Survey on Sero - prevalence of Contagious Bovine Pleuropneumonia in Bambasi district of Benishangul Gumuz regional state, western Ethiopia

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Abstract: Contagious Bovine Pleuropneumonia is disease of cattle caused by *Mycoplasma mycoides subspecies mycoides small colony* and it is one of the most important diseases to cattle health and production in the district. Across-sectional study was carried out from December to January 2017 in six-peasant association of Bambasi district to determine the sero prevalence of contagious bovine pleuropneumonia (CBPP) and to identify the associated risk factor for the occurrence of the disease using a competitive Enzyme Linked Immunosorbent Assay (c-ELISA). In the current study a total of 421 serum sample were collected and tested. The overall sero prevalence of Contagious Bovine Pleuropneumonia was 192/421(42.51%). The sero prevalence of Contagious Bovine Pleuropneumonia at peasant associations level was Shobora (66.34%), Mutsa (43.90%), Mender 45 (24.67%), Mender 52 (52%), Keshmando number 2 (28.57%) and Mender 48 (39.58%). There is statistically significant variation ($p<0.05$) between sero prevalence result of the disease and peasant association ($\chi^2=37.75$, $p=0.00$), and also among age groups significant variation were recorded ($\chi^2=11.01$, $p=0.001$). However, other risk factors such as sex, body conditions and vaccination status were not significant associated ($p>0.05$) with the sero–status of the animal. In conclusion, the overall prevalence of CBPP in Bambasi district was higher as compared to other research reported. Therefore, it needs to design appropriate control and prevention measures to stop further spread of this economically devastating disease**.**

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1. Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious and contagious respiratory disease of *Bovidae* caused by *Mycoplasma mycoides* subsp. *mycoides* "small colony" (*Mmm*SC) with a major impact on livestock production and a potential for rapid spread. It is one of the most important infectious diseases of cattle in Africa. With the imminent eradication of Rinder pest, CBPP has become one of the most important cattle disease presenting significant setback to livestock development in Africa in general and Ethiopia in particular (OIE, 2002).

CBPP is endemic to parts of Africa, parts of India and China; with minor outbreaks in the Middle East. Countries free of CBPP include the US, UK, and Australia. In almost all African countries CBPP is a notifiable disease with official controls on the import of cattle. Despite these control, there are nomadic people which move freely across borders of certain countries such as the Fulani in West Africa and the Maasai in east Africa which may have contributed to CBPP spread. Wars, famine and inadequate financing of veterinary departments have resulted in CBPP running riot in east and central Africa (Radostitis *et al*., 2006). In World Animal Health organization, reported outbreaks of CBPP in 20 countries, with the highest number of cases in Ethiopia. Ethiopia is one of the African countries were CBPP is causing enormous economic losses through cattle morbidity and mortality.

In Ethiopia, the average physical losses from CBPP in terms of cattle deaths are 25,115 heads (8,372 in endemic and 16,743 in epidemic), 1,852 and 13,396 metric tons of beef and milk, respectively. In terms of animal power, averages of 3,135,000 ox days are lost. Ethiopia experiences the largest number of cattle deaths, and reduction in cattle products under both endemic and epidemic conditions compared to the other African countries, due probably to its large cattle population (Tambi *et al.,* 2004). Although there is systematic epidemiological investigation to show the distribution and impact of CBPP in the country, it is considered as the major disease of cattle in the country especially in pastoral and agro-pastoral areas (OIE, 2008).

In some investigation of western Oromia Regional state the overall seroprevalence of CBPP in the study areas was (28.5 %) (Danial G *et al.,* 2016). The finding was similar result to the work of (Ragassa F., 2001) which reported sero prevalence of (28%), in Bodji district of Western Wollegga and in addition, the finding of this result somewhat related to the report of

(Desta B., 1998) which was reported a sero prevalence of 32.5% in Western Ethiopia.

