Sero-epidemiology of Contagious Bovine Pleuropneumonia in the Bench-Maji Zone, southwest Ethiopia

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Abstract

Contagious bovine pleuropneumonia (CBPP) is a highly contagious respiratory disease in cattle that affects close to 30 countries in sub-Saharan Africa. In Ethiopia, it is one of the major diseases causing reduced cattle productivity and lower performance, particularly in the pastoral areas, and poses a threat to the livestock export market. A cross-sectional study aimed at estimating the seroprevalence and assessing the associated risk factors of CBPP was conducted between December 2018 and May 2019. For this purpose, a pre-tested semi-structured questionnaire survey and a serological analysis of serum samples from 715 cattle were carried out in three districts selected from the Bench-Maji Zone. The sera were tested with a competitive enzyme-linked immunosorbent assay (c-ELISA). Accordingly, a total of 162 (22.7%) cattle were tested seropositive. The seroprevalence was 32.3% in Meanitshasha, 19.2% in South Bench, and 2.8% in the Shey Bench district. The study found that breed, district (agro-ecology), and history of the CBPP outbreak were the risk factors for CBPP seropositivity identified by a generalized linear mixed model. The seroprevalence of CBPP was significantly higher in crossbred cattle (Adjusted Prevalence Ratio (APR) = 4.5; p <0.001), cattle from the Meanitshasha (lowland) district (APR = 13.9; p <0.001), from South Bench (midland) district (APR = 6.9; p = 0.001) and herds with a history of CBPP outbreaks (APR = 1.4; p = 0.009). The seroprevalence found in the present study indicates that CBPP is a common threat to cattle production in the Bench Maji zone. Therefore, all actors involved in the livestock sector should work together to achieve the successful implementation of strategies to control the disease. It is also important to note that a well-coordinated approach should be addressed with an effective
vaccination campaign to prevent the further spread of the disease and lower the prevalence of the disease in the area.

**Keywords**: Bench-Maji Zone; Cattle; CBPP; Ethiopia; Prevalence Ratio; Seroprevalence.

**Introduction**

With an estimated human population of over 114 million (Worldometer, 2020), Ethiopia is an agricultural country employing almost 85% of the workforce and generating 40% of GDP and 90% of total export earnings (Asresie and Zemedu, 2015). Livestock is an integral part of agriculture, contributing about 45% of the total value of agricultural production and providing livelihoods for a large part of the population (FAO, 2019). In addition, the country's livestock potential is enormous, with an estimated 70.3 million cattle, 52.46 million goats, and 42.9 million sheep (CSA, 2021). Similarly, livestock is viewed as an asset or an integral part of livelihood as it fulfills multiple functions by providing food, inputs to crop production and soil fertility, cash income, as well as savings, fuel, social functions, and employment (Asresie and Zemedu, 2015).

However, due to a large number of factors, this enormous resource makes a disproportionate contribution to national income (in particular export earnings and food production). Low animal genetic potential for production traits, poor nutrition, and widespread diseases are the main barriers contributing to the low productivity of local breeds (Duguma et al., 2012a; Duguma et al., 2012b). Diseases such as contagious bovine pleuropneumonia (CBPP) cause tremendous disruption to the country's livestock industry and livelihoods by affecting animal health and the production, availability, and quality of animal food (Behnke, 2010).

Contagious bovine pleuropneumonia (CBPP) is caused by *Mycoplasma mycoides* subspecies *mycoides* small colony (*MmmSC*). It is a highly contagious respiratory disease of cattle that affects around 30 countries in sub-Saharan Africa. The Pan African Program for the Control of Epizootics (PACE) had already identified CBPP as the second most common transboundary disease in Africa after rinderpest. Transmission occurs through direct and repeated contact between sick and healthy animals and the principal route of infection is the inhalation of infectious droplets from active or carrier cases of the disease.
(Constable et al., 2017). The disease has a significant economic impact on cattle owners due to high mortality rates, loss of production, disease control costs, loss of weight and workability, delayed marketing, decreased fertility, loss due to quarantine, loss of cattle trade, and reduced investment in livestock production (Tambi et al., 2006; Constable et al., 2017).

