Abstract

The cross-sectional study design was conducted to assess the prevalence and potential risk factors associated with bovine babesiosis and to identify the vectors involved in the transmission of these diseases in and around the Wolaita zone, the case district sodozuria, Southern Ethiopia. It was conducted from November 2018 to April 2019. A simple random sampling technique was used to select sampling units and logistic regression was employed to determine the association between hypothetical risk factors and positive bovine babesiosis. For this purpose, 384 cows were randomly selected and blood was drawn and collected from the ear and jugular veins. Thin smears were measured using Giemsa stain techniques for Babesia detection. Out of 384 blood samples of cattle 56 (14.58%) were infected with Babesia. Two Babesia species (8.07% Babesia bovis and 6.51% B. bigemina) were identified. Even though risk factors like body condition score; medium (P=0.007) and good (P=0.001), packed cell volume (P=0.000) and semi intensive management system (P=0.007) were significantly associated with prevalence of bovine Babesiosis (P<0.05), the risk factors like age, kebele, tick infestation, sex and breed were not significantly associated with prevalence of bovine Babesiosis (P>0.05). The overall 15.1% infestation of cattle with two tick specieses, namely Boophilus decoloratus (6.25%) and Rhipicephalus evertsi (8.85%) was recorded. It was then finally concluded that the prevalence of bovine babesiosis was moderate in this area and that the tick vector was controlled to control bovine babesiosis in the study area.
Introduction

Rapid global change is changing the epidemiology of tick-borne diseases. Tick-borne disease is a complex system that is affected by changes in the ecological processes that affect the biology of mites and thus the epidemiology of tick-borne pathogens [2]. Bovine piroplasmosis is caused by Babesiatiick-borne hemotological protozoa, is the most prevalent in tropical and subtropical countries, and has significant economic consequences worldwide. Bovine Babesiosis is a tick-borne bovine disease caused by the Babesia protozoan parasite, Piroplasmidae, and the phylum Apicomplex, and is generally characterized by significant prevalence and mortality worldwide [1].

More than 100 species of Babesia have been identified, which are traditionally divided into small and large groups based on morphology. The most common species of Babesiaprotozoa that parasitize red blood cells include Babesiabovis, Babesiagemina, Babesiadivergence, and Babesia major. Of these four species, Babesiabovis and Babesiagemina are considered to be the most important species because they are widely distributed in the tropical and subtropical regions of the world and cause enormous economic losses to livestock production [3,4].

Cattle breed Sanga is the most common breed in the common area of northern Namibia. Other races such as Africa, Brahmin and Simbra are also found in the area, but are rare. Sanga is more resistant to parasites and tick-borne diseases and has the ability to reduce the number of ticks on the skin. This reduces the number of blood-sucking parasites in the body [5].

The importance of Babesiadivergens [6] for today's farm animals enterprise in Europe is nearly without a doubt underestimated, as is the opportunity of human infection. In 1981 Purnell wrote “bovine babesiosis resulting from B. divergens, additionally called pink water fever, is taken into consideration the maximum essential tick-transmitted disorder in livestock” [7]. Thailand is a growing agricultural unitstates of America placed in Southeast Asia, wherein the improvement of farm animals enterprise has been hampered through the excessive incidence of tick borne diseases, mainly bovine babesiosis [8,10].

Nowadays Haemoparasites infections are essential public health, veterinary and socio-financial issues in Africa, wherein they impose a burden at the healthcare infrastructure of each animals and animal handlers in endemic areas. Ethiopia with its amazing variant in weather and topology possesses the most important variety of farm animals in Africa and farm animals manufacturing performs a first-rate position with inside the improvement of Ethiopia’s agriculture. The envisioned farm animals populace in Ethiopia is fifty nine million livestock, 35 million sheep, 31 million goats, 2.three million camels, 1.ninety one million horse, 6.seventy five million donkey, 0.35 million mules, 38 million fowl and 5,207, three hundred traditional, intermediate and contemporary-day beehives [10]. These farm animals aid make a contribution 15% to gross home product (GDP) and 33% to agricultural output. Among the farm animals, livestock immediately offer meals along with meat and milk, a non-meals along with cover and in a roundabout way to agricultural manufacturing through presenting important inputs Along with manure for replenishing soil fertility and restoring nutrients, animal traction and electricity for plowing and threshing, growing the productiveness of small holdings [11].

The gift observe become carried out at the describing of the morphology of bovine babesiosis with its causative agents, transmission and supply of infection, it’s distribution and pathogenesis, scientific signs, there threat elements like animal threat elements, surroundings threat elements, pathogenic threat elements and Although how babesiosis may be managed with vaccination and dealt with antiparasitic capsules, the vaccines are stay and feature protection worries and plenty of powerful capsules were withdrawn from the market place because of protection or residue issues [12].

