Seroprevalence and associated risk factors of contagious bovine pleuropneumonia in three districts of Ilu Ababor Zone, Oromia, Ethiopia

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Abstract

Contagious bovine pleuropneumonia is caused by *Mycoplasma mycoides* subspecies *mycoides* small colony *(Mmm SC),* which causes significant economic loss in Sub-Saharan Africa, including Ethiopia. A cross-sectional study using systematic random sampling technique was conducted in Bacho, Bure, and Dorani districts of Ilu Ababor Zone to estimate seroprevalence and identify associated risk factors of contagious bovine pleuropneumonia (CBPP) in apparently healthy cattle with no history of CBPP vaccination. Thus a total of 371 blood samples were collected from the jugular vein of each cattle and tested using competitive Enzyme Linked Immunosorbent Assay (c-ELISA) to detect specific antibodies to *Mmm SC*. Data on potential risk factors for the occurrence of CBPP were collected using a questionnaire survey. Data obtained from both serological and questionnaire surveys were analyzed with SPSS version 20 software. Multivariable logistic regression was used to analyze the association of the exposure variables with *Mmm SC* serostatus, and the strength of the association was assessed using the odds ratio. The overall animal and herd-level seroprevalence of CBPP was 9.7% and 30.7%, respectively. The seroprevalence of CBPP in the Bacho, Bure, and Dorani districts was 0%, 10.8%, and 33.3%, respectively. There was a statistically significant association between Mmm SC antibody and district, age, and body condition score (p <0.03) at the individual animal-level. At the herd-level herd size was significantly associated (p <0.00) with *Mmm* SC antibody. This study indicated that CBPP was prevalent in the Dorani and Bure districts. So, methods should be devised to control the disease in the districts and prevent its spread, because CBPP is a contagious disease.

Keywords: CBPP; c-ELISA; Ilu Ababor; Risk factors; Seroprevalence.

Introduction

Contagious bovine pleuropneumonia (CBPP) is a transboundary animal disease primarily affecting cattle. It is highly contagious and has a high impact on animal production and health in Sub-Saharan Africa. In Ethiopia, field studies have shown that CBPP poses a significant threat to cattle production in different parts of the country (Ebisa *et al.,* 2015; Teklue *et al.,* 2015; Daniel *et al.,* 2016; Fulasa *et al.,* 2020).

Moreover, reports from various researchers in the country indicate considerable threat to the livestock export markets (Kassaye and Molla, 2013; Dele *et al.,* 2014; Atnafie *et al.,* 2015). The disease has a significant economic impact on cattle owners and livestock investments through direct losses (in terms of mortality, reduced milk, traction, loss of weight and workability, delayed marketing, and decreased fertility). Indirect losses are attributed to the cost of control measures and the resulting trade ban, as well as compromising food security through protein loss and the painful suffering of animals. Also, the disease retards genetic improvement (Abera *et al.,* 2016; Constable *et al.,* 2017; Demil, 2017).

There are possible challenges that increase the risk of disease in Ethiopia, which include lack of knowledge of the disease by farmers, vaccine shortage, poor diagnostic assays, management system; limitation of epidemiological information about the disease; concentration of livestock at watering points and grazing areas; and difficulty in controlling cattle movements. As a result, it is one of the most severe threats to cattle health in the study area. This disease continues to pose a threat to the livestock export market and reduce investment in livestock production in Ethiopia (Ebisa *et al.,* 2015).

Various animal diseases with unknown causative agents have been reported in western Oromia, with the primary signs being a respiratory problem, affecting livestock production and productivity and threatening the livelihoods of small-scale farmers. Ilu Ababor zone is one of the zones in western Oromia where there was no study, particularly in the current study districts (Bacho, Bure and Dorani), on the sero-prevalence and risk factors of CBPP, except for the study conducted in 1998, in a neighboring district with similar agro-ecology to the present study areas (Desta, 1998). Also, there was a report of cattle deaths in Bacho, Bure and Dorani districts. Lack of sufficient epidemiological information on livestock diseases in the area, contact of animals at grazing

and watering points which make the ease for disease spread, the difficulty of detecting carrier animals, limited resources to apply slaughter and compensation mechanisms, and the inability to restrict animal movement, all present a challenge for disease control. As a result, this study was planned to assess the magnitude of CBPP in non-vaccinated animals in three purposively selected districts in the southwestern region of Ethiopia.