In Ethiopia, field studies have shown that CBPP poses a major threat to cattle production in different parts of the country (Ebisa et al., 2015; Teklue et al., 2015; Daniel et al., 2016; Abdela and Yune, 2017; Geresu et al., 2017; Mamo et al., 2018; Fulasa et al., 2020). Moreover, reports from various export quarantine centers in the country (Kassaye and Molla, 2013; Dele et al., 2014; Attafe et al., 2015) seem to indicate a huge threat to the livestock export markets. The disease also affects investments in the livestock sector through direct losses (in terms of mortality and reduced milk, live weight, fertility, and traction) and indirectly through the cost of control measures and the resulting trade ban (Abera et al., 2016; Demil, 2017). While the annual financial losses due to CBPP for 12 sub-Saharan African countries are estimated at 45 million euros, Ethiopia loses around 15 million euros annually, more than any of these countries (Tambi et al., 2006). Although prevalence reports differ from place to place and the effects may have changed over time, the occurrence of this disease still requires serious attention and necessitates the development and implementation of a rational control strategy to circumvent the above effects. Therefore, it requires a thorough understanding of the epidemiology of the disease, taking into account the risk factors, the pattern of occurrence, and detailed information specific to different ecological and management systems. Nonetheless, high-quality data on the prevalence and associated risk factors of CBPP in the Bench Maji Zone (southwest Ethiopia) are scarce, except for syndrome-based outbreak reports from MoA. Therefore, this study aims to estimate the seroprevalence in three districts of the Bench Maji Zone and to examine the relationship of identified risk factors to seropositivity.

Materials and methods

Study area

The Bench Maji zone (Figure 1) is located in the southwestern part of Ethiopia in the Southern Nations, Nationalities, and Peoples Regional State (SN-NPRS), about 561 kilometers from Addis Ababa. It is bordered by the Kaffa zone (north), the Debub Omo zone (southeast), the Sheka zone (northwest), the
Gambella Regional State, and the Republic of South Sudan (southwest). The zone lies between 5° 33’ to 7° 21’ north latitude and 34° 88’ to 36° 14’ east longitude (BMZARD, 2014; unpublished report). It has 10 districts (known locally as “woredas”), three of which were selected for this study. These are Meanit-shasha, South Bench, and Shey Bench. The zone has different elevations from 500 to 2500 meters above sea level and has three agro-climatic divisions with 45% lowland, 40% midland, and 15% highland. The average annual rainfall is between 400-2000 mm, while the average annual temperature is between 15 °C and 27 °C. The livelihood of the society in Bench Maji is mainly based on a mixed farming system (arable and livestock), which includes sedentary and agro-pastoral activities as well as pastoralism in the lowlands of the zone.

Figure 1 Map of Bench Maji Zone (BMZARD, 2014; unpublished report)
Study animals

This study included cattle raised on an extensive production system in three selected districts in the Bench Maji zone. Cattle from six months of age (both sexes) without a history of CBPP vaccinations were included. Age was determined according to Pace and Wakeman (2003) and divided into young (<3 years old) and adults (3 years and older) based on the categorization of experimental studies (Bashiruddin et al., 2005). The body condition assessments were carried out in accordance with Agdex factsheet 420/40-1 (http://www.agric.gov.ab.ca/) (Anonymous, 2019) and classified as poor (1 and 2), moderate (3) and good (4 and 5).