However, the distinctive repute of bovine babesiosis isn’t always very well studied in our united states of america; mainly sodozuria Woredas, Wolaita Zone, Southern Regional State and the facts is thus far scanty. Due to its financial importance, there’s a want to set up the true occurrence and distribution of the disorder with inside the observe area.

Therefore, this observe become achieved with the objectives;

- To decide the superiority of bovine babesiosis and its distribution for farmers with inside the observe area.
- To estimate and determine threat elements related to bovine babesiosis in Sodozuria Woreda.

Materials and methods

Study Area

This survey was conducted in the Walaita Zone Districts of Sodo2Zuria Woreda, Southern Nations, Nationalities and People's Regions (SNNPRS). Wolaita Sodo is located 330 km southwest of Addis Ababa, at latitude 8°50' N and longitude 37°45' E. With a total area of 4,541 km², it consists of 18 districts of Ethiopia and two registered towns. It is about 2000 meters above sea level and its altitude ranges from 700-2900 meters. The average annual rainfall is 1014 mm and the average daily temperature is 19.5°C. SodoZuria Woreda has 128,783 cows, 35,290 sheep, 9,013 goats, 8,316 horses and 86,979 poultry (WZLFR Bureau, 2016). Woreda’s agroecology is dominated by midlands, which occupy about 87% of the total area, with the remaining 13% being highlands with steep mountains and slopes. Mount Demote is the highest peak in the zone (above 2800 masl) and is considered the main source of water in the surrounding area [13].

Study population

The study included local and Jersey cattle of various ages, body conditions, and sexes kept under extensive, semi-intensive and intensive management system. The study animals comprised different age groups including both male and female sexes. The ages of the animals were conveniently classified as young (<3 years), adult (4-6 years) and old (>7 years) age categories as described by [15]. The condition of the animal’s body was assessed during sampling and classified as good, medium, and poor according to [14].

Study design

Cross-sectional study was conducted from November 2018 to April 2019 to determine the incidence and potential risk factors for bovine babesia disease in selected areas of the Wolaita Zone in southern Ethiopia. Information regarding age, sex, breed, management system, temperature, tick infestation, origin, and body condition of the animals were recorded during sample collection.

Sample size determination and sampling methods

The study area was selected purposively based on ease of accessibility and transportation while a systematic random
sampling technique was employed to select study units in clinic and simple random sampling technique was employed in farms and small holders. The Sample size was calculated according to the formula given by Thrus field (2007) with 95% CI, since there is no previous similar study was conducted in the same agro-ecology, 50% expected prevalence was taken to include 384 animals in the study. A proportional sampling methods was followed to include representative samples from different districts (Gulgula= 58, of a Gandaba=42, of a Sere=43, Demote=52, Buge Wanche= 64, Delboat Waro= 53, Waaraaz Lasho= 40 and Waacciga Bushe= 32).

\[ n = \frac{1.96^2 (expP) (1 - expP)}{d^2} \]

Where, \( n \) = required sample size 1.96=the value at 50% confidence interval p=expected prevalence of babesiosis d=desired absolute precision level; 0.05 at 95% confidence interval.

**Study methods**

**Sample Collection and Transportation:** Blood samples was drawn from jugular/or ear veins after the site was cleaned, hair removed and disinfected with 70% alcohol. Blood was collected from jugular veins using vacutainer tube with EDTA and vacu-tainer needle and holder. The first drops of blood were taken from ear veins and thin smear preparation was made to confirm the presence of Babesia species under microscope. The collected blood then stored at 4°C till the value of PCV was evaluated. Ticks were collected mainly from ears, rump, perineum and udder from all cattle where ticks could be found, preserved by methanol and put into collecting tubes and transported to Wolaita Sodo Veterinary Parasitology Laboratory for examination of their morphological features of the tick species.

**Laboratory Investigation procedures:** Thin smears had been organized through making use of the slide with blood directly to a clean slide at an angle of 45° after which lightly shifting forward. The slide changed into dried in air and stuck for two minutes in methyl alcohol (absolute methanol). Giemsa staining procedures and microscopic examination of slides was conducted in line with [16]. The slides were immersed in Giemsa stain (1:10 solution) in staining rack for 30 minutes. Then the slides were washed with distilled water to remove excess stain and made air dry. The stained blood smears were examined under oil immersion lens of microscope (100X) for appreciation and identification of different Babesia species according to their morphological characteristics [17]. All collected ticks were examined under the stereo microscope and classified to general levels based on size, mouthparts, presence and absence festoon, presence and absence of the eye and color of the body. Furthermore, different morphology of tick such as shape of Scutum, leg color, body, festoon, eye shape, ventral plates and marginal spot were considered for species level identification according to [18].

