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Prevalence of Bovine Babesiosis, Associated Risk Factors and Tick Species Identification in Galka-Ayo District, Somalia

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ABSTRACT

A cross-sectional study was conducted from June 2021 to November 2021 with the objectives to estimate the prevalence bovine babesiosis and identify the associated potential risk factors and tick species involved in the transmission of this disease in Galka-Ayo district, Somalia. A random sampling technique was employed for selecting the sampling units and the logistic regression analysis was used to determine the association of hypothesized risk factors with positivity for bovine babesiosis. A total of 348 blood samples were collected and examined for the presence of bovine babesiosis by Giemsa staining technique. An overall 11.2% (43/384 babesiosis prevalence was estimated. Except for age and sex, body condition, village and tick infestation showed statistically significant associations (P<0.05) with the occurrence of the disease. The highest prevalence of bovine babesiosis was recorded in Roox (16.94%) and lowest in Agaran (2.70%) villages. However, there was a statistically significant variation in positivity of bovine babesiosis between different localities (P=0.001). According to multivariate logistic regression analysis, the odd of bovine babesiosis occurrence in medium and poor body condition animals were 3.19 and 2.73 times more likely than in good body condition animals, respectively. This difference was found to be statistically significant (P=0.015). A higher prevalence of babesiosis was found in tick-infested (13.91%) than in non-infested cattle (5.08%) and this difference was statistically significant (P=0.001). The mean ± Standard deviation packed cell volume (PCV) was 23.56 ± 4.465 for the overall study animals, 20.56 ± 3.896 for the infected and 24.47 ± 4.429 for the non-infected cattle. There was statistically significant difference in mean PCV value between infected and non-infected cattle (P=0.02). In conclusion, implementing surveillance systems, raising cattle owners' awareness on tick-borne diseases, further future studies using more refined serological and genetic techniques, maintaining enzootic stability, and enhancing pre-munity by improving cares given to calves is essential.

INTRODUCTION

The cattle in Somalia are predominantly of the East African Shorthorn (thoracic humped) Zebu type among which the well-recognized types are Gassara, Dawara, Surqo and Boran breeds. There are some cattle that have the characteristics of cross-breed between the East African Short Horn and the cervicothoracic humped Ethiopian cattle, in that a Surqa breed is a representative of this cross-bred type. In 2013, Somalia had an estimated cattle population of over 5.1 million heads of cattle economic importance for Somalia lies in food safety and milk production. The average estimated milk production per cow is two liters per day with an estimated gate farm price of 1 USD (Ibrahim *et al.*, 2020). Generally, the productivity of livestock in Somalia is low, however, it is possible to increase the situation through improved management practices (Knips, 2004). There are a lot of diseases that may decrease the production and productivity of livestock in Somalia these diseases include babesiosis so is one of the important animal diseases worldwide that can cause a lot of production losses in grazing animals. Haemoparasitic infections are major public health, veterinary and socio-economic problems in African countries including Somalia, where they impose a burden on the healthcare infrastructure of both animals and animal handlers in endemic areas (Ministry of Livestock, 1991).

Bovine babesiosis is a disease that commonly infect cattle, sheep, goats, horses, pigs, dogs and cats and occasionally man. It is a hemiparasitic disease which is considered as the second most common tick-borne parasite of mammals after trypanosome. Babesia bovis and B. bigemina are the main species affecting cattle widely distributed throughout of Somalia and other tropical and subtropical countries they are responsible for high mortality rates up to 50% in susceptible herds and it's known to be transmitted in this country by Rhipicephalus (Mohammed & Elshahawy, 2020). Animals suffering from acute babesiosis shows symptoms such as fever, oculo-nasal discharge, increased heart rate, increased respiratory rate, abnormal mucous membrane color, and low packed cell volume (PCV) values. Although these symptoms are very typical, they are not pathognomonic and animals with chronic infections can be asymptomatic carriers (Abdela *et al.*, 2018). Disease signs vary in severity from silent infection to acute circulatory shock with anemia, depending on susceptibility, immunity, and age of the host and Babesia species and parasite load and bovine Babesia is principally maintained by sub-clinically infected cattle that have recovered from disease (Disassa