Sampling procedure

A multistage sampling technique was used to select districts, kebeles, and herds of cattle. Districts were purposively selected as the primary sampling units, while kebeles and herds were selected at random as the secondary and tertiary sampling units. Kebeles are the smallest administrative units in Ethiopia. The selection of districts was based on agroecology and livestock population. Of the three selected districts, the Meanitshasha district consisting of 31 kebeles represents the lowlands; the South Bench district with 26 kebeles represents the midland, while the Shey Bench district with 20 kebeles represents the highlands, making a total of 77 kebeles. Within each study district, at least 10% of the kebeles were selected that represented the characteristic agroecology of the area. Accordingly, four kebeles from Meanitshasha, three from South Bench, and two from Shey Bench district, for a total of nine, were selected to conduct this study. In this study, a group of cattle grazing on communal land was considered as ‘herd.’ In each Kebele, a list of communal grazing areas (herds) was drawn up in collaboration with community animal health workers. A total of 35 herds with a herd size of 34 to 358 cattle were identified, 25 of which were selected at random. Of these, 12 were from Meanitshasha, nine from the South bench, and four from Shey Bench district. Of the 25 selected herds, 19 (76%) use communal pastures mixed with other herds, while 6 (24%) had their grazing area and were managed without contact with other herds. Individual animals from the herds were selected by a systematic random sampling procedure.
Sample size determination

The sample size required for the study was calculated using the formula by Thrusfield (2018). The necessary parameters were specified for this. This included an expected prevalence of 8.1% based on a previous study (Mamo et al., 2018), a 95% level of confidence, and an absolute error of 2%.

\[ n = \left( \frac{Z^2 \cdot P_{exp} \cdot (1-P_{exp})}{d^2} \right) \]

Where \( n \) is the required sample size, \( d \) is the absolute desired precision, \( P_{exp} \) is the expected prevalence, and \( Z \) is the value from the standard normal distribution that reflects the confidence level used (1.96 for 95%). Accordingly, the sample size was calculated to be 715. The sample size was distributed proportionally to the districts based on the cattle population (i.e., 45% for Meantishasha, 40% for South bench, and 15% for Sheybench). Therefore, a sample size of 322, 286, and 107 cattle, respectively, were considered for the districts of Meantishasha, South bench, and Sheybench. At the herd level, individual cattle were systematically selected for blood sampling as described above.

Questionnaire data collection

During the course of the study, data on potential risk factors such as age, sex, breed, body condition, production system, housing, agro-ecology, introduction of new stock, herd size, trekking, herd contact (watering point, grazing, and market), vaccination, and the history of occurrence of CBPP was collected through face-to-face interview using a pre-tested semi-structured questionnaire.

Blood sample collection

From the selected animals, a 10 ml blood sample was aseptically drawn from the jugular vein using vacutainer tubes and then kept in a slant position in the shade for 24 hours. The sera were collected using cryovials, labeled and transported in the freezer to the Mizan Regional Veterinary Laboratory, and stored at -20 °C until analysis. The serum samples were then analyzed with a competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) kit for the detection of CBPP antibodies according to the manufacturer’s instructions.
Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)

Antibodies against *MmmSC* were detected using a test (CBPP serum competition ELISA/Version: P05410/02) developed by CIRAD-UMR15 (CIRAD/ Institut Pourquier) [http://www.institut-pourquier.fr](http://www.institut-pourquier.fr). The test is based on a monoclonal anti-*MmmSC* antibody (Mab 177/5) (OIE, 2014). Briefly, two plates were used for each sample run, the pre-plate, and the *MmmSC* precoated plates.

Serum samples were diluted and mixed with the specific monoclonal antibody (Mab 117/5) in a dilution plate (“pre-plate”). The resulting mixture was then transferred to the *MmmSC*-coated micro-plate. This enables all of the specific antibodies present in the test sera to bind to the *MmmSC* antigen and thus compete with the Mab for the specific epitope. After washing, an anti-mouse IgG serum conjugated to horseradish peroxidase (HRP) was added. The presence of *MmmSC* specific antibodies in the test sera would displace the Mab and therefore the conjugate may not be able to bind to the Mab (block). Alternatively, the absence of *MmmSC* specific antibodies in the test sera allows the MAb117/5 to bind to its specific epitope, thereby allowing the conjugate to bind freely. After another series of washes, the HRP substrate Tetra methyl Benzedrine (TMB) was added to form a blue compound that turns yellow when the reaction is stopped. The underside of the plate was wiped and the optical density (OD) of each reaction was measured at 450 nm using a plate reader. The color intensity is an inverse measure of the proportion of *MmmSC* antibodies that are present in the test sera; the reactions were monitored using positive and negative control sera supplied with the kit included in each microtiter plate to validate the results. The cut-off point was calculated using the results of a monoclonal control (Cm, 0% inhibition) and a conjugate control (Cc, 100% inhibition) and set to 50%. In addition, the percentage inhibition value (PI) for each sample was calculated using the following formula:

\[
PI = 100 \times \left( \frac{OD \; Cm - OD \; Test}{OD \; Cm - OD \; Cc} \right);
\]

