L/L), and no statistical difference (U = 998, Z = − 1.75, P = 0.80) mean PCV between *Babesia*-seropositive (0.25 l/l) and *Babesia*-seronegative cattle (0.28 L/L).

The overall individual animal-level seroprevalence of *A. marginale* antibodies was 108/127 (85 %; 95 % CI: 77.6 – 90.8 %). The highest seroprevalence for *A. marginale* was estimated in Awdhegle district 60/63 (95.2%, 95 % CI: 86.7 – 99.0%), followed by Agoye 11/18 (61.1%, 95 % CI: 35.8 – 82.7%), and Wanla Weyn 37/46 (80.4%, 95 % CI: 66.1 – 90.6%) (Table 1).

The overall individual animal-level seroprevalence for *B. bovis* was 93/127 (73.2%, 95 % CI: 64.7 – 80.7%). The highest seroprevalence for *B. bovis* was estimated in Awdhegle district 56/63 (88.9 %, 95 % CI: 78.4 – 95.4)%), followed by Agoye 12/18 (66.7%, 95 % CI: 40.9 – 86.7%), and Wanla Weyn 25/46 (54.3%, 95 % CI: 39.0 – 69.1%) (Table 2).

The overall individual animal-level seroprevalence for *B. bigemina* was 94/127 (74%, 95 % CI: 65.5 – 81.4%), The highest seroprevalence for *B. bigemina* was estimated in Awdhegle district 58/63 (92.1%, 95 % CI: 82.4 – 97.4%), followed by Wanla Weyn 28/46 (60.9%, 95 % CI: 45.4 – 74.9%), and Agoye 8/18 (44.4%, 95 % CI: 21.5 – 69.2%) (Table 3).

Cattle reared in Awdhegle were more likely to be seropositive to *A. marginale*, *B. bovis,* and *B. bigemina* than those reared in Afgoye (OR: 12.7, 4.0, and 14.5, respectively). This difference was observed to be statistically significant ($P < 0.05$). No associations between seropositivity for other districts evaluated were found ($P > 0.05$).

Regarding prevalence based on body condition score of the study animals, the prevalence of *B. bigemina* was significantly ($P < 0.05$) highest in poor body condition (94.7%), followed by moderate body conditioned animals (82.0%), and the lowest in animals with good body condition score (63.58%) (Table 3). While, *A. marginale*, and *B. bovis,* were not significantly associated ($P > 0.05$) between body conditions of studied animals.

No associations between seropositivity for *A. marginale*, *B. bovis,* and *B. bigemina* among different groups regarding studied animal age ($P > 0.05$).

Table 1 Sero-prevalence of *Anaplasma marginale* of cattle within each variable studied

Variable		No. Examined	No. Positive	Prevalence%95% CI:	P-value	OR 95% CI:
District	Awdhegle	63	60	95.2 (86.7 – 99.0)	0.001 ($\chi^2 = 15.1$)	12.7 (2.8 – 56.9)
	Wanle Weyn	46	37	80.4 (66.1 – 90.6)	0.10 ($\chi^2 = 2.58$)	2.6 (0.8 – 8.6)
	Afgoye	18	11	61.1 (35.8 – 82.7)		
Body condition	Poor	19	18	94.7 (73.9 – 99.9)	0.17 ($\chi^2 = 1.74$)	3.8 (0.5 – 31.2)
	Moderate	39	33	84.6 (69.5 – 94.1)	0.51 ($\chi^2 = 0.07$)	1.2 (0.4 – 3.4)
	Good	69	57	82.6 (71.6 – 90.7)		
Age	>5	66	57	86.4 (75.7 – 93.6)	0.99 ($\chi^2 = 0.04$)	0.8 (0.09 – 7.1)
	2-5	52	43	82.7 (69.7 – 91.8)	0.99 ($\chi^2 = 0.21$)	0.6 (0.07 – 5.4)
	<2	9	8	88.9 (51.8 – 99.7)		