Materials and methods

Description of the study area

The study was conducted in the Bacho, Bure, and Dorani districts of Ilu Ababor Zone, Oromia regional state, Ethiopia.

Bacho is located at a distance of 620 km from Addis Ababa at southwest direction. The district has 17 peasant associations, and the district is located at 8°16' N latitude and 35°48' E longitude, with an altitude of about 1875 meters above sea level. The minimum and maximum temperature of the area is 23°C and 27°C, respectively. The district has 115, 626 cattle, 18661 sheep, 28981 goats, 8861 equines, 111,220 poultry, 1152 dogs and 115 cats (BaADO, 2021).

Bure district is located in the southwest direction at about 680 km from Addis Ababa at 8°59' N latitude and 37°19' E longitude with altitudes ranging from 1450-1770 meters above sea level with temperatures ranging from 22 to 29°C. The district has 21 peasant associations. The animal population of the district includes 91,101 cattle, 28,298 sheep, 28,370 goats, 163 horses, 159 mules, 4,282 donkeys, 154, 364 Poultry, 1,519 dogs and 152 cats (BuADO, 2021).

Doreni district is located from Addis Ababa at a distance of 584 km in southwest direction at 8°26' N latitude and 35°51' E longitude, with an altitude range from 1845 to 1950 meters above sea level and rainfall of 700 mm. The temperatures of the area range from 23 to 29 °C. The animal population of the district comprises 41,792 cattle, 27,904 sheep, 15,397 goats, 14,846 equines, 76,996 poultry 967, dogs and 97 cats (DADO, 2021).

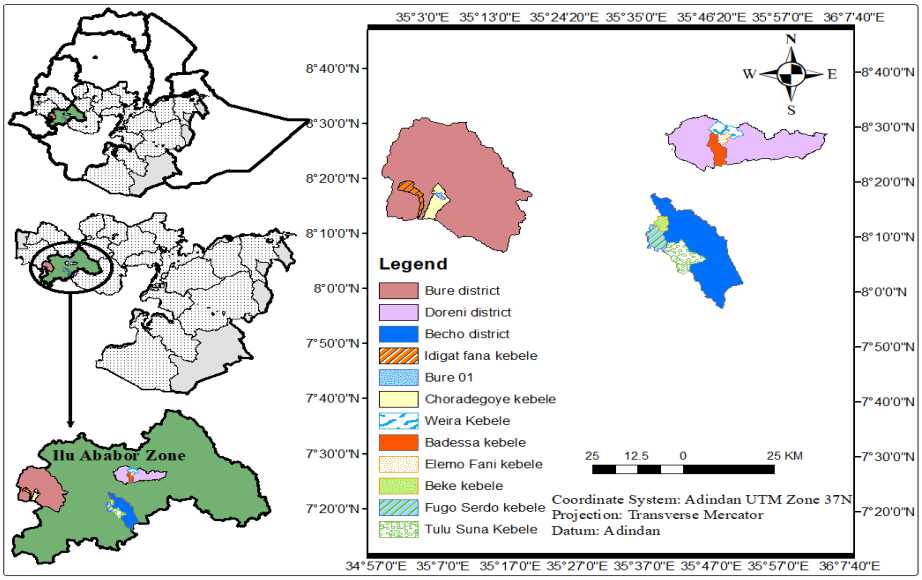

Figure 1. Map of Ethiopia and Oromia National Regional State depicting the study area (Arch GIS 10.4).

Study population and study animals

The study population consisted of all indigenous zebu cattle above six months of age, managed under extensive and semi-intensive management systems, and without a history of CBPP vaccination (information on previous CBPP vaccinations was obtained from the district veterinary office).

Study design and sample size determination

Study design

A cross-sectional study was conducted using a systematic random sampling technique to select the study animals. List of herd distribution and household size were obtained from the peasant associations (PA), and then both blood sample collection and questionnaire surveys were conducted. Pre-tested semi-structured questionnaire was used to collect information on factors influencing the occurrence of CBPP within or between herds using face-to-face interview. The age of animals was determined based on the owners' information and dental eruption,according to De Lahunta and Habel (1986).