Where OD Cm is the optical density for the monoclonal control; OD test is the optical density for the test serum; OD Cc is the optical density for the conjugate control. Finally, the results for serum samples with PI values of less than 50% were interpreted as ‘negative’ and for the presence of *MmmSC* antibodies for the PI above 50% as ‘positive’. The specificity of the test (cELISA) was estimated at 99.9% according to this developed method, but the sensitivity was 63.8% (OIE, 2014).
Data management and analysis

The data collected from the study were entered into the MS Excel 2010 spreadsheet program and then filtered and coded prior to analysis. Statistical analysis was performed with STATA version 14.2 (StataCorp, 4905, Lakeway Drive, College Station, Texas, USA). The overall seroprevalence or the proportion of seropositivity per risk factor was calculated as the number of seropositive animals divided by the total number of animals tested. Since the prevalence obtained in the present study was high (>10%), we estimated the prevalence ratio (PR) and its confidence interval as a measure of the association instead of the odds ratio (OR). This is because, with frequent binary outcomes, usually with a prevalence of more than 10%, the PR may be overestimated by the OR if the PR is greater than 1, or underestimated if the PR is less than 1 (Martinez et al., 2017). We used a generalized linear mixed model (GLMM) in a Poisson link with robust variance to estimate PR and to assess the association of CBPP seroprevalence with different exposure factors. Variables that were significant in the univariable analysis were fitted to a robust multivariable model after evaluating the variables for multicollinearity. In this analysis, the statistical significance was set at $p < 0.05$.

Results

Questionnaire survey

Out of 85 respondents, 78 (91.8%) knew about CBPP and named the local name “Samba” in the South Bench and Shey Bench districts, while “Fofi or fofa” in the Meanitshasha district. They also ranked CBPP first on the list of mentioned cattle diseases in the Meanitshasha and second in the South Bench districts. Of those who recognized CBPP, 36 (46.2%) had reported an outbreak in their herd and described seeing similar signs in the affected group of animals. Accordingly, 27 (35%) reported signs of coughing on exertion, 52 (66.7%) reported foreleg abduction, 39 (50%) extension of the head and neck, and 33 (42.3%) reported mixed clinical signs including difficulty breathing. Moreover, about 44 (56.4%) respondents also mentioned some post-mortem lesions such as the presence of serous fluid and the build-up of fibrin in the thoracic cavity. Furthermore, 33 (42.3%) reported having treated their sick animals with antibiotics, but 12 (15.3%) had been treated with local herbs / traditional remedies, while 36 (46%) sold their animals because they did not respond to any of the treatments. The respondents also stated that their animals make contact with
others at watering points, communal grazing land, in search of water and pasture, and on their way to the market.

**Sero-prevalence of CBPP**

The overall seroprevalence of CBPP at the animal level was 22.7% (95% CI: 19.7-25.9) in the study districts. The seroprevalence was significantly higher in the Meanitshasha district (32.3%), which is in the lowlands than in the South bench (19.2%) district from the midland and Sheybench (2.8%) from the highlands. Of the 25 herds, at least one seropositive animal was detected in 22 (88%). Seroprevalence at herd-level varied from 0% to 100% (Table 1).

