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Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia

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ABSTRACT

Among tick-borne diseases, bovine anaplasmosis and babesiosis are considered to be one of the most important in ruminants worldwide, causing significant economic losses in tropical and subtropical areas. This cross-sectional study was therefore undertaken from November 2016 to April 2017 with the objectives to assess the prevalence and potential risk factors associated with bovine anaplasmosis and babesiosis and also to identify the vectors involved in transmission of these diseases in and around Jimma town, south western Ethiopia. A simple random sampling technique was employed for selecting a sampling unit and logistic regression was used to determine the association of hypothesized risk factors with positivity for bovine anaplasmosis and/or babesiosis. A total of 408 bovine blood samples were examined for the presence of either anaplasmosis or babesiosis by Giemsa staining technique and overall prevalence of 11.7% babesiosis and 6.1% anaplasmosis were determined. Two *Babesia* species (2.2% *Babesia bovis* and 9.8% *B. bigemina*) and two anaplasma species (5.1% *Anaplasma marginale* and 1.2% *A. centrale*) were identified. Even though risk factors like age, body condition, management system, sex and presence of ticks were considered, only age ($p = 0.006$) and body condition ($p = 0.039$) were found to be significantly associated with anaplasmosis. Moreover, multivariable logistic regression analysis showed statistically significant association of babesiosis with age ($p = 0.003$), body condition ($p = 0.012$) and presence of ticks ($p = 0.005$). For both infections the mean PCV of infected animals was significantly ($p < 0.05$) lower than non-infected animals. Similarly, the mean body temperature of infected animals was significantly ($p < 0.05$) higher than non-infected animals. The overall 70.8% infestation of cattle with four tick species, namely *Amblyomma cohaerens* (58.5%) and *A. variegatum* (44.1%), *Rhipicephalus (Boophilus) decoloratus* (50.5%) and *R. evertsi evertsi* (12.9%) were recorded. A significant positive correlation was observed between the presence of *R. evertsi evertsi* ($p = 0.000$) and *R. (B). decoloratus* ($p = 0.000$) on the animals and positivity for bovine anaplasmosis. Besides, *R. (B). decoloratus* was found to be the only tick species which its presence on the animal was significantly correlated ($p = 0.000$) with babesiosis positivity. Conclusively, the study revealed a moderate prevalence of bovine anaplasmosis and babesiosis in the study area which need further investigations using modern serological and molecular techniques for the identification of the carriers the infections and identification of the potential vectors.

1. Introduction

Ethiopia is a resourceful country bestowed with estimated largest livestock population in Africa with cattle being the dominant livestock species accounting for approximately 58 million heads (CSA, 2016). The Livestock sector has a significant role in socioeconomic activity of the country and contributes much to the national economy which can be estimated at 19% of the total gross domestic product (GDP), 45% of the agricultural GDP and about 20% of the country's export earnings (Behnke and Metaferia, 2011). Furthermore, livestock industry is considered as a priority sector for poverty alleviation and there is increasing demand for livestock products from national and regional markets (LMP, 2014). However, development of this sector is hampered by different constraints. The most important bottlenecks are widespread endemic diseases including viral, bacterial, and parasitic infestation (Leta and Mesele, 2013; Abdela, 2017). Besides, lack of appropriate disease control policy, lack of appropriate veterinary services and lack of attention from government is also another constrains (Leta and Mesele, 2013). Of health problem ticks and tick-borne diseases are widely distributed and contribute to important economic losses (Kumsa et al., 2014).

Ticks and tick-borne diseases affect the productivity of bovine in

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tropical and subtropical parts of the world including Ethiopia and leads to a significant negative impact (Sitotaw et al., 2014; Abdela, 2016; Abdela and Bekele, 2016; Abdela and Jilo, 2016). Globally, four main tick-borne diseases (TBDs), namely anaplasmosis, babesiosis, theileriosis, and cowdriosis (heartwater) affect bovines (Jabbar et al., 2015). Among these anaplasmosis and babesiosis are important diseases with greater economic importance in tropical and subtropical regions (Filia et al., 2015; Constable et al., 2017). Babesiosis is a haemoprotozoan and anaplasmosis is a haemobacterial infection of cattle (Dumler et al., 2001). Both diseases have a serious economic impact due to obvious reason of morbidity, mortality and lowered working efficiency (Rahman et al., 2015) and have been reported in Ethiopia (Feleke et al., 2008; Sitotaw et al., 2014; Zerihun et al., 2014; Hamsho et al., 2015; Wodajnew et al., 2015).

The primary causative agent of bovine anaplasmosis is *Anaplasma marginale* and to a lesser extent, by *A. centrale*, a Gram-negative obligate intracellular bacteria parasitizing erythrocytes (Jabbar et al., 2015; OIE, 2015). Almost 20 tick species have been shown to transmit anaplasmosis experimentally (Kocan et al., 2004), and the most important tick genera are *Hyalomma* and *Rhipicephalus* species (Camus et al., 2010), which are widespread in Ethiopia (Sileshi et al., 2007; et al., 2010; Tesema and Gashaw, 2010). The most common causative agent of bovine babesiosis is hemoprotozoa *Babesia bovis* and *B. bigemina*. The principal vectors for this disease is Rhipicephalus (Boophilus) species (OIE, 2013) which are also common in Ethiopia including Jimma (Abebaw, 2004). Animals suffering from acute babesiosis or anaplasmosis can have a variety of symptoms such as fever, oculo-nasal discharge, increased heart rate, increased respiratory rate, abnormal mucous membrane colour, and low PCV values. Although these symptoms are very typical, they are not pathognomonic, and animals with chronic infections can be asymptomatic carriers (Kocan et al., 2010; El-Ashker et al., 2015).

Both anaplasmosis and babesiosis are not well studied in Ethiopia. However, from central Ethiopia recently Sitotaw et al. (2014) reported 1.6% *A. marginale* and 0.3% *A. centrale* using microscopic examination. Recently microscopic examination by Zerihun et al. (2014) reported 5.9% of *Anaplasma* spp. in Illubabor zone, Western Ethiopia. Babesiosis is also another most important TBDs disease in Ethiopia that occurs sometimes in acute forms with serious recognized clinical manifestations (Wodajnew et al., 2015). Recently microscopic examination by Hamsho et al. (2015) and Wodajnew et al. (2015) reported an overall prevalence of 16.9% and 1.5%, respectively.