Sample size determination

The sample size required for the study was determined using the formula given by Thrusfield (2007), with a 5% desired absolute precision, a 95% confidence interval, and an expected prevalence of 32.5% (Desta, 1998).

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

Where N= is the required sample size of the study population, d= is the desired absolute precision, and Pexp= p is the previous/expected prevalence.

Accordingly, the total sample size was computed to be 337 cattle. However, to increase the precision of the outcome, the total number of study animals sampled from all three districts was increased to 371 by 0.1% precision.

Sampling technique

Both purposive sampling and systematic random sampling techniques were employed for the selection of study area and individual animals, respectively. Nine PAs (Bure 01, Core Dagoye and Idigat Fana from Bure district, Elemo Fani, Weira and Badessa, from Dorani district and Fugo Sardo, Tulu Suna, and Bake from Bacho district) were selected based on cattle population, recommendation from zonal and district experts, previous disease history and access to road facility. Accordingly, 45 cattle from Bake, 74 cattle from Fugo Sardo, 50 cattle from Tullu Suna, 53 cattle from Bure 01, 50 cattle from Idigat Fana, 36 from Chore Dagoye, 22 from Baddessa, 18 cattle from Elemo Fani, and 23 from Weira peasant association were selected.

Data collection

Questionnaire survey

A pretested semi-structured questionnaire was developed, and conducted through face-to-face interview using the local language of the community (Afan Oromo). A total of 75 cattle owners were involved in the interview during the sampling of animals to collect relevant data on the presumed risk factors of CBPP.

The questionnaires comprised of questions on factors influencing the occurrence of CBPP, such as animal origin, disease history, herd size, management

system, contact of herds with another herd, the introduction of a new animal, history of commonly observed symptoms and major clinical sign of CBPP; and age and sex of animal were collected. The body condition of the sampled animals was determined following the body condition score (BCS) assessment method set by Nicholson and Butterworth (1986), and the animals were categorized into three categories, namely poor body condition (1-3 BCS), medium body condition (4-6 BCS), and good body condition (7-9 BCS).

Blood sample collection

Blood samples were collected from the jugular vein of each animal using sterile vacutainer tubes following an aseptic procedure, and each sample was appropriately labeled. The blood samples were kept in an inclined position for 3 to 5 hours at room temperature to allow clotting of blood and then centrifuged to separate serum from blood.

The serum was transferred gently into serum storage vials (cryogenic vials) stored in an icebox and transported to the Bedelle Regional Veterinary Laboratory. Upon reaching the laboratory, the serum samples were stored at -20°C until tested. Finally, serum samples were examined for antibodies against CBPP using a c-ELISA, which is based on a monoclonal anti-*Mmm SC* antibody (Mab 117/5) test at Bedelle Regional Veterinary Laboratory.

Laboratory examination

The c-ELISA technique, as recommended by the OIE for CBPP testing, was used to examine the serum samples at Bedele Regional Veterinary Laboratory in accordance with the manufacturer's instructions (CIRAD-EMVT, France) (Amanfu *et al.,* 2000). The c-ELISA test is based on a monoclonal anti-*Mmm SC* antibody known as Mab 117/5 (OIE, 2014). The microplates were coated with *Mmm SC* purified lysate. The sera to be tested were premixed with Mab117/5 specific monoclonal antibodies in a prelate, and the content of the prelate was transferred into the coated microplate.

The immune complex reaction between any *Mmm SC* antibodies found in the serum sample forms an immune complex with Mmm SC 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 an immune complex between *Mmm SC* antigen and antibodies in the sample, Mab117/5 cannot bind to its specific epitopes, and the

conjugate is prevented from binding to Mab117/5. On the other hand, in the absence of Mmm SC antibodies in the test sample, Mab117/5 can bind to its specific epitope, and the conjugate is free to bind to Mab117/5.

The unbound conjugate was washed away, and the enzyme-substrate Tetramethyl Benzedrine (TMB) was added. In the presence of the enzyme, the substrate is oxidized and develops a blue color but changes to a yellow color after the addition of the stop solution. The amount of anti-*Mmm SC* antibodies in the test sample was inversely proportional to the subsequent color development. Finally, the underneath of the plate was wiped, and the optical density (OD) of individual reactions was measured at 450 nm using a plate reader.