Table 2 Sero-prevalence of cattle *Babesia bovis* within each variable studied

Variable		No. Examined	No. Positive	Prevalence%95% CI:	P-value	OR 95% CI:
District	Awdhegle	63	56	88.9 (78.4 – 95.4)	0.03 ($\chi^2 = 5.1$)	4 (1.2 – 14.1)
	Wanle Weyn	46	25	54.3 (39.0 – 69.1)	0.27 ($\chi^2 = 0.8$)	0.6 (0.2 – 1.9)
	Afgoye	18	12	66.7 (40.9 – 86.7)		
Body condition	Poor	19	17	89.4 (66.9 – 98.7)	0.04 ($\chi^2 = 3.8$)	4.2 (0.9 – 19.9)
	Moderate	39	30	76.9 (60.7 – 88.9)	0.18 ($\chi^2 = 1.3$)	1.7 (0.7 – 4.1)
	Good	69	46	66.7 (54.3 – 77.6)		
Age	>5	66	50	75.8 (63.6 – 85.5)	0.34 ($\chi^2 = 0.8$)	0.4 (0.0 – 3.7)
	2-5	52	35	67.3 (52.9 – 79.7)	0.18 ($\chi^2 = 1.7$)	0.3 (0.0 – 2.2)
	<2	9	8	88.9 (51.8 – 99.7)		

Table 3 Sero-prevalence of cattle *Babesia bigemina* within each variable studied

Variable		No. Examined	No. Positive	Prevalence%95% CI:	P-value	OR 95% CI:
District	Awdhegle	63	58	92.1 (82.4 – 97.4)	0.00 ($\chi^2 = 21.0$)	14.5 (3.9 – 53.4)
	Wanle Weyn	46	28	60.9 (45.4 – 74.9)	0.18 ($\chi^2 = 1.4$)	1.9 (0.6 – 5.8)
	Afgoye	18	8	44.4 (21.5 – 69.2)		
Body condition	Poor	19	18	94.7 (73.9 – 99.9)	0.001 ($\chi^2 = 6.8$)	10.2 (1.2 – 81.3)
	Moderate	39	32	82.0 (66.5 – 92.5)	0.03 ($\chi^2 = 3.9$)	2.6 (1.0 – 6.7)
	Good	69	44	63.8 (51.3 – 75.0)		
Age	>5	66	48	72.7 (60.4 – 82.9)	0.27 ($\chi^2 = 1.1$)	0.3 (0.0 – 2.9)
	2-5	52	38	73.1 (58.9 – 84.4)	0.29 ($\chi^2 = 1.0$)	0.3 (0.0 – 3.0)
	<2	9	8	88.9 (51.8 – 99.7)		

Triple infections were most common, with a prevalence of 81/114 (71.1%, 95% CI: 61.8 – 79.2). (Table 3). IgG antibodies to only *A. marginale, B. bovis,* and *B. bigemina* were

detected in 15/114 (13.2%, 95% CI: 7.6 – 20.8), 1/114 (0.09%, 95% CI: 0.02 – 4.8), and 1/114 (0.09%, 95% CI: 0.02 – 4.8) cattle, respectively (Figure 1).