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et al., 2015). Ticks are undoubtedly the most important economically ectoparasites of livestock on a global scale (Kumar *et al.*, 1992). Ticks are obligate, blood-feeding ectoparasites of vertebrates particularly mammals and birds (Wall & Shearer, 2001). The importance of ticks is principally due to the ability to transmit a wide spectrum of pathogenic microorganisms, such as protozoa, rickettsiae, spirochaetes, and viruses (Jongejan & Uilenberg, 1994). The main effect of tick infestation in cattle is mild to severe anemia, loss of appetite, leading to a reduction in growth rate and decreased productivity and tick infestation also results in increased calf mortality (Mohsen *et al.*, 2013). Additionally, ticks are responsible for direct damage to the cattle through their feeding habits. The damage manifested as hiding damage, damage to udders, teats, and scrotum, myiasis due to infestation of damaged sites by maggots, and secondary microbial infections, it may also lead to skin rejection at tannery factories (Urquhart *et al.*, 1996). Ticks which are considered to be most important to the health of domestic animals in Africa comprise about seven genera. Among these ticks, generally, the main ticks found in Somalia include Amblyomma, Boophilus, Haemaphysalis, Hyalomma, and Rhipicephalus. Specifically, Boophilus and Rhipicephalus are factor for bovine babesiosis (Kassa, 2005).

This disease causes economic loss towards livestock production and it has a high mortality rate, not only from mortality or production losses but also through its impact on the international cattle market, severe illness, weight loss, and pregnant cows may cause problems (Annetta, 2003). And despite the widespread distribution of various tick species across Somalia, particularly Galka-Ayo district, little is known about the spread of harmful germs and parasites carried by ticks, such as babesiosis. Despite their important role in disease transmission, no research on these diseases has been conducted, and none have been enabled to analyze the association of distinct tick species with disease occurrence. Thus, there is paucity of information on bovine babesiosis at a regional level and in particular Galka-Ayo district. In order to address the above problems, a study is required to fill the gaps in knowledge about bovine babesiosis and its vectors, as well as to create baseline information that can be used to develop efficient disease control and prevention methods. Therefore, the objective of this study was quantifying the Prevalence of bovine babesiosis, associated risk factors and to determine the packed cell volume (pcv) of infected and non-infected cattle in Galka-ayo District, Somali.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in Galka-Ayo district of Mudug Region of Puntland state of Somalia (Figure 1). Galka-Ayo is situated in the north-central part of Somalia and is one of the most developed towns in the region and also is the regional capital city. Galka-Ayo district is located 750 km north of Muqdisho capital city of Somalia, in the west with bordered by the Somali Regional State of Ethiopia and in the East Indian Ocean. Geographically Galka-Ayo is divided into four main quarters like Garsoor, Israac, Horumar, and Wadajir. Puntland State controls Israac, Garsoor, and Horumar to the north while Galmudug state controls the Wadajir village to the south, the city has grown considerably in recent times and serves as a commercial hub. The climate is generally semi-arid with annual average rainfalls ranging from 200 mm to 300 mm with bimodal pattern. The average temperature is 32.7°C and the altitude of the area is 302 meter above sea level (Muchiri, 2007). According to the UNDP it has a human population of 750,667 in 2018. Livestock herding is a prevalent profession of the rural population with free-range pastoralist and agro-pastoralist management as the most dominant production system.

Study Design

A cross-sectional study was conducted from June 2021 to November 2021 to estimate the prevalence of bovine babesiosis, identify associated risk factors and tick species of identification in Galka-Ayo district.

Sampling Method and Sample Size Determination

Indigenous cattle of different sex and age groups, body conditions, kept under extensive and semi- intensive management system was used for this study. The sampling method employed in this study was a three-stage sampling method. The primary study units were villages and accordingly three villages were selected based on convenience sampling strategy cattle population, the willingness of pastoralists, and accessibility to vehicles. As secondary stages, herds of cattle residing within the selected villages were considered and from Godod village 11 cattle herds, Agaran 11 cattle herd and Rooh 10 herd cattle were chosen randomly. Respectively, finally, from each herd animals were selected and included for the study. From each cattle herd 12 animals randomly, selected. To determine the sample size of bovine babesiosis, an expected prevalence of 50% was taken, since no previous study was done in this area.

The required samples for the study were calculated according to the formula given by (Thrustfield, 2007). With 95% confidence level and 5% absolute precision. Therefore, a sample size of 384 was considered for this study.

n = 1.962 Pexp (1-Pexp)/d2

Where,

n = required sample size,

Pexp =expected prevalence,

d = required precision

Data Collection

During each field work, the selected herds were visited for sample collection. Simultaneously, the breed, sex, age, body condition score of the sampled animal were recorded in a format prepared for this purpose. The body condition score determined based on anatomical parts and flesh fat cover at different parts (Nicholoson and Butterworth, 1986). Animals were conveniently classified as young (<3 years), adult (4-6 years) and old (> 7 years) age categories as described by Delahunta and Habel (1986).