Therefore, Benshangul Gumuz Regional State is one of western part of Ethiopia which is bordering to western wollega of the farming communities there are different animal diseases of unknown etiologic agent which are often reported, affecting production and productivity of livestock and threatening the livelihood of small scale farmers in the area. Among the main problems reported massive cattle death attributable to diseases of unknown etiologic agent. The disease that caused massive cattle death at that time was tentatively. So far there was no systematic study conducted to look into the status of this economically important disease in the study area.

There for, the objectives were:-

- To estimate the sero-prevalence of contagious bovine pleuropneumonia in Bambasi district.
- To identify the associated risk factors for the occurrence of CBPP in the study area.

2. Materials And Methods

2.1. Study area

The study was conducted in Bambasi District from (Dec - Jan2017), which is one of district in the Benshangul Gumuz Regional State. The district is located at 45km far from Assosa, the capital city of Benshangul Gumuz Regional State. The district has an altitude ranging from 1350-1400 meter above sea level and the average annual temperature ranging from21^{0}c - 35^{0}c. The district is bordered on the south by Mao komo special district, on the southwest by Assosa district, on the North West by Oromia, and on the north Odablidglu district. It is divided into 42 peasant association. Bambasi District is characterized by a mixed crop-livestock farming system, in which herds are sedentary and large. Cattle are mostly of the local breed. Animals are used for agricultural activities, milk, meat production and manure. The district has a total population of 38,964 cattle, 3,739 Sheep, 11,990 goat, 4,467 equine 41,438 poultry and 23,423 bee hives (Bambasi district office of livestock and fishery development, 2017). Livestock production is a major component of the livelihood system and provides draught power, food and income. Geographically, it is located at 9.45- 9.75^{0}N and longitude of 34.35 - 34.88^{0}E east longitude, constituting an area of 2210.16 km^2 with human population of 62,693.

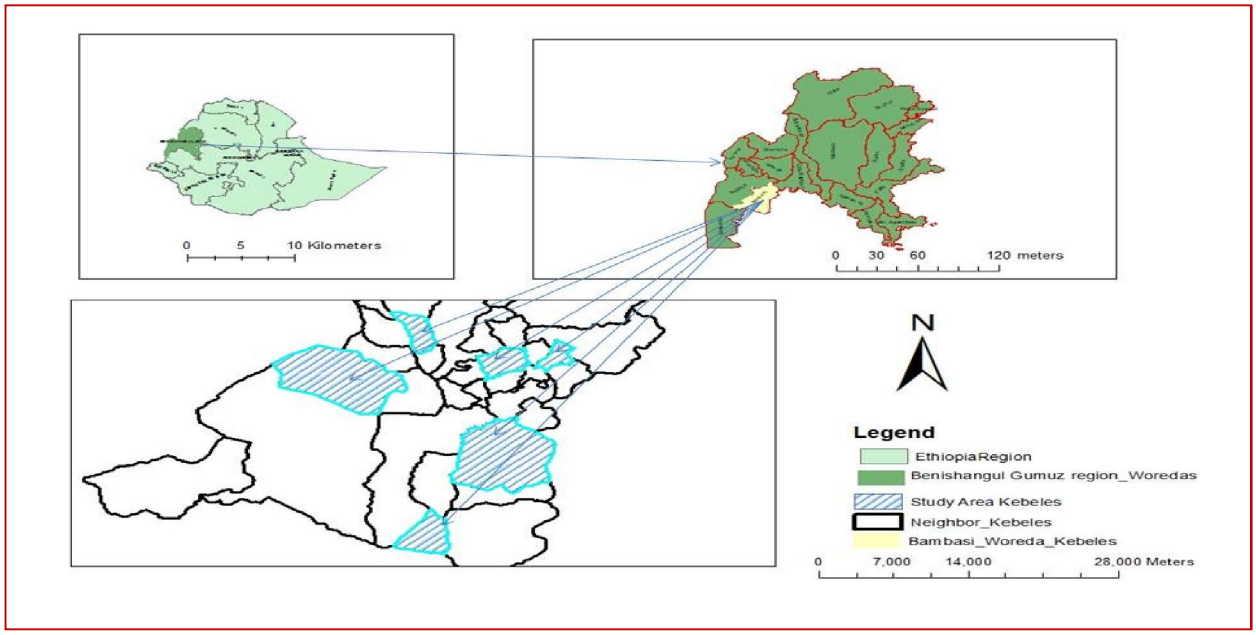

Fig. 1 Map of Bambasi District and study peasant association

2.2. Study animals

The study animals include all cattle populations which are kept under extensive husbandry systems. Both sex and age group above six month old cattle were sampled. Study animals related information on each cattle (such as sex, age, body condition) were recorded at the time of sample collection.