**Data management and analysis**

The data collected was coded, entered into a Microsoft Excel spreadsheet, and analyzed by STATA (version 13). Descriptive statistics was used to estimate the frequency and percentages of bovine babesia, tick species. A chi-square \( \chi^2 \) test was used to assess the association between possible risk factors and the development of disease in the study area. Multivariate logistic regression was used to estimate the magnitude of various risk factors for disease occurrence. Statistical significance was found at \( P <0.05 \).

**Results**

The overall prevalence of bovine babesiosis was 14.84%. Regarding to age, highest infection rates was observed in young cattle (17.9%) followed by old (15%) and adults (14.02%). The prevalence of bovine babesiosis was 63.16%, 26.1% and 7.9% in poor, medium and good body conditioned animals respectively. The association was statistically significant \( P <0.05 \). The detail on the occurrence of bovine babesiosis across different breeds, sex, management and location is indicated (Table 1). The occurrence of Rephicephulus evertsi was 8.85% (34/384) and Boophilus decoloratus was 6.25% (24/384) (Table 2). Two species of Babesia were identified with the prevalence of 8.07% (31/384) and 6.51% (25/384) for B.bovis and B.bigemina respectively (Table 3).

**Table 1:** Prevalence of babesia on the basis of age, breed, sex, body condition and management system.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Positive</th>
<th>Prevalence (%)</th>
<th>( \chi^2 )</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>277</td>
<td>41</td>
<td>14.8</td>
<td>0.0014</td>
<td>0.970</td>
</tr>
<tr>
<td>Cross</td>
<td>107</td>
<td>16</td>
<td>14.95</td>
<td>0.8426</td>
<td>0.359</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>114</td>
<td>14</td>
<td>12.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>270</td>
<td>43</td>
<td>15.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body condition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>19</td>
<td>12</td>
<td>63.16</td>
<td>54.4024</td>
<td>0.000</td>
</tr>
<tr>
<td>Medium</td>
<td>88</td>
<td>23</td>
<td>26.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>277</td>
<td>22</td>
<td>7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>73</td>
<td>13</td>
<td>17.8</td>
<td>0.6530</td>
<td>0.721</td>
</tr>
<tr>
<td>Adult</td>
<td>271</td>
<td>38</td>
<td>14.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>40</td>
<td>6</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>356</td>
<td>50</td>
<td>14.04</td>
<td>2.6492</td>
<td>0.266</td>
</tr>
<tr>
<td>Semi intensive</td>
<td>25</td>
<td>6</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive</td>
<td>3</td>
<td>1</td>
<td>33.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>57</td>
<td>14.84%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Prevalence of babesia on the basis of tick species.

<table>
<thead>
<tr>
<th>Species of tick</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rephicephlusevertsi</td>
<td>34</td>
<td>8.85</td>
<td>8.85</td>
</tr>
<tr>
<td>Boophilusdecoloratus</td>
<td>24</td>
<td>6.25</td>
<td>15.10</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>15.10</td>
<td>23.95</td>
</tr>
</tbody>
</table>

**Table 3:** The prevalence of babesia on the basis of babesia species identified.

<table>
<thead>
<tr>
<th>No. of animals examined</th>
<th>Babesia species</th>
<th>Positive</th>
<th>Prevalence</th>
<th>Cumulative frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>384</td>
<td>Babesia bigemina</td>
<td>25</td>
<td>6.51</td>
<td>91.93</td>
</tr>
<tr>
<td></td>
<td>Babesia bovis</td>
<td>31</td>
<td>8.07</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>14.58</td>
<td>191.93</td>
<td></td>
</tr>
</tbody>
</table>
In this study, the overall prevalence rate of bovine babesiosis was found to be 14.58% out of which two species of Babesia comprising of B. bovis (8.07%) and B. bigemina (6.51%) were identified using Giemsa stained microscopic examination which coincides with the earlier prevalence 17% B. bovis and 16% B. bigemina from Malaysia as reported by [19]. This is may be due to higher concentration of the former parasite in the capillary veins and veins than the latter parasite which evenly distributed in the whole blood vasculature.