Blood Sample Collection and Transportation

The whole blood samples were collected from the jugular vein according to the OIE method (OIE, 2010). Approximately, 3-5 ml of blood was taken using labeled disposable syringe after disinfecting the site of injection. Immediately, the blood was transferred into test tubes containing Ethylene Diamine Tetra-acetic Acid (EDTA) (Nayel *et al.*, 2012). After labeling, the collected blood samples were transported to Galka-Ayo Research Laboratory with an icebox, where thin blood smears preparation and PCV determination were performed.

Collection and Transportation of Ticks

Collections of ticks were conducted after restraining of the animals. The ticks were manually collected from the body surface by using hands from their attachment sites. And examined for the presence ticks on five regions of the body surface, namely; head, neck, thoracic, abdominal and back region (tail region and udder/scrotum), both on the right and left sides. The collected ticks were preserved in labeled plastic containers containing 70% ethanol. After labeling the universal bottles collected ticks were transported to Galka-Ayo Research Laboratory, while other data were written on a specified register format prepared for this particular purpose (date, address, sex, age, and body condition).

Laboratory Procedures

Blood Film Examination

Giemsa staining procedures and microscopic examination of dry glass slides with blood on the clean slide at the angle of 45° and then gently moving forward as described by Kessell (2015). After labeling of the slides with sample code, the thin smear was air-dried and fixed with absolute methyl alcohol for 10 minutes. Staining was achieved by applying Giemsa stain 10% for 30 minutes. Finally, the smear was washed by tap water to remove extra stain and slides were examined microscopically using an oil immersion lens (×100) of a light microscope according to (Zafar *et al.*, 2006). The parasites species were identified according to the characters distinguished by their size, shape, position and location described by Soulsby (1982).

Determination of Packed Cell Volume

Packed cell volume was measured using the microhaematocrit centrifugation technique as described by Brar *et al.* (2011). The capillary tubes were filled in blood up to three-quarters of the microhaematocrit capillary tubes. One end of these tubes was sealed with a crystal seal and then placed into a micro-haematocrit centrifuge for five minutes at 1200 rpm. The capillary tube was removed from the centrifuge, placed on a haematocrit reader and the PCV was recorded. Packed cell volume value of <24 was considered anemic, whereas a value of ≥ 24 was regarded normal (Kessell, 2015).

Tick Identification

The ticks were placed onto petri-dishes and examined under stereomicroscope to identify and differentiate at the tick species level with main identification features which include color, size and shape of mouthparts, scutum, anal groove, festoon, punctuation, and legs of the ticks according to Walker *et al.* (2003) and Onkello-Onen *et al.* (2006).

Data Management and Analysis

The data obtained from the field were recorded in a notebook and later stored and managed in Microsoft Excel spreadsheet 2013 and transferred to Statistical Package for the Social Sciences (SPSS) version 20.0 for analysis. The prevalence of bovine babesiosis was calculated as a proportion of affected animals out of the total animal examined. The association between bovine babesiosis and localities was analyzed using chi-square. Using the logistic regression model, the correlation of bovine babesiosis with various independent factors (age, sex, body condition, district, and tick infestation) was analyzed. Univariable logistic regression analysis was performed and odds ratios (OR) and CI 95% (95% confidence intervals) were used to evaluate the association of strength between risk factors and positivity for bovine babesiosis. Potential risk factors with P≤0.25 in the univariate analysis were subjected to multivariable logistic regression analysis. The student's t-test was employed to compare the mean PCV of the infected and non-infected animals. Furthermore, the Spearman correlation coefficient was used to analyze the relationship between different tick species and positivity for bovine babesiosis. The confidence level (CL) for all analyses was set at 95%, while the significance level was set at P≤0.05.