The result is expressed in percentage of inhibition by comparing the optical density in the test well with the optical densities in the Mab control wells. The percentage inhibition (PI) value for each sample was calculated by the following formula.

$$PI = \frac{(OD\ Mab - OD\ test\ serum) \times 100\%}{(OD\ Mab - OD\ Conjugated)}$$

Where OD Mab denotes the optical density of a monoclonal antibody, OD Test serum denotes the optical density of a test serum, and OD conjugate denotes the optical density of a conjugate.

Samples with a percentage inhibition of ≤ 40% were considered negative for the presence of Mmm SC antibodies. In comparison, samples with a percentage inhibition of 40% >, less than 50% were considered doubtful, and those with a percentage inhibition of ≥ 50% were considered positive (OIE, 2014).

Data management and analysis

All collected data was entered and coded into a Microsoft Excel spreadsheet and imported to the SPSS version 20 software for analysis. The total seroprevalence of individual animals was calculated by dividing the number of c-ELISA positive samples by the total number of animals tested. Herd level seroprevalence was calculated by dividing the number of positive herds by the total number of herds tested. If at least one animal in a herd was seropositive, the herd was considered seropositive.

Risk factors which, showed *p* values less than 0.25 in the univariable logistic regression were chosen for the final multivariable logistic regression analysis. The Odds Ratio (OR) was used to assess the strength of the association between the risk factors and the occurrence of the disease. Pearson correlation coefficients were used to test for co-linearity among the variables. Then, the final multivariable model was created using backward elimination method.

Ethical clearance

Ethical clearance for the study was obtained from the ethical review committee of Mamo Mezemir Campus School of Veterinary Medicine, Ambo University. The aim of the study was explained, and permission was obtained from animal owners prior to sample and data collection. Blood samples were collected by the researcher following good animal handling practices to minimize animal suffering.

Results

Seroprevalence of contagious bovine pleuropneumonia (CBPP)

Of the total of 371 sampled animals, 36 animals were seropositive for CBPP. The overall animal-level seroprevalence of CBPP was 9.7% (95% CI: 6.7-12.7). Out of the total 75 sampled herds, 23 herds were found to be infected, and the overall herd-level seroprevalence of CBPP was 30.7% (95%CI: 20.5–42.4).

Risk factors associated to seroprevalence of CBPP

Animal-level risk factors

From individual animal-level risk factors across the study district, significantly higher seroprevalence was observed in the Dorani district (33.3%) than in Bure (10.8%) and Bacho district (0%). This difference might be due to number of study animals, climate condition, socio-economic factors, and trade-routes. The study also showed that seropositivity was higher in females (10.9%) than in males (6.9%). Lower seroprevalence was observed in the young age (6 months to less than equal to 3 years) group (2.5%) than the adult (greater than three years) age group (14.9%). Poor body condition animals (14.9%) had a higher prevalence as compared to good (2.1%) body condition score animals. The seroprevalence of CBPP was highest in animals bought from a local market (11.6%) as compared to their source origin (9.3%) (Table1).

Table 1. Univariable logistic regression analysis of animal-level Seroprevalence.

Risk factors	Categories	Animals tested	№ positive (%)	95%CI	OR (95%CI)	*p* value
District	Bacho	169	0 (0.0)	0-2		
	Bure	139	15 (10.8)	6.0-17		
	Dorani	63	21 (33.3)	22-46	3.7 (1.9- 8.7)	0.002
Sex	Male	116	8 (6.9)	3.0-13		
	Female	255	28 (10.9)	7.0-15	1.6 (0.7-3.7)	0.220
Age	Young	157	4 (2.5)	1.0-6.0		
	Adult	214	32 (14.9)	10-20	6.7 (2.3-19.4)	0.000
BCS	Good	47	1 (2.1)	0.0-11		
	Medium	117	4 (3.4)	1.0-9.0	1.6 (0.7-14.9)	0.666
	Poor	208	31 (14.9)	10-20	4.9 (1.7-14.4)	0.003
Animal Origin	Born	302	28 (9.3)	6.0-13		
	Bought	69	8 (11.6)	5.0-22	1.3 (0.56-2.9)	0.557