Despite the widely distribution of several tick species in all agro-ecological zones of Ethiopia (Abera et al., 2010; Tomassone et al., 2012; Ayalew et al., 2014), including Jimma (Abebaw, 2004; Yitbarek, 2004), little is known about the occurrence of tick-borne pathogenic bacteria and parasite like anaplasmosis and babesiosis. A few studies undertaken on these diseases were also failed to assess the correlation of different tick species with disease occurrence despite its significant role in disease transmission. Thus, there is scarcity of information on bovine anaplasmosis and babesiosis at national level and in Jimma in particular. In view of addressing the problem, the objectives of the present research is to bridge the information gap on bovine anaplasmosis and babesiosis and their vectors helping to generate base line data that may assist for designing effective disease control and prevention strategies.

Therefore, the objectives of this study were:

- To determine the prevalence and risk factors associated with bovine anaplasmosis and babesiosis in and around Jimma town.
- To identify different tick species associated with occurrence of bovine anaplasmosis and babesiosis in and around Jimma town.

2. Materials and methods

2.1. Study area

The study was conducted from November 2016 to April 2017 in and around Jimma town which is the town is located in the south western part of the Ethiopia in Oromia regional state (Fig. 1). Jimma town is found at distance of about 352 km from Addis Ababa, the capital city of Ethiopia. Geographically, it is located at 7°13′ and 8°56′N latitude and 35°52′ and 37°E longitude. The area has an altitude ranging between 880 and 3358 m above sea level. The annual rainfall is ranging between 1200 mm–2000 mm; and the annual temperature of the area ranges 7 °C–30 °C. Farmers in the area practices mixed crop-livestock agriculture. The zone is one of the major coffee growing areas in southwest part of Ethiopia. Furthermore, the zone is well known by livestock production which can be estimated at about 2,212,962 cattle, 866,561

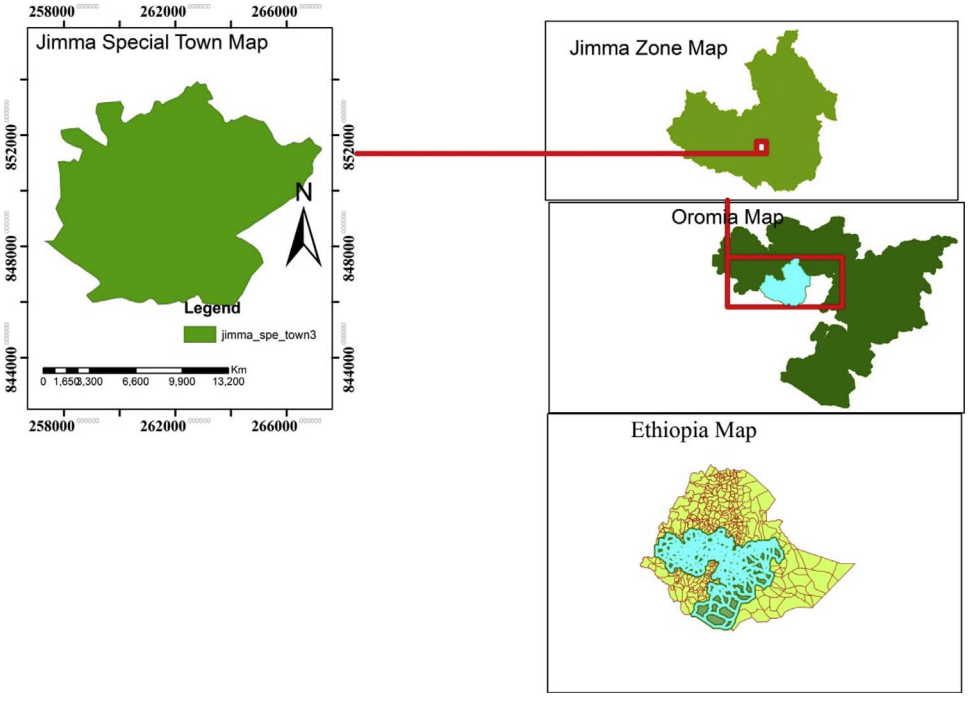

Fig. 1. Map of the study area.

sheep, 457,311 goats, 96,782 horses, 17,644 mules, 77,767 donkeys, 1,951,129 poultry and 546,722 beehives (CSA, 2016).

2.2. Study population

Study population comprise of cattle brought to Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) open air veterinary clinic from Jimma town and surrounding peasant association. The population consist of cattle owned by small holder farmers that are managed under extensive and semi-intensive management system. A local of cattle with different age, sex and body conditions (poor, medium and good) were included in the study.

2.3. Study design and sampling methods

A cross-sectional study was conducted from November 2016 to April 2017 to determine the prevalence and risk factors associated with bovine anaplasmosis and babesiosis in correlation with vectors identification in and around Jimma town. The study animals were selected by using simple random sampling method from animals coming to JUCAVM open air veterinary clinic regardless of their health status. Before collection of samples, relevant data such as age, sex, body condition, rectal temperature and management system were recorded. The age of the animals was determined based on owners' information and as described by De-Lahunta and Habel (1986). Animals were conveniently classified as young (≤3 years), adult (3–6 years) and old (≥6 years) age categories. Body conditions were also classified in to poor, medium and good according to body condition scoring method by Nicholson and Butterworth (1986).

2.4. Sample size determination

The required sample size for the study was determined by the formula given by Thrusfield (2005) based on the expected prevalence (50%) of anaplasmsosis and babesiosis and the 5% desired absolute precision and 95% confidence interval (CI). The following formula was used to determine the sample size.

$$N = \frac{1.96^2 \times Pexp\,(1 - Pexp)}{d^2}$$

Where, N = required sample sizeP exp = expected prevalence = 50% d = desired absolute precision = 5%1.96 = the value of z at 95% confidence interval

Accordingly, the required numbers of animals were 384. However, to increase precision the sample sizes were increased and total of 408 animals were included in the study.

