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Contagious bovine pleuropneumonia: Seroprevalence and its associated risk factors in selected districts of Afar region, Ethiopia

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Abstract

Background: In pastoral and lowland areas of the country particularly in Afar region, studies suggested higher prevalence of contagious bovine pleuropneumonia (CBPP) than mid and highland agro-ecologies. Though CBPP is a prime constraint to cattle productivity in the region, research outputs pertaining to CBPP are unavailable compared to highland areas. Thus, the objectives of the current study were to determine seroprevalence of CBPP and assess risk factors in selected districts of Afar region.

Methods: A cross-sectional study was conducted on cattle aged 6 months and above from February 2018 to January 2019 in selected districts of the region. A total of 420 blood samples were collected and sera were separated for further serologic analysis. Using competitive enzyme linked immunosorbent assay (c-ELISA), antibodies against *Mycoplasma mycoides subspecies mycoides small colony* (MmmSc) were detected at National Veterinary Institute, Ethiopia. Data were analysed using Stata version 14.0.

Result: Of 420 samples tested by c-ELISA, 158 samples were found to be positive for CBPP providing an overall seroprevalence of 37.6%. Among the three risk factors considered (age, sex and district) assessed, only two (age and district) were found to be associated significantly with the disease ($p < 0.05$) at 95% CI and p-value less than 5% applying logistic regression.

Conclusion: The study has revealed a higher prevalence of CBPP over the study areas urging a coordinated act to be set in place.

KEYWORDS
Afar region, cattle, CBPP, risk factors, seroprevalence

List of Abbreviations: CBPP, contagious bovine pleuropneumonia; c-ELISA, competitive enzyme linked immunosorbent assay; CSA, Central Statistical Agency; FAO, Food and Agricultural Organization; FMD, foot and mouth disease; MmmSc, *Mycoplasma mycoides subspecies mycoides* small colony type; NGOs, nongovernmental organizations; NVI, National Veterinary Institute; OIE, Office of International Des Epizootics; PCR, polymerase chain reaction; PFE, Pastoralist Forum Ethiopia; SNNP, Sothern Nations and Nationalities People; SPSS, statistical package for social science

1 | BACKGROUND

Livestock significantly contribute to the world economy, household income, food security, draft power for crop cultivation, high-quality animal proteins and vitamins (meat and milk), manure, hide and skin and sustain livelihoods (Bonnet et al., 2011). Major constraints to low productivity of cattle include low genetic potential of the animals, poor nutrition and prevailing diseases (Belay et al., 2012). Among the prevalent diseases, foot and mouth disease, contagious bovine pleuropneumonia (CBPP), lumpy skin disease, trypanosomsis, external parasites and tick borne diseases are indicated. CBPP is a transboundary cattle disease and categorized as the only bacterial disease in the OIE list A diseases (OIE., 2000). In Africa, CBPP was introduced first into South Africa in 1854 through importations of cattle and spread to other countries in the region (Andrews et al., 2004). The disease is endemic in almost all African countries and remained to be a threat to the livestock in the region (Tambi et al., 2006).

CBPP is a highly infectious, acute, subacute or chronic septicaemic cattle disease affecting the lungs, pleura and occasionally joints in calves (Tambi et al., 2006) caused by *Mycoplasma mycoides subspecies mycoides* small colony (OIE., O.I.D.E., 2008). The principal transmission of CBPP is by the inhalation of infective droplets from active animals or carrier cases of the disease (Radostits & Gay, 2008). CBPP is clinically characterized by anorexia, fever, weakness, emaciation, dyspnoea, polypnoea, cough and nasal discharges. CBPP is diagnosed by clinical signs, pathologic lung lesions, identification and isolation of the agent, immunoblotting, serology and PCR techniques (Goffe & Thiaucourt, 1998). CBPP causes reduced productivity, disruption of trade, causes suffering, disrupts food supplies system, retards genetic improvement, increases disease control costs, reduces draft power, causes direct losses, adds labour costs and profit losses (Tambi et al., 2006).

