

Materials and Methods

Study Area

The study was carried out in Guto Gida and Diga district, which is located in East Wollega, Oromia regional state of Ethiopia. The districts are 2,088 meters above sea level in latitude 9° 5'N and longitude 36°33' East. Nekemte is 331 kilometers west of Addis Ababa, the nation's capital. The lengthy summer rainy season (June to September), the short rainy season (March to April), and the dry winter seasons rotate in the climate (December to February). The ranges for daily temperatures and yearly rainfall are correspondingly 1450 to 2150mm and 15 to 27°C.

The region has a total land area of roughly 729,725 hectares, of which 3366,220 hectares are used for crop production, 184,412 hectares are used for animal grazing, 256,901 hectares are covered in forest, and 20,492 hectares are used for other purposes.

Study Design

A cross-sectional study design was employed to determine the prevalence of bovine babesiosis and associative risk factors in selected district of East Wollega Zone of Oromia regional state, Ethiopia.

Sample Size Determination

SAMPLE SIZE DETERMINATION

The total sample size was determined according to the formula given by Ruseld given below; using a five percent desired absolute precision, 95% confidence interval and 50% expected prevalence. Accordingly, the total sample size would be 384.

$$N = \frac{1.96^2 P \text{exp} (1 - P \text{exp})}{d^2}$$

Where N = required sample size, P exp= expected prevalence, d = desired absolute precision (0.05). Accordingly, 384 animals were included in this study.

SAMPLE SIZE DETERMINATION

Cattle presented to Nekemte veterinary clinic of Guto Gida district and Diga veterinary clinic of Diga district were included in the study. Nekemte and Diga veterinary clinics were selected based on purposive sampling because of the number of cattle presented to the clinics and their accessibility. Purposive sampling technique was utilized to obtain study animals from both Nekemte and Diga veterinary clinics. During sampling a clinical sign like change in urine colour, yellowish and paleness of visible mucous membrane and tick infestation was considered as criteria to purposively selected animals. Accordingly, 219 animals were selected from Nekemte veterinary clinic and 165 were selected from Diga veterinary clinic.

Sample Collection and Processing

The blood sample was collected either from the jugular vein or ear vein after proper restraining of the animal according to Urquhart. Before blood collection, the area of blood collection by puncture was cleaned; the hair was removed and disinfected with 70% alcohol. Blood sample was collected from the jugular vein by a heparinized vacutainer tube and by hematocrit capillary tube from ear vein and transported to Wollega University Veterinary Parasitology laboratory in ice box. Age, sex and body condition score of the studied animals were recorded during sampling. The ages of the animals was determined based on owners' information and as described by De-lahunta and Habel and also the body condition of the animals was determined according to

Nicholson and Better worth.

Microscopic Examination

Thin and thick blood smears on clean and dry glass slides were prepared from the blood samples. The thin blood smear films were air-dried and fixed in absolute methanol for 2-5 minutes and stained with Giemsa for 30 minutes, washed with tap water to remove an extra stain, and air-dried slides were examined under the oil immersion lens of a light microscope. Morphological characteristics of *Babesia* species were identified according to key Soulsby. Thick films were made by placing a small droplet of blood onto a clean glass slide and spreading this over a small area using a circular motion on the corner of another slide, air-dried and stained in Giemsa. This is a more sensitive technique for the detection of *Babesia* species, as RBCs are lysed and parasites concentrated, but species differentiation is more difficult. PCV was measured to know the level of anemia for each individual animal. PCV was measured by filling blood into a hematocrit capillary tube up to ¾ its volume and sealing in soap and placing in a hematocrit centrifuge for 15000rpm per 5 minutes. After centrifugation measured by PCV recorded.

Data Collection and Analysis

Data collected were recorded properly and entered into a Microsoft Excel spreadsheet and analyzed using SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA) coded. Data were summarized using descriptive statistics. The associations between the prevalence of Bovine *Babesia* infection and the associated risk factors (age, sex and body condition score) were evaluated using logistic regression analysis. Student's t-test was applied to examine the differences in mean PCV values among *Babesia* infection status. Analysis of variance (ANOVA) was applied to compare the mean PCV values of infected animals with different *Babesia* species. Differences were considered to be significant as $P < 0.05$ at 95% confidence interval.

RESULTS

In this study, 384 cattle were examined to determine the prevalence of bovine babesiosis. The majority of the animals were local breeds (91.9%) and female (69.8%) animals. Most cattle had poor body conditions and the majority of them were (86.7%) reared under an extensive management system. Among the 384 cattle examined 20 (5.2%) were found to be infected with *Babesia*. The dominant *Babesia* species identified in the study area was *Babesia bovis* 15 (3.91%) followed by *Babesia bigemina* 5 (1.30%). A univariable logistic regression analysis was carried out to examine the associations of age, sex, breed, body condition score, and management system categories of the cattle with the prevalence of *Babesia* Table 1. The prevalence of babesiosis was higher in cross breed 19 (5.4%) cattle than in local 1 (3.2%) breed of cattle but, the prevalence was not significantly varied among breed of cattle ($P > 0.05$). A univariable logistic regression revealed that cattle of poor body condition score were more likely to be affected by *Babesia* than good-conditioned animals (OR = 6.25; 95% CI: 0.74%-53.1%). The odds of the infection of babesiosis in poor conditioned cattle were 6.25 times more likely than in cattle of good body condition score with 0.74%-53.1%. N, Number examined; BCS, Body condition score; †, Reference category; MLE, Maximum Likelihood Estimate; SE, Standard Error; OR, odds ratio; CI, confidence interval

Prevalence

In this study, the overall mean packed cell volume (PCV) value of the sampled animals was $24.6\% \pm 1.8$. The mean PCV values among

Babesia infected and uninfected cattle variables were compared using student's t test (Table 2). The mean PCV values of examined animals significantly ($P < 0.05$) varied with infection status. The mean PCV value of *Babesia* infected ($22.3\% \pm 1.3$) cattle was significantly ($P < 0.05$) lower than that of uninfected ($24.7\% \pm 1.7$) animals.

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The overall prevalence of Bovine babesiosis in the selected district of East Wollega was 5.2%, in which is markedly lower than the previous findings of 12.8% (50/390) reported from in and around Jimma Southwestern Ethiopia, 16.9% (65/384) reported from Teltele district, northwest Borana zone, southern Ethiopia. On the other hand, it is higher than the report of Wodajnew and Sitotaw who reported a prevalence of 1.5% (6/402) and 0.9% in and around Assosa Woreda Benishangul Gumuz regional state Western Ethiopia and at Bishou respectively. This variation in the prevalence of bovine *babesiosis* may be caused by various factors, including animal husbandry practices, anti-parasitic drug use for vector control, parasite variation in carriers of the disease over time, test sensitivity, distribution th,

SPSS: Statistical Package for Social Science

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the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Haramaya University College of Veterinary Medicine approved this study. A formal letter was written to the district veterinary clinics. The study purpose was explained, and the permission to get sample from animals was granted.

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The authors declare that they have no conflicts of interest.

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E N and J G were involved in the design of the study; E N. collected data. A E and E N analyzed the data and drafted the manuscript. E N, J G, and A E revised the manuscript; all authors approved the final manuscript.

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