2.5. Collection and transportation of blood sample

The blood samples were taken from ear vein for detection of babesiosis and from the jugular vein for detection anaplasmosis and PCV determination for each study animals. Before blood collection from ear vein, the area of puncture was cleaned, hair removed and disinfected with 70% alcohol. Then smaller marginal ear veins were punctured with sterile lancet and the first drops of blood was taken for thin smear preparation for detecting babesiosis. Furthermore, using sterile disposable needle blood samples were also collected from the jugular veins of the each study animals in EDTA containing vacutainers. After labelling the collected blood samples were transported Jimma University veterinary parasitology and pathology laboratory with ice box, where blood smears preparation and PCV determination were performed. In contrast to *B. bovis, A. marginale* does not accumulate in capillaries and the blood drawn from the jugular is suggested to be preferable (OIE, 2015). Thus, the blood drawn from jugular vein was used for preparation of thin smear for detection of anaplasmosis.

2.6. Clinical examination

With the objective of a clearer judgment of the health status of each study animal additional clinical examinations were also made. The rectal temperature was taken from each study animals that were blood sampled. Temperatures between 38.0–39 °C were considered as normal. Animals with temperatures > 39 °C were considered to have fever and those with temperatures ≤ 39.0 °C were classified as non-febrile as described.

2.7. Collection and identification of ticks

For identification of genus and species of ticks, all body surfaces particularly the known tick feeding regions of all animals that were blood sampled were thoroughly examined visually and by palpation in order to establish the presence or absence of ticks. were available up to six tick and above were randomly collected from the predilection areas by hand and using special forceps, holding the basis capitulum and screwing so as not to lose the mouthparts of the ticks. The collected ticks from each animal were placed into separate pre-labelled small plastic tubes with 70% ethyl alcohol and transported to Jimma University veterinary parasitology and pathology laboratory for identification. The ticks were identified using stereomicroscope and classified to genera levels based on size, mouthparts, presence and absence festoon, presence and absence of the eye and colour of the body. Furthermore, different morphology of tick such as shape of scutum, leg colour, body, festoon, eye shape, ventral plates and marginal spot were considered for species level identification according to Walker et al. (2003).

2.8. Preparation and microscopic examination of blood smear

Two thin blood smears were prepared for each animal using blood drawn from the ear vein and jugular vein for detection of babesiosis and anaplasmosis, respectively. The smears were prepared by applying the slide with blood on to a clean slide at an angle of 45° and then gently moving forward as described by Kessell (2015). After labelling with sample code and air-drying they were fixed in methyl alcohol (absolute methanol). Fixed slides were stained in 10% Giemsa stain for 30 min. The smears were washed with tap water to remove extra stain and air dried. A total of 816 blood smears examinations were performed with light microscope under oil immersion (100× magnifications) and blood pathogens were identified as described by OIE (2013) for *Babesia* species and OIE (2015) for anaplasma species.

2.9. Determination of packed cell volume

One of the typical symptoms of anaplasmosis and babesiosis is anaemia (OIE, 2013; OIE, 2015). Therefore, hematocrit values can serve as a reliable and significant measurement parameter for the health status of the animal. Thus, Parallel to tick identification the packed cell volume (PCV) of all study animals was determined. PCV was determined using microhaematocrit centrifugation technique as described by Brar et al. (2011). Briefly, using the blood drawn from the jugular vein the capillary tubes were filled to 3/4th of its length. One end of these tubes was sealed with crystal seal and then placed into a micro-haematocrit centrifuge for five minutes at 1200 rpm. Afterwards the PCV was determined from the reading expressed as percentage of packed red cells in the total volume of blood components with the aid of a Microhematocrit scale. A PCV value of < 24 was considered as anaemic whereas ≥24 was considered as non-anaemic as described by Kessell (2015).

2.10. Data management and analysis

The data were entered and managed in a Microsoft Excel spread sheet 2010 and analysed using statistical package for social Sciences

(SPSS) version 20. The prevalence of bovine anaplasmosis and babesiosis were determined as a proportion of affected animals out of the total animal examined. The association of bovine anaplasmosis and babesiosis with different independent variables (age, sex, body condition, management system and presence of ticks) was analysed using logistic regression. Univariable logistic regression analysis was performed and OR (odds ratios) and CI 95% (95% confidence intervals) were used to quantify the association between risk factors and positivity for bovine anaplasmosis and babesiosis. Potential risk factors with P-value of < 0.25 in the univariate analysis were checked for multi-collinearity using Kruskal gamma statistics. However, none of the variables were found to be collinear since all variables showed gamma value ranged between −0.6 and +0.6 and thus all were subjected to a multivariable logistic regression analysis. The goodness of fit of the model was assessed by Hosmer and Lemeshow test. There was insignificant difference between the observed and predicted values. The values of Hosmer and Lemeshow test for anaplasmosis and babesiosis were (χ^2 = 3.745, P = 0.879) and (χ^2 = 1.429, P = 0.985), respectively. Therefore, the model was fitted well with the data. The 95% confidence interval of OR and p-values were used to describe statistical significance associations and value of $p < 0.05$ was considered as significant. The difference between mean PCV% and mean rectal temperature of infected and non-infected cattle were compared by Student's t-test. Moreover, for assessing the correlation of different tick species with positivity for bovine anaplasmosis and babesiosis spearman correlation coefficient was employed.

3. Results

3.1. Species of bovine anaplasmosis and babesiosis identified and its prevalence

A total of 408 cattle were examined for babesiosis and anaplasmosis and overall prevalence of 6.1% (25/408) and 11.7% (48/408) were found for bovine anaplasmosis and babesiosis, respectively. Regarding the species identified *Babesia bigemina* and *Anaplasma marginale* were recorded to be the most frequently identified species with prevalence rate of 9.8% and 5.1%, respectively (Table 1). The list prevalent species found were *B. bovis* (2.2%) and *A. centrale* (1.2%). There was one animal which was positive for both *A. centrale* and *A. marginale*. Similarly the co-infection of *B. bovis* and *B. bigemina* were recorded in one animal. Moreover, the co-infection of anaplasmosis and babesiosis were detected in 2 cattle. Fig. 2 shows the photograph of some the tick borne pathogens and ticks identified

3.2. Prevalence of bovine anaplasmosis and babesiosis according to localities

Table 2 presents the prevalence of anaplasmosis and babesiosis by localities in the study area. The highest prevalence of bovine anaplasmosis was recorded in Babela kosa (8.7%), whereas the lowest prevalence was detected in Ifa bula (2.3%). However, there were no statistical significant difference between different localities and positivity for bovine anaplasmosis (χ^2 = 6.99, P = 0.637). The highest and the lowest prevalence of bovine babesiosis were recorded in Babela kosa (25%) and Frustale (3.2%), respectively. This difference was also not statistically significant (χ^2 = 13.434, p = 0.144).