Herd-level risk factors

The risk factors associated with the seroprevalence of CBPP at the herd level were herd size, management, introduction of new animals from the market, herd contact site, and season. Results of the study indicated that animals from large herd sizes showed a higher seroprevalence (50%) than medium herd sizes (19%) and small herd sizes (10%). From the management system, a prevalence of 35.2 % was obtained in herds reared under an extensive management system compared to a prevalence of 19% in herds reared under a semi-extensive management system. The seroprevalence of CBPP in herds with a history of new animal introduction, 35.3%, was higher than the prevalence of 20.8% in herds without the introduction of a new animal. Herds that had contact with other herds at the grazing site had shown lower seroprevalence, 28.5%, than herds that had contact with other herds at the watering and grazing site, with prevalence of 32.5%.

Table 2. Univariable logistic regression analysis of herd level risk factors.

Risk factors	Categories	Animals tested	№ positive (%)	95% CI	OR (95% CI)	*p* value
Herd size	Small	20	2 (10.0)	1-32		
	Medium	21	4 (19.0)	5-42	2.1 (0.34-13.1)	0.419
Management	Semi extensive	21	4 (19.0)	5-42		
	Extensive	54	19 (35.2)	23-49	2.3 (0.7-7.8)	0.181
New animal introduction	No	24	5 (20.8)	7-42		
	Yes	51	18 (35.3)	22-50	2.1 (0.66-6.5)	0.210
Herd contact area	Grazing place	35	10 (28.5)	15-46		
	Grazing and watering	40	13 (32.5)	19-49	1.2 (0.45-3.2)	0.713
Season	Rainy season	27	6 (22.2)	9-42		
	Dry season	48	17 (35.4)	22-51	1.9 (0.65-5.6)	0.240

Multivariable logistic regression analysis indicated that cattle from Dorani district were about four times (OR: 4.1, 95% CI:1.9-8.7, $p = 0.00$) more likely to have CBPP seropositivity of Mmm SC antibody circulation than cattle from Bacho districts. Adult cattle were five times (OR: 5.2, 95% CI: 1.2-19.2, $p = 0.01$) more likely to have CBPP than young. Poor body condition animals were two times (OR: 2.1, 95% CI: 1.5-8.6, $p = 0.03$) more likely to have CBPP seropositivity than good body condition score animals (Table 3).

Table 3. Multivariable analysis of potential risk factors for contagious bovine pleuropneumonia at individual animal level.

Risk factors	Categories	Animals tested	№ positive (%)	OR (95% CI)	*p* value
Districts	Bacho	169	0 (0)		
	Bure	139	15 (10.8)		
	Dorani	63	21 (33.3)	4.1(1.9-8.7)	0.00**
Age	Young	157	4 (2.5)		
	Adult	214	32 (14.9)	5.2(1.2-19.2)	0.01*
BCS	Good	47	1 (2.1%)		
	Medium	117	4 (3.4)		
	Poor	208	31 (14.9)	2.1(1.5-8.6)	0.03*

PA: peasant association; OR: odd ratio; CI: confidence interval; *: statistically significant.

From herd-level risk factors, a large herd size was two times (OR: 2.2, 95% CI: 1.1-15.6, p = 0.00) more likely to have CBPP seropositive than a small herd size.

Discussion

In this study, the overall animal-level seroprevalence of CBPP was 9.7%. A relatively similar result was reported by different investigators in different parts of Ethiopia and other countries: 8.6% in the Gudeya Bila and Boneya Boshe districts of the Eastern Wollega zone (Neggasa *et al.,* 2020), 8.7% in Bishoftu (Teklue *et al.,* 2015), 9.4% in Borena (Ahmed 2004), 9.5% in export quarantine center of Adama (Kassaye and Molla, 2012), 10% in Dassenech district of South Omo zone (Molla and Delil, 2014), 8.1% in the Gimbo district of southwest Ethiopia (Mamo *et al.,* 2018), 9.7% in Southwestern Kenya (Schnier *et al.,* 2006), 8.1% in Mali (Sery *et al.,* 2014), 8% in northeast states of Peninsular Malaysia Pertanika (Zarina *et al.,* 2016) and 10.65% in the Kwara state of Nigeria (Olabode *et al.,* 2013).