Previous studies have also indicated that cattle infected with B. bovis remain carriers for long periods, while those infected with B. bigemina remain carriers for only a few months. This finding was higher than the previous findings 6.6% from Malak and Agency [20] and 9.9% from study conducted in Khyber Pakhtunkhwa, Pakistan [21]. However, this result was lower than the earlier reports from Malaysia (42%) [19], Nevertheless the present finding was also 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 and the prevalence of 26.6% from a cattle rise nearby forest in Salakpra Wildlife Sanctuary in Kanchanaburi province (Nongnuch, et al., 2013).

The variations in the prevalence of bovine babesiosis might be due to different factors like management condition of the focus area, use of acaricides during tick infestation, farming system and proper use of antiparasitic drugs, fluctuations of parasites during chronic course of the disease and in carriers animals, sensitivity of test used, distribution of infected vector and accessibility of animals to wildlife sanctuary and parks and forest area harboring the Babesia vectors [22]. Other cause of variation may be due to different geographical conditions and or due to different breeds of cattle studied [23].

The highest prevalence of bovine babesiosis was recorded in Gulgula peasant association (PA) 13/58 (22.4%) and lowest prevalence occurred in delboatwara 6/53 (11.3%) among the study areas considered for this study. The possible explanation for this might be associated with that sodo zuria PA mainly contains gulgula pastoralists who have wide ranging land and keep their cattle far away to areas having forests and bushes which is believed to be the most suitable for the vector of the Babesia but the seven remaining PAs are mainly predominated by the mixed farming system who are mainly agro industries practicing both crop and trading production. The latter study areas also practice keeping their cattle near to their cultivated land using agricultural by-product. This could reduce the probability of their cattle exposure to high tick infestation and accessibility to tick infested area. This finding concurs with the study conducted in yabello and Jimma that the prevalence of infection in poor body conditioned animals as compared to medium body condition and lack enough body potential to build resistance with age advancement.

The prevalence of the disease based on the body condition of the animals was 7.9% (22/277), 26.1% (23/88), 63.16% (12/19) for good, medium and poor scoring respectively with significant association (p<0.05). This could be due to the fact that animals with poor body condition have lower immunity which encourages infection of animal by different organisms like Babesia. In addition, during this study period it was very common to see high burden of ectoparasite (ticks) in animal with poor body condition and this can increase rate of infection from Babesia. The proportion of tick infestation was higher in poor body conditioned as compared to medium body conditioned and good body conditioned animals. This was due to poor body conditioned animals are less resistant to tick infestation and lack enough body potential to build resistance with age advancement.

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 [27]. In the present study, the mean PCV of babesiosis infected animals (0.397601-0.6058854) was significantly (p = 0.000) which is lower than (p<0.05). This result in line with the finding of [28] 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 piroplasms inside the erythrocytes and destruction of large numbers of these erythrocytes by the parasite thereby resulting in hemglobinaemia and consequently hemoglobinuria [29].

Based on management system prevalence of bovine babeiosis was 50/356 (14.02%) in extensively managed, 6/25 (24%) under semi-extensive management system and 1/3 (33.3%) in intensive management system. Prevalence of bovine babesiosis based on management system has no statistically significant difference in extensive and intensive (P>0.05), but it was sig-
significant (p=0.007) in semi intensive management system lower than (p<0.05).

Conclusion and recommendations

In summary, the available results show that bovine babesiosis was moderately prevalent in the study area. *B. bovis* and *B. bigemina* were identified as the species responsible for bovine babesiosis with greater prevalence of *B. bovis*. This study has clearly identified a need for more farmer education and awareness about tick borne diseases. Effective management of this disease is important not only for the zoonotic nature of disease under study, but also for adversely affecting animal production.

- Regular strategic prophylactic treatments and the use of acaricides need to be enhanced to control Babesia parasites.
- Further attention should be paid to the integrated management options by using one or more ways to achieve good results, including selection of resistant cattle breeds, proper grazing management in local pastures, predator use, vaccination, and good nutritional levels to get good performance of productive breeds in the area.
- Relevant government officials, non-governmental organizations, and experts need to work together to develop and implement rigorous guidelines for the proper management of livestock and common ectoparasites in general and tick in particular.
- Further studies to elucidate the effects and epidemiology of tick-borne diseases using immunological methods to implement better control measures for bovine ticks and tick-borne diseases and to validate this study must be carried out.

Ethics approval and permission to participate

The Wolaita Sodo University of Research Ethics Committee granted this project ethical approval. A verbal agreement was sought before collecting samples from animal owners, and proper sanitary precautions were taken for blood collection and taking tick samples from the animals. The study’s goal was presented to the owners, and the Wolaita Sodo University of Research Ethics and Review Committee accepted the approach of oral informed consent outlined in the paper.

Acknowledgements

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References