Table 1
Species of TBPs identified and their prevalence.

Genus	Species	Number infected	Prevalence at species level	Over all prevalence
Anaplasma	*Anaplasma marginale*	21	5.1%	6.1%
	Anaplasma centrale	5	1.2%	
Babesia	*Babesia bigemina*	40	9.8%	11.7%
	Babesia bovis	9	2.2%	

3.3. Risk factors associated with bovine anaplasmosis and babesiosis

Different risk factors which include age, sex, body condition, management system and tick infestation were considered in univariable logistic regression analysis. Among these factors, age, body condition and tick infestation were found to be significantly associated with positivity for anaplasma and/or *Babesia* infection ($p < 0.05$). On the other hand, sex and management system was found to have non-significant ($p > 0.05$) effects on occurrence of these infections. Among the factors considered in the initial univariable analysis sex and management type were dropped from further analysis. Thus, age, body condition and tick infestation ($p < 0.25$) were subjected to final multivariable logistic regression model.

The prevalence of bovine babesiosis based on sex of the study animals was found to be insignificantly higher in male (12.1%) than female (11.4%) (COR = 0.93, CI = 0.512-1.709, p = 0.828) (Table 3). Moreover, 5.7% and 6.5% prevalence of bovine anaplasmosis was recorded in female and male, respectively. The odd of occurrence of anaplasmosis in male were 1.15 time more likely than in female. However, this difference was not statistically significant (COR = 1.15, CI = 0.51–2.60, p = 0.720).

The highest prevalence of bovine babesiosis was recorded in old (19.8%) and adult animals (9.1%) than young (3.8%) which were 4.46 and 2.22 times more likely to be positive for babesiosis than young animals, respectively. This difference was observed to be statistically significant (P = 0.012) (Table 4). Similarly, significant (P = 0.006) highest prevalence of bovine anaplasmosis was found in old animals (12.5%) followed by adult (3.2%) and least in young animals (0.9%). Multivariable logistic regression analysis showed that old and adult were 11.32 and 3.11 times more likely to be positive for anaplasmosis than young animals, respectively.

Regarding prevalence based on body condition score of the study animals, the prevalence of bovine anaplasmosis was significantly (P = 0.039) highest in poor body condition (10%) followed by medium body conditioned animals (2.6%) and the lowest in animals with good body condition score (1.7%). Similarly 3.5, 5.8 and 18.6% of bovine babesiosis prevalence was recorded in good, medium and poor body conditioned animals, respectively. The odd of babesiosis occurrence in poor and medium body conditioned cattle were 4.63 and 1.41 times more likely than good body conditioned cattle, respectively. This difference was found to be statistically significant (P = 0.003).

Univariable logistic regression analysis showed that the risk of anaplasma infection were insignificantly highest in extensively managed cattle (6.6%) than semi-intensively managed cattle (3.9%) (COR = 1.727, CI = 0.503–5.925, P = 0.385). Likewise, the highest prevalence of bovine babesiosis was recorded in extensively (12.6%) managed cattle than semi-intensive (7.8%) (COR = 1.727, CI = 0.503–5.925, P = 0.385) (Table 3).

Regarding the prevalence of anaplasmosis in tick infested and non-tick infested cattle the higher prevalence was found in tick infested (7.9%) cattle than non-tick infested (1.6%) cattle (AOR = 3.218, CI = 0.723–14.324). This variation was statistically significant during univariable logistic regression analysis (p = 0.030) (Table 5). However, during multivariable logistic regression analysis the tick presence was found have non-significant (p = 0.125) effect on occurrence of anaplasma infection in cattle (Table 6). The prevalence of babesiosis in tick infected cattle (16.2%) was significantly higher than non-tick infested cattle (0.8%). The risk of occurrence of babesiosis in tick infested cattle was 17.46 times more likely than non- tick infested cattle (AOR = 17.46, CI = 2.356–129.543, p = 0.005).