1.1 | CBPP status in Ethiopia

No recorded document as to when and how the CBPP exactly entered to Ethiopia (Amanfu, 2009) and it is an important economic disease (Thomson., 2005). CBPP is endemic in Eastern Africa including Ethiopia (Lesnoff et al., 2004). The highest number of outbreaks reported in 20 African countries was recorded in Ethiopia according to OIE (OIE., O.I.D.E., 2008). An economic loss analysis in 10 African countries, including Ethiopia, has estimated an annual loss of 14,987,000 million Euros attributed to CBPP threat, as shown in a study conducted by Tambi et al. (2006). In Ethiopia, CBPP has been reported in different regional states of Ethiopia with an overall seroprevalence like 7.13% in Afar, 1.29% in Amhara, 12.05% in Benishangul Gumuz, 19.72% in Gambella, 5.17% in Oromia, 5.44% in Southern Nations Nationalities and People (SNNP), 0.9% in Somali and 6.11% in Tigray (Darsema, 2011).

1.2 | CBPP in arid and semi-arid areas: The case of Afar region

CBPP is reported to be a major constraint to cattle production in the arid and semi-arid pastoral areas (Kairu-Wanyoike et al., 2013). CBPP has continued to devastate cattle of the region on which many people are dependent in the lowlands. Significantly higher seroprevalence was found in animals in the lowland than highland and mid highland agroecologies (Mamo, 2016). The persistence of CBPP in pastoral areas could be attributed to the migratory lifestyle of pastoralists leading in-turn for uncontrolled movement of cattle with continuous mixing at grazing fields, watering points and difficulty accessing vaccination services (Amanfu, 2009). Though CBPP is a prime constraint to cattle productivity in Afar region, research outputs pertaining to CBPP are unavailable compared to highland areas of the country. Therefore, the objectives of the current study were to determine seroprevalence of CBPP and assess risk factors of the disease.

2 | MATERIALS AND METHODS

The study was conducted in three selected districts of Afar region, namely Dubti, Asaita and Chifra. All are situated in Zone one of Afar region. The Afar region is one of the nine federal states of Ethiopia located in the northeastern part of the country. The region is geographically located between 39°34' and 42°28' East Longitude and 8°49'and 14°30'North Latitude. The region comprises 5 administrative zones, 32 districts and 331 kebeles, 28 towns, and 401 rural and urban kebeles.

2.1 | Study design

A cross-sectional study design was applied to determine the seroprevalence of CBPP and associated risk factors in the selected study sites.

2.2 | Study population

All indigenous Afar cattle aged 6 months and above reared by pastorals and agropastorals in the selected sites were used for the study.

2.3 | Sampling technique and sample size determination

Random sampling techniques were applied for selection of study animals (cattle) and study areas. Study kebeles or peasant associations, households and study units/individual cattle were selected using simple random sampling technique. As no previous study conducted on CBPP in cattle found in the selected areas, the present study has considered 50% expected prevalence, 95% confidence level and

5% absolute precision or marginal error. Based on these assumptions, the total number of animals to be included in the study got determined using the formula given by Thrusfield (2007):

$$n = \frac{1.96^2 \times P_{exp} \times (1 - P_{exp})}{d^2},$$

where n is the required sample size, d is the desired absolute precision and P_{exp} is expected prevalence (50%).

Based on the formula, the total sample size was computed to be 384 cattle to be selected from all three districts. To minimize chance and increase precision of the outcome, the total number of study animals was increased to 420. Proportionally, a total of 128, 130 and 162 were collected from Asaita, Dubti and Chifra districts, respectively, based on density of cattle population in the districts. The ages of the cattle were grouped into young (1–2 years), adult (3–8 years) and old (> 8 years) according to Abera et al. (2010).

TABLE 1 Summary of descriptive statistics

Variable	Samples tested	c-ELISA test result		Percentage (%)
		Negative (*n*)	Positive (*n*)	
Sex				
Female	377	234	143	34.0
Male	43	28	15	3.6
District				
Dubti	130	89	41	9.8
Asayita	128	59	69	16.4
Chifra	162	114	48	11.4
Age				
Young	48	29	19	4.5
Adult	249	170	79	18.8
Old	123	63	60	14.3

2.4 | Methodology

A total of all 420 sera samples each amounting to 8–10 ml of whole blood were collected from jugular vein of cattle into disposable plain vacutainer tubes using 21 Gauge needle. Just following collection, vacutainer tubes were labelled and transported to laboratory and kept overnight at room temperature to allow the blood clot. Correspondingly, each sample was identified along with age, study district and sex. Coded sera were transferred to cryogenic vials and stored in −20°C refrigerator at Samara University Veterinary Medicine Microbiology Laboratory to the time of transportation to National Veterinary Institute (NVI) for sera analysis.