RESULTS AND DISCUSSION

Prevalence of Bovine Babesiosis According to Localities

A total of 384 blood samples were collected and examined using a thin blood smear and an overall prevalence of bovine babesiosis 11.2% (95% CI: 8.2% - 14.8%) was recorded in the study areas. Table 1 shows the prevalence of bovine babesiosis in the study area by locality. The prevalence in each village (subdistrict) was determined to be 12.75% in Godod, 16.94% in Roox and 2.70% in Agaran of Galka-Ayo District. The highest prevalence of bovine babesiosis was recorded in Roox (16.93%) and the lowest in Agaran (2.70%) villages. However, there was a statistically significant association between bovine prevalence positive and localities (Fischer's exact value =12.52, P=001).

Table 1: Prevalence of bovine babesiosis according to localities

Locality	Number examined	Number positive	Prevalence (%)	Fischer's exact value	p-value
Agaran	111	3	2.70	12.52	0.001*
Godod	149	19	12.75		
Roox	124	21	16.94		
Total	384	43	11.2		

**Significant*

Risk Factors for Bovine Babesiosis

In univariable logistic regression, risk factors such as age, sex, body condition, district, and tick infestation were analyzed. Among these factors, body condition, district, and tick infestation were found to be significantly associated with positivity for Babesia infection ($P<0.05$). On the other hand, age and sex were found to have non-significant ($P>0.05$) effects on the occurrence of these infections. However, all risk factors in the initial univariate analysis were passed to the final multivariable logistic regression model to control other confound factors.

Based on the sex of the study animals, the prevalence of bovine babesiosis was determined to be higher in males (12.07%) compared the females (10.82%). The odd of occurrence of babesiosis in males were 1.13 times more likely than in females. However, there was no statistically significant difference between the two groups female and male (AOR=1.14; CI=0.55-2.24; P=0.731) (Table 3). This finding is not in- line with the report of Kocan *et al.* (2010) who found a higher prevalence of babesiosis in females 11.2% compared to male cattle 6.96%. Moreover, the higher prevalence of tick-borne diseases in male animals may be due to the fact that male animals are subjected to trek long journey for drought purposes and stressful work that suppress the immune system of the animals.

Old (12.43%) and young (12%) animals had the highest prevalence of bovine babesiosis than the adult (9.55%) animals, which were 1.47 and 1.12 times more likely to be positive for babesiosis than young animals, respectively. This difference was not statistically significant (AOR=1.47; 95% CI=0.23-1.98; P=0.478) (Table 3). This result was not in the line with the finding of Abdela *et al.* (2017), from Ethiopia who reported the highest prevalence in adult animals (7.5%) followed by young animals in (3.3%) while the lowest was found in old animals (2.5%) and Ayaz *et al.* (2014) in Pakistan who reported in young animals with 14.4% followed by adult animals, 13.7% while the lowest was found in old animals (6.8%). However, the results of this study were concurring with Amorim *et al.* (2014) from Brazil who reported a high prevalence in young 5.4% followed by adult animals with 4.3% while the lowest was found in old animals.

The prevalence of bovine babesiosis was significant (P=0.015) based on the body condition score of the study animals. The highest prevalence was recorded in animals with a poor body condition (14.16%) followed by medium body conditioned animals were (13.89%) and the lowest in animals with good body condition scores were (5.51%). According to multivariable logistic regression analysis, the odd of babesiosis occurrence in medium and poor animals were 3.19 and 2.73 times more likely than good animals positive for babesiosis, respectively.

Regarding the prevalence of babesiosis in tick-infested and non-tick-infested cattle the higher prevalence was found in tick-infested (13.91%) cattle than non-tick infested (5.08%) cattle. The risk of occurrence of babesiosis in tick-infested cattle was 4.64 times more

Likely than non- tick- infested cattle. This difference was statistically significant (AOR=4.64; 95% CI=1.85-11.62; P=0.001) (Table 3).

Table 2: Univariable logistic regression analysis of risk factors associated with bovine babesiosis

Risk factors	Categories	Number examined	Number positive	Prevalence (%)	COR (95%CI)	P-value
Village	Agaran	111	3	2.70	Ref.	
	Godod	149	19	12.75	5.26(1.52-18.26)	0.009*
	Roox	124	21	16.94	7.34(2.13-25.35)	0.002*
Sex	Female	268	29	10.82	Ref.	
	Male	116	14	12.07	1.13(1.75-2.23)	0.722
Age	Young	50	6	12	Ref.	
	Adult	157	15	9.55	1.30(0.28-2.12)	0.619
	Old	177	22	12.43	1.04(0.40-2.73)	0.935
Body condition	Good	127	7	5.51	Ref.	
	Medium	144	20	13.89	2.76(1.13-6.76)	0.026*
	Poor	113	16	14.16	2.83(1.12-7.15)	0.028*