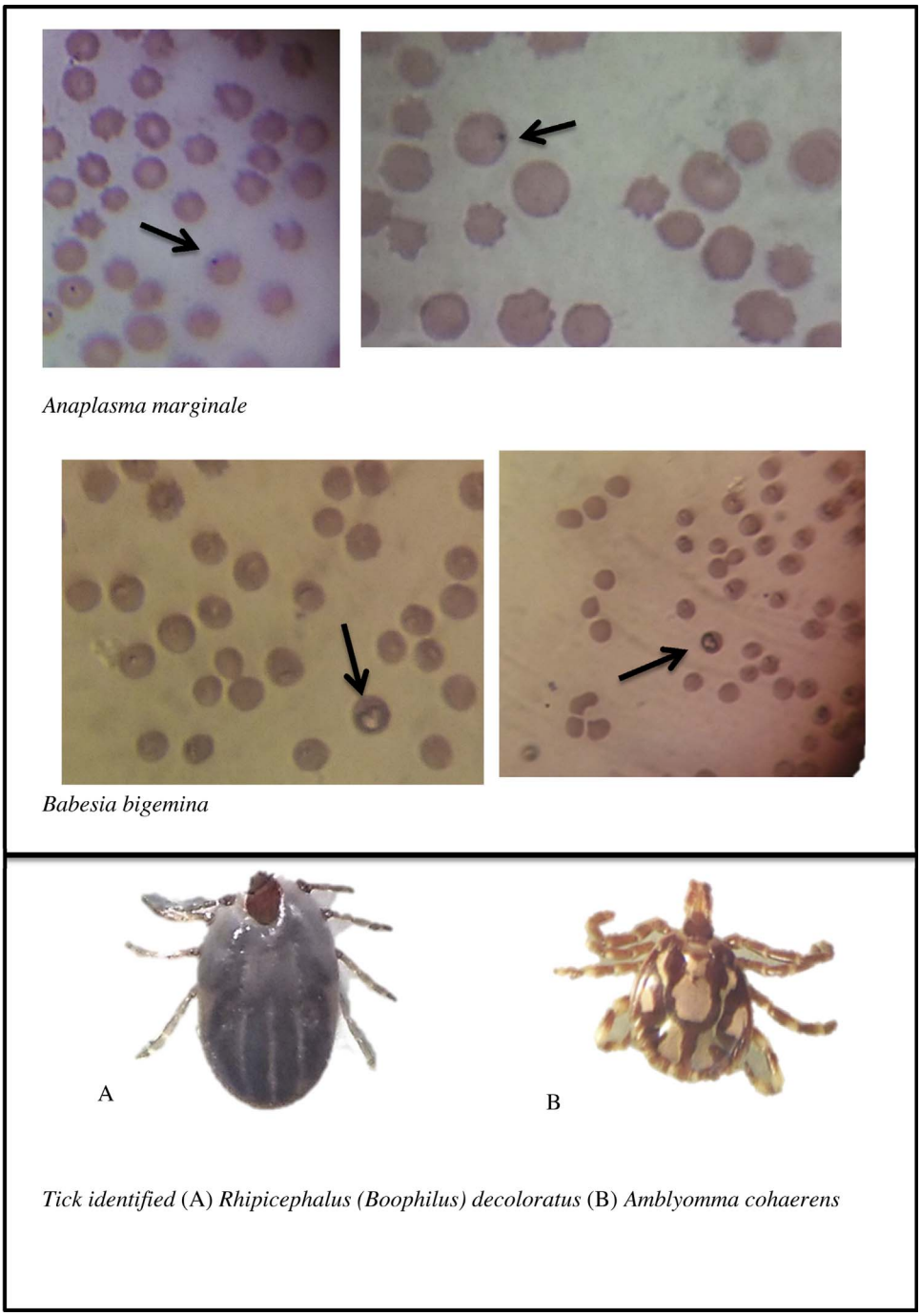

Fig. 2. Photograph of some the tick borne pathogens and ticks identified.

3.4. Tick identified and its correlation with bovine anaplasmosis and babesiosis

The study showed 289/408 (70.8%) infestation of cattle with tick. The overall, four tick species belonging to the Amblyomma and Rhipicephalus genera, namely *Rhipicephalus (Boophilus) decoloratus* (50.5%), *Rhipicephalus everetsi everetsi* (12.9%) *Amblyomma cohaerens* (58.5%) and *A.variegatum* (44.1%) were found. A significant positive correlation was observed between the presence *R. evertsi evertsi* on the animal and positivity for bovine anaplasmosis (P = 0.000). Moreover, the correlation of the presence of *R.(B).decoloratus* on the animals and positivity for bovine anaplasmosis was found to be statistically significant (P = 0.000). However, the correlation of bovine anaplasmosis positivity with the presence of Amblyomma species on the animals was found to be not significant (Table 7). Regarding the correlation of bovine babesiosis with tick species, the only tick species which its presence on the animal was significantly correlated with babesiosis positivity was *R. (B).decoloratus* (P = 0.000). There were no a significant correlation between Amblyomma species and *R. evertsi evertsi* presence on the animals and positivity for bovine babesiosis (Table 8).

3.5. Association of bovine anaplasmosis and babesiosis with PCV

The overall mean ± SD PCV of the study animals was 23.81 ± 4.645 (Table 9). Using the PCV value cut off of 24% (Kessell, 2015) 75% of babesiosis infected and 40.2% of babesiosis non-infected animals were found anaemic. Moreover, 84% of anaplasmosis infected and 37.8% of anaplasmosis non-infected animals were found anaemic. The mean PCV of babesiosis infected animals (20.79 ± 3.294) was significantly (P = 0.000) lower than non-infected animals

Table 2
Prevalence of bovine anaplamosis and babesiosis according to localities.

Locality	Number examined	Anaplasmsosis		Babesiosis	
		Number positive	Prevalence	Number positive	Prevalence
Babela karra	45	3	6.6%	8	17.7%
Babela kosa	44	6	13.0%	11	25%
Bosa addis ketema	43	2	4.6%	4	9.3%
Bosa kitto	44	2	4.5%	5	11.3%
Ifa bula	43	1	2.3%	4	9.3%
Kofe	39	1	2.5%	5	12.8%
Mendara kochi	34	2	5.8%	3	8.8%
Sato samara	42	3	7.1%	4	9.5%
Somodo	37	2	5.4%	3	8.1%
Furustale	37	3	8.7%	1	3.2%
Overall	408	48	11.7%	25	6.1%

χ^2 for anaplasmosis = 6.99, P-Value = 0.637, χ^2 for babesiosis = 13.434, P-Value = 0.144.

(24.21 ± 4.654). The mean PCV of anaplasmosis infected cattle (20.44 ± 3.787) was also significantly (P = 0.000) lower than non-infected animals (24.03 ± 4.615).

3.6. Association of bovine anaplasmosis and babesiosis with body temperature

The overall mean ± SD body temperature of the study animals was 38.870 ± 0.7799. The majority of the study animals (74.2%) had a normal temperature with values ranging from 38.0 to 39 °C. From the whole study animals 75% and 23.2% of anaplasmosis infected and non-infected cattle were found to be febrile, respectively. 52% babesiosis infected animals and 22.2% of non-infected animals were also found to be febrile. The mean body temperature of anaplasmosis infected animals (39.620 ± 0.7837) was significantly (P = 0.000) higher than non-infected animals (38.821 ± 0.7552). Likewise, the mean body temperature of babesiosis infected cattle (39.252 ± 0.8121) was significantly (P = 0.000) higher than non-infected animals (38.819 ± 0.7623) (Table 10).

4. Discussion

Anaplasmosis and babesiosis are important tick borne diseases which have worldwide distribution with greater economic importance in tropical and subtropical regions (Rahman et al., 2015; Filia et al., 2015) and have been reported in Ethiopia (Sitotaw et al., 2014). Cross-sectional study was undertaken to assess the prevalence and potential risk factors associated with bovine anaplasmosis and babesiosis. To the best of our knowledge this is the first study in south-western Ethiopia that asses bovine anaplasmosis and babesiosis simultaneously in correlation with vectors identification.