However, the overall seroprevalence of the present study was lower than previous research reports which include 96% in Western Gojam and 74% in Borena Zone (Yigezu and Roger, 1997), 66.3% in Banja of western Gojam (Gashaw, 1998), 46% in Konso of SNNP (Roger and Yigezu, 1995), 39% in Somali Regional State (Gedlu, 2004), 37.6% in the selected districts Afar region (Negash and Dubie, 2021), 32.5% in Ilu ababor of western Ethiopia (Desta, 1998), 31.8% in Amaro district of SNNP region (Ebisa *et al.,* 2015), 28.5% in western Oromia (Daniel *et al.,* 2016; Mersha, 2016), and 25.3% in Sidama Zone (Malicha *et al.,* 2017).

On the other hand, the present result was higher than several earlier reports from various parts of the country, including a 0.4% prevalence from the export quarantine center in Adama (Erimiyas *et al.,* 2014), 4% from Adama and its surroundings (Kassaye and Molla, 2012), 6.14% from Southern Ethiopia (Asmamaw, 2003), 1.39% from the Bale zone (Lemu and Worku, 2017), and 3.4% from South Wollo zone (Juhar, 2020). The variation of seroprevalence reported might be due to differences in the degree of cattle movement, population density (degree of confinement or crowding), sample size, and the types of tests used to determine the seroprevalence.

District had statistical association with *Mmm SC* antibody and this result agreed with previous studies conducted in different parts of Ethiopia like in western Oromia by Mersha (2016), in the Bako Tibe and Ilu Galan districts of West Shewa Zone by Fulasa *et al. (*2020), in Bench-Maji Zone of southwest Ethiopia by Kebede *et al.* (2022), in East Wollega Zone by Wakgari *et al.* (2018) and a study in the Afar region by Negash and Dubie (2021), where district/location affected seroprevalence. The statistically significant association between district and *Mmm SC* antibody circulation might be due to the presence of large herd sizes, and contact of animals at grazing and watering points in the study area.

In this study adult age group had significantly (p<0.05) higher occurrence of CBPP which agreed with several earlier reports from different corners of Ethiopia (Ebisa *et al.,* 2015; Teklue *et al.,* 2015; Mersha, 2016; Wakgari *et al.,* 2018; Juhar, 2020; Mersha *et al*., 2020; Negash and Dubie, 2021) several African countries (Thomson, 2005; Matua-Alumira *et al.,* 2006; Schnier *et al.,* 2006); Mtui-Malamsha, 2009; Elhassan and Elsadig, 2012; Swai *et al.,* 2013; Suleiman *et al.,* 2015; Alhaji and Babalobi, 2016)), where all noted the significant association of adult age (p<0.05) with occurrence of CBPP disease. This might be due to the fact that adult animals move long-distances in search of pasture and water, and they contact with herds from other households.

Animals with poor body condition had significantly higher seropositivity to Mmm SC antibodies which agreed with observations of several previous studies from various parts of Ethiopia (Biruhtesfa *et al.,* 2015; Ebisa *et al.,* 2015; Juhar, 2020; Neggasa *et al.,* 2020; Kebede *et al.,* 2022), and elsewhere in Africa (Mtui-Malamsha, 2009; Suleiman *et al.,* 2015), where animals with lower BCS had significantly higher seroprevalence. This might be due to the fact that animals with poor body condition are likely to have poor immunological response (resistance) to the infectious agent compared to animals with good body condition (Radostits *et al.,* 2007).

Conclusions

The findings showed an overall seroprevalence of 9.7% at the individual animal and 30.6% at the herd-level. Seropositivity was recorded in the Dorani and Bure districts, with a prevalence of 33.3% and 10.8%, respectively. Dorani district, adult animals and poor body condition score were the risk factors identified as having a considerable association with the prevalence of CBPP in the

study area. Therefore, regular vaccination, in seropositive areas, especially for young animals, should be given, and cattle in poor body condition should be fed well in order to help them develop resistance to diseases, including CBPP.

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