**Table 1. Sero-prevalence of CBPP in cattle in three districts of Bench Maji Zone**

<table>
<thead>
<tr>
<th>District</th>
<th>Herd level</th>
<th>Animal level</th>
<th>Prevalence (%)</th>
<th>Prevalence (95% CI)</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meanitshasha</td>
<td>12</td>
<td>12</td>
<td>100 (73.5-100)</td>
<td>322</td>
<td>104</td>
</tr>
<tr>
<td>South bench</td>
<td>9</td>
<td>8</td>
<td>88.9 (44-99)</td>
<td>286</td>
<td>55</td>
</tr>
<tr>
<td>Sheybench</td>
<td>4</td>
<td>2</td>
<td>50 (8.4-91.6)</td>
<td>107</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>22</td>
<td>88 (66.7-96.4)</td>
<td>715</td>
<td>162</td>
</tr>
</tbody>
</table>

*95% confidence interval

**CBPP risk factors**

Using the GLMM analysis, the potential variables assessed for their association with CBPP seroprevalence were age, sex, breed, BCS, herd size, district, herd history of the CBPP outbreak, and long-distance trekking. In the univariable analysis, all the hypothesized risk factors except age and sex were found to be significantly \( p < 0.05 \) associated with the seroprevalence of CBPP (Table 2). Therefore, the multivariable model was adjusted for the breed, BCS, herd size, district, history of the CBPP outbreak in herds, and long-distance trekking. Accordingly, the variables identified as significantly \( p < 0.05 \) associated with CBPP in the final model were breed, district (agro-ecology), and history of CBPP occurrence in the herd. The final model showed that the prevalence of CBPP in crossbred animals was 4.5 times higher \( p < 0.001 \) than local and
1.43 times higher in cattle selected from herds with a history of CBBP outbreaks \((p = 0.009)\) than in cattle without an outbreak history. In addition, the prevalence in South Bench \((p = 0.001)\) and Meanitshasha \((p < 0.001)\) was 6.85 and 13.9 times higher, respectively than in the Sheybench district (Table 3).

Table 2. Univariable analysis of CBPP seroprevalence with different risk factors using Poisson regression with robust variance

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>No. tested</th>
<th>No. Positive</th>
<th>Prevalence (%)</th>
<th>Unadjusted PR*</th>
<th>95% CI for PR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Young</td>
<td>145</td>
<td>29</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>570</td>
<td>133</td>
<td>23.3</td>
<td>1.2</td>
<td>0.82 - 1.67</td>
<td>0.399</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>115</td>
<td>24</td>
<td>20.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>600</td>
<td>138</td>
<td>23</td>
<td>1.1</td>
<td>0.75 - 1.62</td>
<td>0.621</td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td>Local</td>
<td>674</td>
<td>143</td>
<td>21.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross</td>
<td>41</td>
<td>19</td>
<td>46.3</td>
<td>2.2</td>
<td>1.52 - 3.13</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>Good</td>
<td>316</td>
<td>58</td>
<td>18.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>298</td>
<td>71</td>
<td>23.8</td>
<td>1.3</td>
<td>0.95 - 1.77</td>
<td>0.098</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>101</td>
<td>33</td>
<td>32.7</td>
<td>1.8</td>
<td>1.24 - 2.56</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Herd size</td>
<td>Small</td>
<td>270</td>
<td>34</td>
<td>12.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>231</td>
<td>54</td>
<td>23.4</td>
<td>1.9</td>
<td>1.25 - 2.74</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>214</td>
<td>74</td>
<td>34.6</td>
<td>2.7</td>
<td>1.91 - 3.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>District (agro-ecology)</td>
<td>Shey Bench</td>
<td>107</td>
<td>3</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South bench</td>
<td>286</td>
<td>55</td>
<td>19.2</td>
<td>6.9</td>
<td>2.19-21.48</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Meanitshasha</td>
<td>322</td>
<td>104</td>
<td>32.3</td>
<td>11.5</td>
<td>3.73-35.57</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>History of CBPP outbreak</td>
<td>No</td>
<td>530</td>
<td>97</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>185</td>
<td>65</td>
<td>35.1</td>
<td>1.9</td>
<td>1.47 - 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Long-distance trekking</td>
<td>No</td>
<td>499</td>
<td>93</td>
<td>18.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>216</td>
<td>69</td>
<td>31.9</td>
<td>1.7</td>
<td>1.31 - 2.24</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
*Prevalence ratio
Table 3. Factors associated with CBPP seroprevalence in a GLMM model