In this study, overall prevalence of 11.7% and 6.1% babesiosis and anaplasmosis were recorded, respectively. The result of bovine anaplasmosis were found to be in agreement with the finding of the Zerihun et al. (2014), who indicated 5.9% prevalence of anaplasma species in Western Ethiopia by microscopic examination. Moreover, this finding was in approximate to the finding of Paul et al. (2016) who reported 5.8% prevalence of anaplasma species in Maiduguri, Nigeria. Although, we cannot compare direct detection of parasites (microscopy) with serology (which only demonstrates exposure with no implied infection status), this result was found to be low when compared to result of Solomon et al. (1998) who reported 94.68% anaplasmosis seroprevalence in Didtuyura ranch and Sileshi (1996) who reported 99% prevalence of antibodies against *A. marginale* in different ecological zones in Ethiopia. This difference could be due to the sensitivity of diagnostic test used since examination of blood smears is not reliable for detecting pre-symptomatic or carrier animals.

The result of bovine babesiosis was found to be relatively higher than the finding (1.5%) and Sitotaw et al. (2014) (0.6%). Nevertheless, the present finding was lower than the previous reports in Teltele district, Borena Zone, 16.9% (Hamsho et al., 2015). This difference could be attributed to less sensitivity of diagnostic method used and vector control difference between different areas.

In our study, the prevalence of *B. bigemina* (9.8%) was higher than *B. bovis* (2.2%). This finding disagrees with the finding of different scholars from different areas of Ethiopia. The study conducted in Teltele district, Borena zone reported 9.9% *B. bovis* and 7.03% *B. bigemina* (Hamsho et al., 2015). The study in Assosa district also reported 1.24% *B. bovis* and 0.248% *B. bigemina* (Wodajnew et al., 2015). However, the current finding could be supported by the result of Sileshi (1996) who stated that the infection with *B. bigemina* is widespread in Ethiopia and *B. bovis* is of recent origin in the livestock disease scenario of the country. According to Sileshi (1996) the wide spread nature of *B. bigemina* in Ethiopia can be associated with its vectors *R.(B). decoloratus* which is the most common and widespread one-host cattle tick in Ethiopia (Regassa, 2001; Sileshi et al., 2001; Tesema and Gashaw, 2010). Moreover, the probability of mechanical transmission is slight with *B. bovis* and higher with *B. bigemina* (Constable et al., 2017). Thus, the predominance of *B. bigemina* infection detected in this study is not surprising since distribution of both *B. bovis* and *B. bigemina* is determined by the distribution of their tick vectors. The current study supported this finding by dominance of vector of *B. bigemina* (*R.(B). decoloratus*) identified in the study area.

Increasing age has been frequently reported to be associated with increased TBDs prevalence (Atif et al., 2013; Ibrahim et al., 2013; Maharana et al., 2016). The result of this study also showed as there was a clear gradient of age effects where the risk of exposure increased

Table 3
Univariable logistic regression analysis of risk factors associated with bovine babesiosis.

Risk factors		No. Examined	No. positive (prevalence%)	COR(95% CI)	p-value
Age	Young	104	4(3.8)	Ref.	0.001
	Adult	153	14(9.1)	2.51(0.80–7.87)	
	Old	151	30(19.8)	6.19(2.11–18.18)	
Sex	Male	198	24(12.1)	0.93(0.512–1.709)	0.828
	Female	210	24(11.4)		
Body condition	Poor	199	37(18.6)	6.167(1.438–26.443)	0.000
	Medium	153	9(5.8)	1.687(0.353–8.061)	
	Good	56	2(3.5)	Ref.	
Management	Extensive	332	42(12.6)	1.69 (0.691–4.132)	0.25
	Semi-intensive	76	6(7.8)		
Tick infestation	Absent	119	1(0.8)		0.002
	Present	289	47 (16.2)	22.91(3.124–168.138)	

COR = Crude odd ratio, CI = Confidence Interval, ref = reference category cell.

Table 4
Final multivariable logistic regression model output of factors associated with bovine babesiosis.

Risk factors		Number Examined	Number positive (prevalence%)	AOR(95% CI)	p-value
Age	Young	104	4(3.8)	Ref.	0.012
	Adult	153	14(9.1)	2.22(0.690–7.148)	
	Old	151	30(19.8)	4.46(1.478–13.477)	
Body condition	Poor	199	37(18.6)	4.63(1.047–20.465)	0.003
	Medium	153	9(5.8)	1.41(0.288–6.77)	
	Good	56	2(3.5)	Ref.	
Tick infestation	Absent	119	1(0.8)		0.005
	Present	289	47 (16.2)	17.46 (2.356–129.543)	

AOR = Adjusted odd ratio, CI = Confidence Interval, Ref.= reference category cell.

with age in both infections (anaplasmosis and babesiosis). The prevalence of bovine anaplasmosis was found to be significantly (p = 0.006) associated with age being highest in older animals (12.5%) followed by adult (3.2%) and least in young (0.9%). This result is in agreement with the finding of the study in Machakos County, Kenya (Wesonga et al. (2017), in Punjab, Pakistan (Atif et al., 2013), in Tunisia (M'ghirbi et al., 2016) and in Morocco (Hamou et al., 2012).

Similar to bovine anaplasmosis significantly (P = 0.012) highest prevalence of bovine babesiosis was recorded in old and adult animals which were found to 4.46 and 2.22 times more likely to be positive for babesiosis than young animals, respectively. This result in line with the finding of studies conducted in different areas of Ethiopia (Wodajnew et al., 2014; Wodajnew et al., 2015; Hamsho et al., 2015) and elsewhere in the world (Ayaz et al., 2013). However, this finding disagree and Fereig et al. (2017) who identified that young animals are more susceptible to *Babesia* infection when compared to adult. The lower prevalence of anaplasma and *Babesia* infection in young animals compared to adults and old could be attributed to restricted grazing of young animals which tends to reduce their chance of contact with the vectors of these diseases and more sustained exposure of old to vectors. Moreover, lower prevalence detected in young animal could be attributed to passive colostrum immunity since in endemic areas, the young animal first acquires immunity passively, in the colostrum of the dam and, as a result, often suffers only transient infections with mild clinical signs (Taylor et al., 2007).