2.5 | Competitive enzyme linked immunosorbent assay

As recommended by OIE for CBPP test, competitive enzyme linked immunosorbent assay (c-ELISA) technique was applied at National Veterinary Institute (NVI) serology laboratory, based on the manufacturer's instruction (CIRAD-EMVT, France) (Amanfu et al., 2000). C-ELISA test is based on a monoclonal anti-MmmSC antibody named Mab 177/5. Technically, using microplates precoated with MmmSC purified lysate, test samples were premixed with the specific monoclonal antibody Mab117/5 in a separate plate and the mix was transferred to the precoated microplate with MmmSC antigen. Any MmmSC-specific antibodies present in the sample will form an immune complex with MmmSC antigen coated on the microplate competing with Mab117/5 for the specific epitope. Following the wash of unbounded material, an anti-mouse antibody enzyme conjugate was added. In the presence of immune complex between MmmSC antigen and antibodies from the sample, Mab117/5 cannot bind to its specific epitope and the conjugate is blocked from binding to Mab117/5. On the other hand, in the absence of MmmSC antibodies in the test sample, Mab117/5 can bind to its specific epitope and the conjugate is free to bind to Mab 117/5. Unbound conjugate was washed away and enzyme substrate Tetra methyl Benzedrine was added. In the presence of the enzyme, the substrate is oxidized and develops a blue colour, which becomes yellow after adding the stop solution. Subsequent colour development was inversely proportional to the amount of anti-MmmSC antibodies in the test sample. In the end, optical density (OD) of individual reactions was measured at 450 nm using a plate reader and samples having OD values greater 50 or more were considered positive.

2.6 | Data management and analysis

Data were coded and fed into Microsoft Excel for further statistical analysis. Applying Stata version 14.0 (Stata Corp, Texas, USA, 2015) descriptive statistics was computed to calculate the overall prevalence of CBPP and other prevalence associated with risk factors. Association between risk factors and the disease positivity was assessed using Chi-square ($\chi 2$). Bivariate logistic regression was computed to estimate the magnitude association between risk factors and the disease. Risk factors having significant association with the disease were further analysed by multivariate logistic regression analysis using 95% confidence level (CI) and p-value less than 0.05.

3 | RESULTS

3.1 | Descriptive findings

Descriptive statistics was employed to calculate the proportion of risk factors (season, sex, age and district) with respect to test result. The total collected sera were tested using c-ELISA. Accordingly, the total number of positive samples for each variable category with its respective percentage has been computed and summarized (Table 1).

TABLE 2 Bivariate and multivariate logistic regression analyses

	c-ELISA test result		Binary logistic regression analysis		Multivariate logistic regression analysis	
Variable	Negative	Positive	COR (95%CI)	*p*-value	AOR (95%CI)	*p*-value
Age						
Young	29	19	0.6878 (0.349–1.355)	0.280	0.923 (0.429–1.981)	0.837
Adult	170	79	0.488 (0.313–0.759)	0.001	0.607 (0.362–1.0173)	0.058
Old	63	60	1		1	0
District						
Dubti	89	41	1		1	0
Asaita	59	69	2.537 (1.528–4.217)	0.000	2.509 (1.505–4.185)	0.000
Chifra	114	48	0.9139 (0.554–1.508)	0.725	1.089 (0.619–1.913)	0.768

Of all serum samples tested ($n = 420$) by c-ELISA in search of *Mycoplasma mycoides subspecies mycoides* small *colony type* (MmmSc)-specific antibody, 158 serum samples were found to possess MmmSc-specific antibody. Hence, the overall seroprevalence of CBPP over the three study districts was calculated to be 37.6% with 95% CI (32.97–42.27).

3.2 | Association of risk factors and CBPP

Only two risk factors (age, $p = 0.036$) and district, $p = 0.000$) were found to be associated with CBPP. However, sex has no significant association with CBPP occurrence ($p = 0.696$). Further, using 95% CI and $p < 0.05$, bivariate logistic regression analysis was computed for age and district to estimate the magnitude of association (crude odds ratio = COR) with the disease (Table 2).