2.3. Study design

A cross-sectional type of study was carried out from (December - January 2017) in Bambasi district, to estimate the sero- prevalence of CBPP and to identify the associated risk factor in the study area. At the beginning of the study momentary description and discussion about the study was done with the district

animal health experts and all data are collect accordingly.

2.4. Sample size and sampling method

The number of animals sampled in the study area is estimated by using the formula described by (Thrusfield, 2005) with 95% confidence interval.

$N=1.96^2 \times p\ (1-p) \times 100/\ D2 = 384$ sample for serological examination

Where N is number of animals sampled, P is the expected prevalence of the disease and D is precision level (0.05). Expected prevalence of the disease was taken as 50% since no CBPP study conducted in the study area and to get the maximum sample size (384). To increase the precision sample size was inflated to 421. From each herd 20% of animals were selected using the random sampling procedure (Thrusfield, 2005).

The area would be purposively selected based on certain criteria i.e. cattle population, accessibility, outbreak and in addition the status of CBPP is not known yet in the area. A purposive sampling method would be followed and first peasant associations were selected from the list peasant associations. From the selected PAs animals sera samples would be collected along with other data/information to see the associated risk factors. Six peasant associations are purposively selected from the peasant association list and from each peasant association herds (households) are also selected randomly.

3. Laboratory methods

3.1. Sample collection

Animals are restrained by owners and 5-10 ml of blood sample was collected from the jugular vein using vacutainer tubes under aseptic condition. The samples are kept under the shade in a slant position for twenty four hours. The sera sample were transferred to serum tubes, labeled with a code and kept at - 20 ^0_C until they are tested. Corresponding to each sample code, the age, breed, body condition, site and sex of every animal's information are collected and registered on a separate case book. Therefore, in this study, PAs, age, sex, body condition and vaccination status were considered as risk factors.

3.2. Laboratory diagnosis

A total of 421 serum samples were collected from the study area Bambasi district and submitted to Assosa Regional Animal Health Diagnostic Laboratory (ARAHDL), and sera are examined for the presence of specific antibodies against *Mycoplasma mycoides* sub species *mycoides* small colony type by using competitive enzyme linked immune sorbent assay (c-ELISA).

Microplates are coated with an MmmSC purified lysate. Samples to be tested are premixed with a specific monoclonal Antibody Mab 117/5 in a separate plate (pre plate) and content of the "pre plate" is transferred into the coated microplate. Any *MmmSc* specific antibodies present in the sample will form an immune –complex with *MmmSc* antigen coated micro plate, competing with Mab 117/5 for the specific epitopes. After washing away unbound material, an anti- mouse Antibody enzyme Conjugate is added.

In presence of immune-complex between *MmmSC* antigen and Antibodies from the sample, Mab 117/5 can bind to its specific epitopes and the conjugate is free to bind to Mab 117/5. Unbound conjugate is washed away and enzyme substrate is oxidized (TMB) is added. In presence of enzyme, the Substrate is oxidized and develops a blue compound becoming yellow after blocking. Subsequent color development is inversely proportional to the amount of anti-*MmmSc* Antibodies in the test sample.

3.3. Data analysis

The collected data were stored in Microsoft office excel 2007 spreadsheet. Statistical analyses were performed using STATA version 11 software. The overall sero-prevalence of CBPP was determined using descriptive statistics. Sero-prevalence was calculated by dividing the number of positive test results by the total number of animals tested. Chi-square test was used to determine association between explanatory variables and the sero-status of the animals. In all analyses confidence level of 95% and p–value of 0.05 was used for statistical value.