This study showed a significant association of positivity for anaplasmosis and babesiosis with the body condition of the study animals. Poor and medium body conditioned cattle were 4.40 and 1.22 times more likely to be positive for anaplasmosis than good body conditioned cattle, respectively. The odd of babesiosis occurrence in poor and medium body conditioned cattle were also 4.63 and 1.41 times more likely than good body conditioned cattle, respectively. A similar observation has been reported by other scholars (Sitotaw et al., 2014; Hamsho et al., 2015; Wodajnew et al., 2015). This difference could be due to the fact that animals with poor body condition have lower

Table 6
Final multivariable logistic regression model output of risk factors associated with bovine anaplasmosis.

Risk factors		Number Examined	Number positive (prevalence %)	AOR(95% CI)	p-value
Age	Young	104	1(0.9)	Ref.	
	Adult	153	5(3.2)	3.11 (0.354–27.302)	0.006
	Old	151	19(12.5)	11.32(1.470–87.286)	
Body condition	Poor	199	20(10.0)	4.40 (0.562–34.532)	0.039
	Medium	153	4(2.6)	1.22 (0.13–11.46)	
	Good	56	1(1.7)	Ref.	
Tick infestation	Absent	119	2(1.6)		
	Present	289	23(7.9)	3.218 (0.723–14.324)	0.125

AOR = Adjusted odd ratio, CI = Confidence Interval, ref = reference category cell.

Table 7
Correlation of bovine anaplasmosis with different tick species.

Tick species	Categories	Number examined	Number positive (prevalence)	Correlation coefficient	P-value
R. (B). decoloratus	Absent	202	3 (1.4%)	0.192	0.000
	Present	206	22 (10.6%)		
R. evertsi evertsi	Absent	355	11(3.0%)	0.327	0.000
	Present	53	14(26.4%)		
A.cohaerens	Absent	169	23(13.6%)	−0.034	0.400
	Present	239	2(0.8%)		
A. variegatum	Absent	228	16(7.0%)	−0.112	0.492
	Present	180	9(5%)		

Table 5
Univariable logistic regression analysis of risk factors associated with bovine anaplasmosis.

Risk factors		No. Examined	No. positive (prevalence%)	COR(95% CI)	p-value
Age	Young	104	1(0.9)	Ref.	
	Adult	153	5(3.2)	3.48(0.40–30.22)	
	Old	151	19(12.5)	14.82(1.95–112.58)	0.001
Sex	Male	198	13(6.5)	1.15(0.51–2.60)	0.720
	Female	210	12(5.7)		
Body condition	Poor	199	20(10.0)	6.14(0.80–46.83)	
	Medium	153	4(2.6)	1.477(0.161–13.500)	0.012
	Good	56	1(1.7)	Ref.	
Management	Extensive	332	22(6.6)	1.727 (0.503–5.925)	0.385
	Semi-intensive	76	3(3.9)		
Tick infestation	Absent	119	2(1.6)		
	Present	289	23(7.9)	5.058 (1.173–21.806)	0.030

COR = Crude odd ratio, CI = Confidence Interval, ref = reference category cell.

Table 8
Correlation of bovine babesiosis with different tick species.

Tick species		Number examined	Number positive (prevalence)	Correlation coefficient	P-value
R.(B). decoloratus	Absent	202	3(1.4%)	0.316	0.000
	Present	206	45(21.8%)		
R. evertsi evertsi	Absent	355	42(11.8%)	−0.005	0.915
	Present	53	6(11.3%)		
A. cohaerens	Absent	169	34(20.1%)	0.014	0.784
	Present	239	14(5.8%)		
A. variegatum	Absent	228	24(10.5%)	−0.003	0.957
	Present	180	24(13.3%)		

Table 9
PCV (mean ± SD) of infected and non-infected animals.

Diseases	Infection status	No. of animals	PCV (mean ± SD)	P-value
Anaplasmosis	Infected	25	20.44 ± 3.787	0.000
	Non- Infected	383	24.03 ± 4.615	
Babesiosis	Infected	48	20.79 ± 3.294	0.000
	Non- Infected	360	24.21 ± 4.654	
	Total	408	23.81 ± 4.645	

Table 10
Temperature (Mean ± SD) of infected and non-infected cattle.

Disease	Infection status	No. of animals	Temperature (Mean ± SD)	P-value
Anaplasmosis	Infected	25	39.620 ± 0.7837	0.000
	Non-Infected	383	38.821 ± 0.7552	
Babesiosis	Infected	48	39.252 ± 0.8121	0.000
	Non-Infected	360	38.819 ± 0.7623	
	Total	408	38.870 ± 0.7799	

immunity which encourages infection by different organisms like anaplasma and *Babesia*. However, further longitudinal study is needed to determine whether the body condition is the consequence the disease or predisposing factors since the scope of the present study design does not allow investigation of such relationship.

Even though not significant, the study showed the higher risk of anaplasma and *Babesia* infection in extensively managed cattle than semi-intensively managed cattle. This finding concurs with the finding of Angwech et al. (2011) and Wesonga et al. (2017) who reported a higher prevalence of infection with tick-borne disease in extensively managed cattle. The management system effect on TBD pathogen positivity emanates from the nature of exposure from the vectors. Thus, this ranges from unrestricted tick exposure in extensive management systems through restricted exposure in semi-intensive, where animals are alternately open grazed and stall fed. The current findings was consistent with this phenomenon basically due to these differential exposures since animals raised under open grazing (extensive) management are frequently exposed to infected ticks and thus, are more likely to be positive for TBD pathogen. The non-significance difference recorded in this study might be associated with disproportionality of sample of each managements system.

Availability of vectors is one of the potential risk factor for both *Babesia* and Anaplasma infections (Constable et al., 2017). The multivariate logistic regression analysis showed that risk of babesiosis was significantly (p = 0.005) higher in tick infested (16.2%) cattle than non-tick infested (0.8%) cattle. A similar observation was reported by Costa et al. (2013). Similarly the higher prevalence of anaplasmosis was also recorded in tick infested cattle. The association of bovine anaplasmosis with presence of ticks was statistically significant during univariable logistic regression analysis. However, tick presence was kicked off from potential risk factors of anaplasmosis during the multivariable logistic regression analysis and found to have a non-significant (p = 0.125) effect on disease occurrence. Although this finding was unexpected, the possible explanation was the effect of the additional mechanical transmission by blood-sucking arthropods and fomites for Anaplasma infection (OIE, 2015; Constable et al., 2017; Dantas-Torres et al., 2017; Dantas-Torres and Otranto, 2017). This form of mechanical transmission is considered to be the major route of dissemination of bovine anaplasmosis in areas of Central and South America and Africa where tick vectors do not occur (Figueroa et al., 1998).