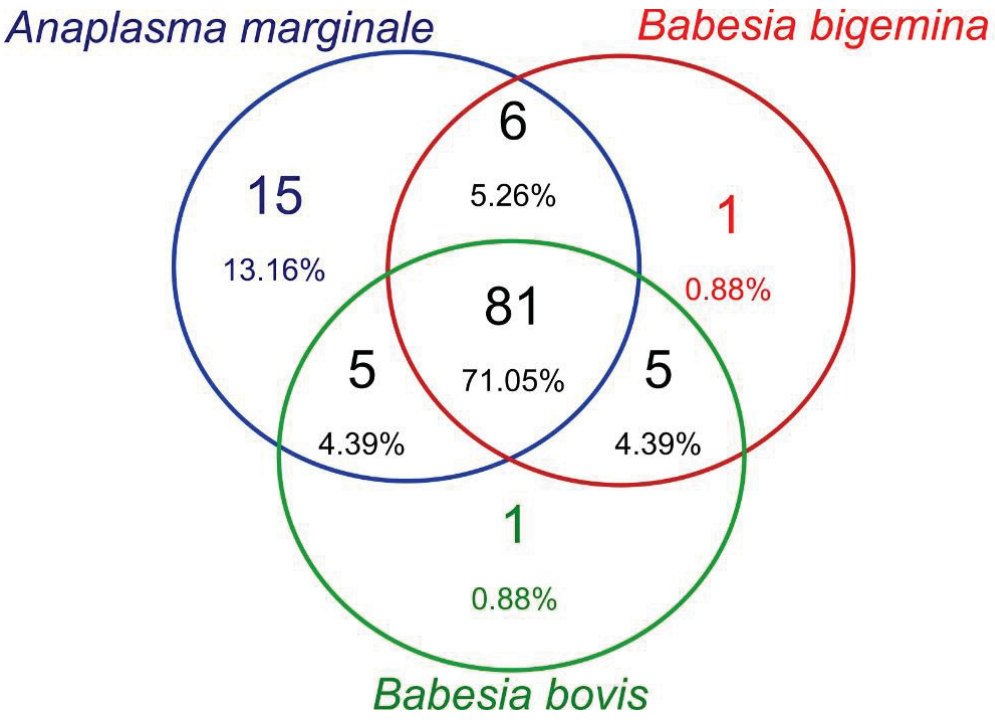

Figure 1 Single and coinfections (n = 114)

4.5 DISCUSSION:

To the author's knowledge, this study was the first of its kind since the civil war 1990s to use a serological technique for the detection of *Anaplasma marginale*, *Babesia bovis,* and *Babesia bigemina* in cattle and assess these results for potential associations with epidemiological data collected from cattle in Somalia. This was done to improve the results from previous studies in Somalia where only microscopic and serological techniques were used to determine the prevalence of anaplasmosis and babesiosis infecting cattle.

This study provided a preliminary assessment of serum antibody prevalences to *A. marginale, B. bovis*, and *B. bigemina* infections in three districts of the Lower Shabelle region, Somalia. Cattle in the traditional mixed farming system in the Lower Shabelle region showed evidence of varied exposure to the TBD infections studied.

In the present study, only 30 cattle were found infested by ticks. The absence of ticks on the majority of sampled animals may indicate the time of sampling, which was the dry season, and it is characterized by high temperature and lack of rain that directly impact the population of ticks and flies (Ogden and Lindsay 2016). Six tick species which includes *Rhipicephalus pulchellus, Amblyomma gemma, Amblyomma lepidum, Hyalomma marginatum, Hyalmomma rufipes,* and *Rhipicephalus pravus* were identified in the present study. The identified tick species were known to be present in different areas of Somalia (Pergam, 1976; Schoepf et al., 1984; Eschborn, 1993).

Herein, overall 85 %, 73.2%, and 74% of cattle from the Lower Shabelle region of Somalia were positive for *A. marginale, B. bovis*, and *B. bigemina* antibodies, respectively. Interestingly, the prevalence found in this study was higher than previous studies on cattle from Somalia, which have shown prevalence rates ranging from 0.8% to 72.4% of *Anaplasma* spp. infection, and lower than prevalence rates ranging from 0% to 91.3% of *Babesia* spp. infection by microscopical and serological techniques (Heuer et al., 1990a; Osman et al., 2020a,b; Caille, 1987; Schoepf et al., 1984). The difference in prevalence may be due to variation in climate, study design, cattle management system, ectoparasite control measures, and laboratory protocols (Hove et al., 2018; Kamani et al., 2022).