Independently analysed by binary logistic regression, age and district were found to be associated significantly with the disease. To compute real significant contribution of associated risk factors without compounding effect on the other, multivariate logistic regression analysis was employed with 95%CI and $p < 0.05$, only district has been associated significantly ($p = 0.000$) with the disease but age has no significant impact on CBPP occurrence among different age group. Those animals found in Asaita district were 2.54 times more likely at risk of acquiring CBPP as compared to those animals found in Dubti district.

4 | DISCUSSION

Based on the magnitude of the current overall prevalence observed (37.6%) over the three selected Afar districts, CBPP could be considered as a prime concern for cattle health and productivity in the particular study districts as well in Afar region. It could also be inferred that CBPP is more prevalent in Asaita district (16.4%) as compared to Dubti (9.8%) and Chifra (11.4%) districts. It could be due to ecological and seasonal difference and herd immunity as well in the study districts.

In Ethiopia, the prevalence of CBPP varies from the lowest prevalence of 0.4% reported by Alemayehu et al. (2014) in Borena Zone to the highest CBPP prevalence in Ethiopia (96%) reported by Yigezu and Roger(1997) from Western Gojam. Similarly, the magnitude of CBPP prevalence from different parts of Ethiopia varies extremely. According to reports of various outbreaks, national serological surveillance and research results from 1997 to 2010, CBPP has been confirmed to be present in almost all regional states of Ethiopia. Compared to the overall CBPP prevalence of 37.6% reported by the current study in Afar region, the findings from other lowland areas of Ethiopia were observed to be lower. A study performed by Roger and Yigezu (1995) indicated a 5.1% CBPP prevalence in North Omo, which was far more lower compared to the current report in Afar region. Similarly, another study carried out by Issa (2004) reported a 5.1% prevalence of CBPP in Borena pastoral areas, which was more than seven times lower compared to the report of Afar region. Similarly, 10.3% prevalence in Somali region by Mekonnen (2004), 4% prevalence on export quarantine centres in and around Adama by Kassaye and Molla (2013), 11.0% prevalence in Southern Tigray by Teshale et al. (2015), 28% prevalence from Bodji district of Western Wollega by Fikru (2001) and 9.1% from Northwest Ethiopia prevalence by Gashaw (1998) were reported in which all had recorded a far more lower prevalence comparatively.

However, the current finding closely agrees with the reports of Gedlu (2004), who reported 39% prevalence from Somali Regional State. Similarly, the current report also closely agrees with the report of 32.5% prevalence in Western Ethiopia (Desta, 1998). Conversely, the current finding was lower than other prevalence reports: From Western Gojam and Awi Zone, Gashaw (1998) has reported 66.3% in Banja district and 41.7% in Dangila district. Similarly, a study has reported 56% CBPP prevalence in North Omo (Dejene, 1996), and other similar finding has reported a prevalence of 48% in Ilu Ababor and Wellega (Western Ethiopia) (Desta, 1998). Moreover, Roger and Yigezu (1995) reported 46% CBPP prevalence in Konso of SNNP, and Yigezu and Roger (1997) reported 74% CBPP prevalence in Borena Zone and 75% prevalence of CBPP in Western Wellega zone.

As indicated by different scholars, the prevalence of CBPP varies from area to area in Ethiopia as well the across African continent in general. The variation of findings on CBPP seroprevalence in different parts of Ethiopia could be due to variation in temporal and spatial

distribution of the disease agroecological system, animal management (husbandry practices), biological or breed difference, communal grazing areas, production system, cattle population density, herds size, number of examined animals, livestock movement and sensitivity of serological tests used to evaluate the seroprevalence (Daniel et al., 2016; OIE., O.I.d.E., 2014).

In the current study, district, age and sex were considered as risk factors. Accordingly, their association and strength of association with disease was statistically computed. Based on the statistical computations, study district was found to have statistically significant association ($p = 0.000, \chi 2 = 20.924$) with the disease. Similarly, age of animals was also significantly associated ($p = 0.006, \chi 2 = 100.292$) with the disease seropositivity. On the other hand, sex ($p = 0.696, \chi 2 = 0.153$) had no significant association with the disease.