Even though four tick species which includes *R. (B) decoloratus*, *R. evertsi evertsi*, *A. cohaerens* and *A.variegatum* were identified in the current study, the method we used was not in accordance with standardized method, including scanning and collecting all ticks from one body side. Thus, this limits us in the interpretations of tick burden. However, the identified tick species were known to be present in different areas of Ethiopia and has been reported from the current study area previously (Abebaw, 2004; Yitbarek, 2004). Since the presence of *Babesia* and anaplasma infection in cattle is broadly related to presence of suitable vectors (ticks), this study correlated the presence of different tick species identified with disease positivity and found a significantly positive correlation of anaplasma infection with presence of *R. (B).decoluratus* and *R. evertsi evertsi* on the animals, suggesting that this tick could be the vector for this disease. This finding was in agreement with what was previously shown by several scholars (Walker et al., 2003; Kocan et al., 2004; 2010) who stated *R. (B.).decoluratus* and *R. evertsi evertsi* as most important vectors of bovine anaplasmosis. Moreover, the study showed a significant (P = 0.000) positive correlation of bovine babesiosis infection with *R. (B).decoloratus* presence on the animal. This tick is known to transmit bovine babesiosis in different area of the world (Walker et al., 2003; Constable et al., 2017; Dantas-Torres et al., 2017 Dantas-Torres and Otranto, 2017) including Ethiopia (Sileshi, 1996). Though we did not detect the pathogens in tick, the correlation observed in this study might be attributed to its vectors capacity.

Increase in body temperature is frequently mentioned as the main consequence of bovine anaplasmosis and babesiosis (Constable et al., 2017; Dantas-Torres et al., 2017). In our study, the mean body temperature of anaplasmosis infected animals (39.620 ± 0.7837) was significantly (P = 0.000) higher than non-infected animals (38.821 ± 0.7552). Likewise, the mean body temperature of babesiosis infected animals (39.252 ± 0.8121) was significantly (P = 0.000) higher than non-infected animals (38.819 ± 0.7623). The reported clinical finding come in agreement with what was previously described by Brown and Torres (2008), Mahmmod (2014), Aktas and Özübek (2015) and El-Ashker et al. (2015). The detected fever could be attributed to response to the effect of unspecific toxic substances produced during the metabolism of *Babesia* on thermoregulatory (Radostits et al., 2007). In anaplasmosis infected cattle infected erythrocytes are removed by phagocytosis in the reticular endothelial system, with release of acute-phase inflammatory reactants and the consequent development of fever (Constable et al., 2017).

The PCV of individual animals is a useful indicator of anaemia which is recognized as the most important consequence of several tick born disease including babesiosis and anaplasmosis in cattle (Kocan et al., 2010; Aubry and Geale, 2011; OIE, 2013, 2015). In the present study, the mean PCV of babesiosis infected animals (20.79 ± 3.294) was significantly (p = 0.000) lower than non-infected animals (24.21 ± 4.654). This result in line with the finding of Mahmmod (2014) and Marufu et al. (2010) who reported a significantly lower mean PCV in babesiosis infected cattle than non-infected cattle. The significance difference in mean PCV of the two groups could be attributed to the severe haemolytic process associated the presence of *Babesia* piroplams inside the erythrocytes and destruction of large numbers of these erythrocytes by the parasite thereby resulting in

hemoglobinaemia and consequently hemoglobinuria (Dantas-Torres et al., 2017).

Similar to babesiosis, the mean PCV of anaplasmosis infected cattle (20.44 ± 3.787) was significantly (P = 0.000) lower than non-infected animals (24.03 ± 4.615). This result in line with the finding of El-Ashker et al. (2015) and Marufu et al. (2010) who reported a lower PCV in anaplasmosis infected cattle. The difference in mean PCV of anaplasmosis infected and non- infected cattle could associated with the ability of anaplasma to invade erythrocytes, undergoes cycles of replication which is then principally cleared from the body by macrophages of the reticuloendothelial system in the spleen. The removal of these infected erythrocytes by phagocytosis results in development of anaemi (Kocan et al., 2010). By and large the present finding supports the general understanding that anaemia is an important outcome of bovine anaplasmosis and babesiosis. The detection of anaemia in non-infected animals indicates the existence of other anaemia causing factors like helminthosis and malnutrition in the study area besides which need to be explored by future studies.

5. Conclusion

The present study provides basic information on the prevalence and associated risk factors of bovine babesiosis and anaplasmosis in correlation with vectors identification in and around Jimma town. The study revealed a moderate prevalence of bovine anaplasmosis (6.1%) and babesiosis (11.7%) by Giemsa staining technique. Two *Babesia* species, namely *B. bovis* (2.2%) and *B. bigemina* (9.8%) and two anaplasma species, *A. marginale* (5.1%) and *A. centrale* (1.2%) were identified. The main risk factors found to be significantly associated with bovine babesiosis were age, body condition and tick presence on the animals. However, only age and body condition were found to be significantly associated with bovine anaplasmosis. The main tick species which its presence was found to be significantly correlated with positivity for bovine anaplasmosis were *R. evertsi evertsi* and *R.(B). decoloratus,* whereas bovine babesiosis positivity was found to be only correlated with *R.(B). decoloratus* presence on the animals. Moreover, the study disclosed high body temperature and low PCV in cattle infected with anaplasmosis and/or babesiosis. 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 becoming a nuisance, but allows enough ticks to remain for infection to occur at an early age so that the animals become protected against the diseases. Furthermore, farmers should be educated on the effect of ticks and tick borne diseases and also on tick control measurement in order to establish or maintain enzootic stability. Further investigations should also be conducted using modern serological and molecular techniques for the identification of the carriers of these tick-borne pathogens. There is also the need for detection of tick borne pathogens in tick so that it helps in knowing which tick species can harbour specific pathogen and their respective vector capacity.

Conflict of interest

The authors declare that they have no conflict of interest.

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