In the present study, cattle were more exposed to *A. marginale* compared to *B. bigemina* (74%). Both *A. marginale* and *B. bigemina* parasites are vectored by the same tick (*R. decoloratus*). The distribution of *B. bigemina* in cattle can only closely correlate to the vector ticks by absolute biological necessity unless the cattle are moved to new locations where the tick can survive. In this study, the higher *A. marginale* sero-occurrence could most likely be due to transmission dynamics of *A. marginale* by biting flies as well as ticks (Potgieter and Stoltsz, 2004).

With the assumption that endemic stability and instability are characterized by the high and low prevalence of serum antibodies to TBD infections (>70 and <30%, respectively) (Norval et al. 1992), the overall findings in this study suggest an indication of endemic stability for both *A. marginale, B.bovis,* and *B. bigemina* infections. However, this endemic stability results from a complex interaction of several factors, including zebu cattle's high innate resistance to TBD infections, their ability to develop immunity to TBD infections quickly and effectively, suitable ecological factors for vectors, and regular transmission to the host population, which boosts immunity on a regular basis (Norval et al. 1992).

A higher prevalence of both infections (anaplasmosis and babesiosis) was observed among <2 years cattle compared to the 2-5 years and >5 years ones; this is in accordance with Swai et al. (2013), who observed that the prevalence of *A. marginale* decreases with age, and Amorim et al. (2014), Ola-Fadunsin et al. (2018), and Fereig et al., (2017), who identified that young animals are more susceptible to babesia infection when compared to **the** adult. However, in contrast, Atif et al. (2018), Wesonga et al. (2017), Abdela et al. (2018), and Urdaz-Rodriguez et al. (2019) indicated that the seroprevalence of *A. marginale* increased with age. The measure of seroprevalence, however, must be treated with caution as it is not an indicator of active infection, and it is expected that the older animals will acquire antibody titers over prolonged periods of exposure.

In the present study slightly higher infection rate was recorded in poor body conditions compared to the moderate and good body conditions of the study animals. A similar observation has been reported by other scholars (Sitotaw et al., 2014; Hamsho et al., 2015; Wodajnew et al., 2015, Abdela et al., 2018). This difference could be because animals with poor body conditions have lower immunity which encourages infection by different organisms like *Anaplasma* and *Babesia*. However, further longitudinal studies are needed

to determine whether the body condition is the consequence of the disease or predisposing factors.

In conclusion, this study shows that *A. marginale*, *B.bovis,* and *B. bigemina* are endemic among cattle in the Lower Shabelle region, Somalia. To our knowledge, this is the first study to confirm infections with *A. marginale, B.bovis,* and *B. bigemina* in Somali cattle using serological techniques since Civil war 1990s. Our data indicate the disease is endemic in the country, and effective control strategies are necessary to reduce economic losses, as the country is moving towards self-sufficiency in livestock production for meat, dairy, and related products. Further large-scale studies and sustainable control programmes against T&TBDs are needed in the country.

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4.7 Appendix:

Figure 2 *Rhipicephalus pulchellus*(Male and Female)

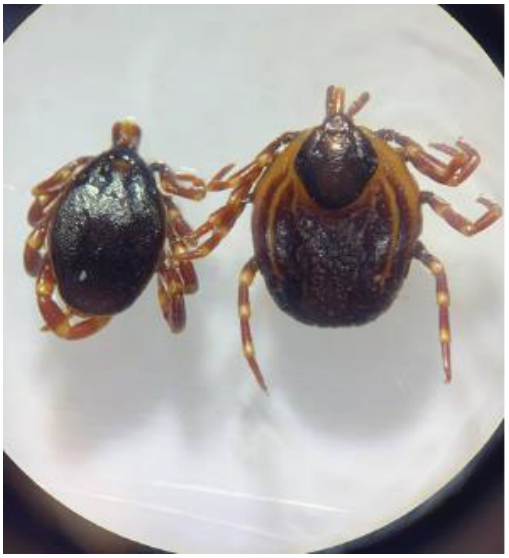

Figure 3 *Hyalomma marginatum* (Male and Female)

Figure 4 *Amblyomma lepidum* (Male and Female)

Figure 5 *Hyalomma Rufipes* (Female)