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>Adjusted PR*</th>
<th>95% CI for PR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>Local</td>
<td>4.5</td>
<td>3.05 - 6.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Cross</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>District (agroecology)</td>
<td>Shey Bench</td>
<td>6.9</td>
<td>2.14 - 21.91</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>South bench</td>
<td>13.9</td>
<td>4.19 - 46.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Meanitshasha</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of CBPP outbreak</td>
<td>No</td>
<td>1.4</td>
<td>1.09 - 1.87</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Prevalence ratio

Discussion

The present study indicated that CBPP posed a potential threat to cattle production in various districts of the Bench Maji zone in southwest Ethiopia. The results of the questionnaire survey showed that the disease was one of the main problems faced by the cattle farmers. Farmers in the study area described the local names of CBPP, clinical signs, and postmortem lesions in their local languages that are very similar to signs and lesions of the disease already documented in the literature. The fact that the respondents recognized the disease to the extent mentioned is an indication of the farmers’ indigenous knowledge that they were able to name the diseases and their clinical signs in the local languages. The increased ability to detect the disease could be due to the endemic nature of the disease in the study area, with new cases being recorded regularly due to frequent outbreaks.

In the present study, the respondents gave CBPP the highest-ranking among the other major infectious diseases that were observed in the study area. Therefore, this finding agrees with that of Teklu et al. (2015) and Yohannes (2007), who topped the CBPP ranking first, followed by CCPP, anthrax, blackleg, and pasteurellosis as the most important infectious diseases observed in their respective study areas. Similarly, Gedlu (2004) reported that CBPP ranks first in the Somali regional state, and Birhanu (2014) found that CBPP ranks sec-
ond among the most common infectious animal diseases affecting the livestock trade in Ethiopia.

A significant number of respondents (42.3%) in the current study indicated antibiotic treatments in their animals suspected of having CBPP, but some later sold them for a lack of response. In line with this, elsewhere in Ethiopia, a large proportion of respondents (69.0%), who also used antibiotics to treat cattle with suspected CBPP, reported similar information (Huebschle et al., 2004). The increased use of antibiotics by the respondents can be an attempt to alleviate the suffering of the affected cattle. However, complete elimination of the causative organism cannot be achieved and, due to the increase in carriers that could spread infections and resistant strains to antibiotics, offers the chance of a potential pool of diseases (Huebschle et al., 2004; Yaya et al., 2004; Ayling et al., 2005; Mariner et al., 2006).

The current finding of an overall seroprevalence of 22.7% using the c-ELISA in cattle in this study indicates the above facts. Reasons for this can be persistent infections, contact between cattle from different herds at grazing and watering points, incomplete and irregular control measures as well as the introduction of infected cattle, which promote the transmission of infection. It could also be a consequence of the nature of the disease, if carriers are present in some herds, thereby maintaining the infection and gradually spreading the disease (Aliy et al., 2017). The current seroprevalence is quite comparable to that of other studies reported using c-ELISA from different parts of Ethiopia, particularly 25.3% in the Sidama Zone (Malicha et al., 2017) and 28.5% in the west of Oromia (Daniel et al., 2016). However, it is below 39% in the Somali Regional State (Gedilu, 2004), 31.8% in the Amaro Special District (Ebisa et al., 2015), and 56% in the North Omo Zone (Dejene, 1996). This difference may be due to agro-ecology, herd dynamics, or the production system involved. Conversely, it is higher than many other previous reports such as 8.7% in Bishofu (Atnafie et al., 2015), 8.1% in Gimbo district (Mamo et al., 2018), and 10% in the Dassenech district in the South Omo zone (Molla and Delil, 2015). This variation could also be attributed to the population density, the number of animals examined, the animal movements, and the sensitivity of the serological tests used.

This study also showed that breed is a significant predictor of seropositivity. The likelihood of CBPP seropositivity in Holstein-Friesian x Zebu crosses was 4.5 times higher than that of pure indigenous Zebu cattle. This shows that
pure local breeds are more resistant to CBPP than those carrying exotic blood. Breed differences in susceptibility to CBPP have also been reported elsewhere (Billy, 2014; Geresu et al., 2017).