The current study has revealed the seroprevalence of CBPP at the districts to be 9.8%, 16.4% and 11.4% for Dubti, Asaita and Chifra, respectively, indicating differences with statistically significant variation ($p = 0.000$) in the prevalence of antibodies among the districts. Other studies have reported similar findings (Daniel et al., 2016; Mersha, 2016). The significant variation among study districts might be attributed to the presence of large number of livestock population within the districts, the presence of communal grazing and watering areas, degree of confinement/crowding, husbandry practices, agroecologic difference and degree of cattle movements (OIE., O.I.d.E., 2014).

The current study has indicated infection rates of 4.5%, 18.8% and 14.3% for young, adult and old age groups, respectively. Unlike sex, age had statistically significant association ($p = 0.006, \chi 2 = 100.292$) with CBPP occurrence. However, multivariate logistic regression analysis showed that the significant association of age with disease found by bivariate logistic regression analysis was found to be nonsignificant (Table 2) implying that age has no real contribution to CBPP occurrence.

In the current study, the prevalence of CBPP in young animals was lower than both adult and old animals. In adult animals, the prevalence was observed to be higher than young animals. The higher prevalence in adults would be attributed to the fact that young animals do not move far away from houses; therefore, there is less chance to come into contact with infected animals. In addition, it is reported that young animals are more susceptible to acute forms of CBPP than adult cattle and thus acutely infected young animals may die of CBPP and not be available for testing. The current finding agrees with reports of Bashiruddin et al. (2005), who reported that with age, variation infection resistance could also vary. According to Bashiruddin et al. (2005), animals less than 3 years of age are less resistant to CBPP by experimental studies. In two separate experiments, it was shown that cattle over 3 years of age were more resistant to CBPP infection than younger animals. In addition, the present result is also in close agreement with previous reports by Swai et al. (2013), who reported that sero-positivity in adults was relatively higher in young animals.

The prevalence of CBPP in adult cattle was higher as compared to old and young animals. However, the prevalence of CBPP in young animals was still lower than older cattle comparatively. The reduction in prevalence in old and young animals may be due to developed resistance and acquired immunity, respectively.

In the current study, infection rate by sex was computed to be 3.6% and 34.0% for male and female, respectively. However, there was no statistically significant difference with sex ($p = 0.696, \chi 2 = 0.153$). Even if there was no statistically significant difference ($p > 0.05$), the higher prevalence of CBPP in female cattle was in agreement with reports of Schnier et al. (2006), who reported a significantly higher prevalence in female animals. Similarly, the existence of difference in the prevalence of CBPP between male and female was also reported by Teshale et al. (2015). The variation in the prevalence between male and female animals could be due to sampling error or the nonproportionality of samples collected, level of stress and degree of contact with young susceptible young/calves. Similarly, the current finding agrees with the report of Mersha (2016). However, the insignificant ($p < 0.05$) difference of seroprevalence between sex may be due to similar exposure of animals to the disease since the disease is contagious to all animals in the herd and can be affected and a single diseased animal can serve as continuous source of infection to the whole herd. The disease is mainly transmitted from animal to animal in aerosols. This organism occurs in saliva, urine, foetal membranes and uterine discharges (Radostits & Gay, 2008). This could play a great role in the uniformity of infection in both sexes.

5 | CONCLUSION

The current study has provided convincing evidence against CBPP in Afar region. The magnitude of the overall seroprevalence of the disease over the three selected districts suggests higher prevalence indicating the magnitude of economic challenge posed on cattle owners. Furthermore, the study has identified contributory risk factors for the disease occurrence. Of the three risk factors assessed, age and study sites (districts) have indicated to have significant association with seropositivity of the disease. In conclusion, the study findings strongly urge a prompted and coordinated interventional to be taken forward.

DECLARATIONS

Ethics approval and consent to participate.

As the work or research was not experimental, no ethical approval was needed. However, to take blood from animals, the oral consent of the animal owners was obtained.

CONFLICT OF INTEREST

The authors declared that they had no competing interests.

AUTHORS' CONTRIBUTIONS

TD was involved in data collection, proposal review, assisted in data analysis, and preparation of manuscript. WN contributed by preparing proposal, data collection, data analysis, and article write up.

DATA AVAILABILITY STATEMENT

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

PEER REVIEW

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