Tick infestation	Absent	118	6	5.08	Ref.	
	Present	266	37	13.91	3.02(1.24-7.36)	0.015*

**Significant; COR=Crude odd ratio; CI=Confidence Interval; Ref=reference categories*

Table 3: Final multivariable logistic regression model output of factors associated with bovine

Risk factors	Categories	Number examined	Number positive	Prevalence (%)	AOR (95%CI)	P-value
Village	Agaran	111	3	2.70	Ref.	
	Godod	149	19	12.75	6.22(1.75-22.12)	0.005*
	Roox	124	21	16.94	10.96(3.09-38.85)	0.001*
Sex	Female	268	29	10.82	Ref.	
	Male	116	14	12.07	1.14(0.55-2.34)	0.731
Age	Young	50	6	12	Ref.	
	Adult	157	15	9.55	1.47(0.23-1.98)	0.478
	Old	177	22	12.43	1.12(0.32-2.51)	0.831
Body condition	Good	127	7	5.51	Ref.	
	Medium	144	20	13.89	3.19(1.25-8.13)	0.015*
	Poor	113	16	14.16	2.73(1.02-7.29)	0.045*
Tick infestation	Absent	118	6	5.08	Ref.	
	Present	266	37	13.91	4.64(1.85-11.62)	0.001*

Tick Identified and Association between Bovine Babesiosis

Tick infestation was found in 266 (69.27%) of the animals studied. A. cohaerens (52.86 %), A. variegatum (71.87%), R. (B.) decoloratus (45.1 %), and R. everetsi (16.4 %) were recognized as tick species belonging to the Amblyomma and Rhipicephalus genera. Using Pearson Chi-square analysis has been utilized to measure of the association between the bovine babesiosis and different tick species. The Chi-square analysis has showed there was association between presence A. cohaerens on the animal and prevalence bovine babesiosis (p=0.001). According, A. variegatum and prevalence bovine babesiosis there was no association between. Furthermore, and also there was relationship between R. (B.) decoloratus and prevalence bovine babesiosis (p=0 .001)

Table 4: Association between bovine babesiosis and different tick species

Tick species	Categories	Positive	Negative	Number examined	χ^2 -value	P-value
A. cohaerens	Present	28	175	203	10.40	0.0001*
	Absent	15	166	181		
A. variegatum	Present	31	245	276	0.001	0.973
	Absent	12	96	108		
R. decoloratus	Present	30	143	173	11.95	0.001*
	Absent	13	198	211		
R. evertsi	Present	16	47	63	15.28	0.001*
	Absent	27	294	321		

**Significance, A= Amblyomma, R= Rhipicephalus*

Association of Bovine Babesiosis with Packed Cell Volume (PCV)

In the compare mean analysis, the overall mean± Standard deviation PCV of the study animals was 23.56 ± 4.465 (Table 5). Using the PCV value cut off of 24% (Kessell, 2015) the mean PCV value for the infected cattle was 20.56 ± 3.896 SD while the mean PCV value for non-infected cattle was 24.47 ± 4.429 SD. There was statistically significance mean PCV value of infected and non-infected animals (P=0.02), (Table 5).

Table 5: The mean and standard deviation of packed cell volume of infected and non-infected cattle

Disease	Condition	Number of Cattle	Mean PCV	SD	P-value
Babesiosis	Infected	43	20.56	3.896	0.02*

	Non-infected	341	24.47	4.429	
	Total	384	23.56	4.465	

SD = Standard deviation; PCV=Packed cell volume

CONCLUSION

In conclusion, the present study provides basic information on the prevalence and associated risk factors of bovine babesiosis in correlation with vectors identification in Galka-Ayo district. The study revealed prevalence of bovine babesiosis (11.19%) by the Giemsa staining technique. The main risk factors found to be significantly associated with bovine babesiosis were body condition, district, and tick presence on the animals. The main tick species whose presence was found to be significantly correlated with positivity for bovine babesiosis were A. cohaerens and A. variegatum. Moreover, the current low awareness or knowledge of the livestock owners about the diseases transmitted by ticks could be attributed to a lack of treatments and a shortfall of control strategies in animals and resulting in significant economic loss and increased occurrence of the diseases. In order to minimize losses attributed to ticks and tick-borne disease in the area strategic tick control techniques should be implemented, as it is a level of control that prevents ticks from becoming a nuisance.

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