This study also confirmed that seropositivity for CBPP was significantly linked to agro-ecology (districts), with seropositivity in cattle from Meanitsha (lowlands) and Southbench (midlands) being 13.9 and 6.9 times higher, respectively, than cattle from Sheybench (highlands). This is in line with Abdela and Yune (2017), where seroprevalence is significantly associated with agroecology, as reports of the highest seroprevalence of CBPP come from lowland areas of Ethiopia where 40% of the livestock are kept, as does Alhaji et al. (2016). The occurrence of large numbers of cattle in Meanitsha (lowlands), followed by South Bench (midlands), and least of all in Sheybench (highlands) in the current study area and the corresponding recorded results suggest the above idea. Cattle in the low and midland areas tend to move around in search of pasture and water, creating closer contact with others at grazing and watering points depending on pasture availability, especially in the dry season, while cattle in the highlands are kept near homesteads and have little contact with others. This has already been demonstrated by Thiaucourt et al. (2004) as documented factors of the greatest importance for the rate of spread of CBPP, such as contact density, infection intensity, and the number of susceptible animals in a certain ecology, which could well agree with the current result.

The present study also identified a previous outbreak of CBPP in a herd as a risk factor for CBPP seropositivity. It was found that seropositivity was higher in herds with a history of CBPP infection. Cattle selected from herds with a history of a CBPP outbreak were 1.43 times more likely to detect CBPP antibodies than cattle from herds with no history of an outbreak. It is stated that when CBPP does occur in a herd, 25% of infected animals remain as recovered carriers with or without clinical signs and may shed *Mycoplasma* in “lungers” for up to two years after recovery (Constable et al., 2017). Therefore, it is common to find more seropositive cattle when testing herds with a history of CBPP outbreaks.

**Limitation of the study**

It is worth noting that the diagnostic method used, the limited number of crossbred animals that were sampled, and the low proportion of animals taken from non-communal grazing lands are the main limitations of this study. Re-
Regarding the diagnostic technique, we considered the c-ELISA as a screening test to classify cattle as seropositive or negative for CBPP exposure. Although the c-ELISA is known to be more sensitive in detecting cattle with chronic infections, it tends to miss recently infected animals. To counter this problem, an attempt was made to carry out an immunoblotting test in parallel with the c-ELISA, but unfortunately, no test option was made available to us. Therefore, our data is based solely on the results of the c-ELISA test and we encourage readers to consider this. For this purpose, the use of an additional test should be considered in future studies. The other limitation of the present study is that it was conducted in the dry season and consequently the seroprevalence obtained may not reflect the actual extent of the disease in the area. In contrast, previous studies in the country have shown that the disease had a prevalence of almost twice as high in the wet season of the year compared to the dry season (Molla and Delil, 2015). Therefore, future studies in the current area should consider different times of the year to get a true picture of the disease.

Conclusions

By and large, the present study has shown that CBPP appears to be fairly common in the study area and requires due attention. For this reason, all stakeholders, including animal owners, veterinarians, and the government, should work together to successfully implement control measures in this area, especially in the lowlands. Systematic disease surveillance, mass vaccination, and control of animal movements or herd contact are therefore recommended as practicable strategies to reduce the prevalence of the disease in the Bench-Maji zone to a negligible level.

Ethics approval and consent to participate

The Hawassa University Research Ethics Review Committee ruled that no formal ethics approval is required to conduct this research. However, before conducting the study, informed consent was obtained from the owners of the cattle herders who participated in this study.

Availability of supporting data

The datasets used and/or analysed during the current study are available from the authors on reasonable request.
Conflict of interests

The authors declare that there is no conflict of interest.

Authors' contributions

WK conceived the study, collected all necessary data, performed the laboratory work, and drafted the paper, while JBH designed the study. Both RA and JBH were involved in the data analysis, the interpretation of the results, and the critical revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors thank Mizan Regional Veterinary Laboratory for providing laboratory and field facilities and Hawassa University for providing financial support. The owners of all herds of cattle who participated in this study are warmly thanked for their cooperation in collecting samples.

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