4. Result

4.1. Prevalence of contagious bovine pleuropneumonia using c-ELISA

In the current study the sera were collected from 421 cattles among 6 peasant association and examined by using competitive enzyme linked immune sorbent assay (c-ELISA). The result of this examination reveals that 192 animals were appeared positive for disease among sampled animals.

The overall sero-prevalence of contagious bovine pleuropneumonia in the study area was (42.51%) (192/421). From the six peasant association the result indicate that (66.34%), (43.90%), (24.67%), (52%), (28.57%), (39.58%) animals were positive for the disease in Shobora, Mutsa, Mender 45, Mender 52, Keshmando no.2, and Mender 48 respectively. The highest sero-prevalence of CBPP was observed in Shobora (66.34%) whereas the lowest sero-prevalence (24.67%) in mender 45 peasant association of Bambasi district was diagnosed. There is significant variation (χ^2 =37.75, $p<0.05$) in contagious bovine pleuropneumonia sero-prevalence among peasant association shown in (Table.1).

Host related potential risk factors like sex, age, body condition and vaccination status of animal were associated with contagious bovine pleuropneumonia

sero status of animal. In the current study, there is no statistically significant association amongst (sex, body condition and vaccination status and the serological finding of the animals ($p>0.05$) but there is statistically significant difference in age categories ($p<0.05$) shown in (Table. 2).

Table 1. Sero –prevalence of CBPP disease in individual animal in the sampled peasant- association of the study area (Dec-Jan 2017).

S.N	Site (PA)	Number of animal tested	Number of Positive	Prevalence (%)	χ^2	(p-value)
1	Shobora	104	69	66.34%	37.75	0.000
2	Mutsa	82	36	43.90%		
3	Mender 45	77	19	24.67%		
4	Mender 52	75	39	52%		
5	Keshmando number 2	35	10	28.57%		
6	Mender 48	48	19	39.58%		
	Total	421	192	42.51%		

Table 2. Sero -prevalence of CBPP disease in cattle by age, sex, body condition and vaccination status analysis for the association between CBPP and individual animal risk factor using chi- square test (Dec-Jan 2017)

S.N	Risk factor	Categories	Number of animal tested	Number of positive	Prevalence %	χ^2	(p-value)
1	Age	Adult	302	153	50.66%	11.01	0.001
		Young	119	39	32.77%		
3	Sex	Male	121	51	42.14%	0.81	0.366
		Female	300	141	47%		
4	Body condition	Good	21	10	47.61%	2.66	0.264
		Medium	158	64	40.505		
		Poor	242	118	48.76%		
5	Vaccination status	Yes	125	50	40.60%	0.000	0.999
		No	296	135	45.66%		

Table 3. Prevalence of suspected CBPP case at veterinary health post (clinic record) in different peasant association in the study area from (2015-2017)

S.N	Peasant association	Year	Total number of population	Suspected CBPP case	Prevalence (%)
1	Shobora	2015	240	10	(4.16%)
		2016	310	17	(5.48%)
		2017	370	32	(8.64%)
2	Mutsa	2015	2300	9	(0.39%)
		2016	1982	0	0
		2017	2100	21	(1%)
3	Mender 45	2015	1750	0	0
		2016	1160	45	(3.87%)
		2017	1844	20	(1.08%)
4	Mender 52	2015	1132	0	0
		2016	1160	16	(1.37%)
		2017	1914	10	(0.52%)
5	Mender 48	2015	1226	0	0
		2016	1095	0	0
		2017	1325	7	(0.52%)
6	Keshmando mender 2	2015	700	0	0
		2016	750	0	0
		2017	800	8	(1%)

From the above retrospective data of the animal health post record the disease is highly prevalent in Shobora peasant association in the three consecutive (2015-2017) year. This indicated that the current study which is confirmed by c-ELISA is similar which is higher in Shobora. Because of the first out break or

introduction of the disease is seen in that peasant association of relatively susceptible population. Similarly there is a case in other peasant association in different year (Table 3).

5. Discussion

In the current study, contagious bovine pleuropneumonia was major cattle health problem in the study district. A total of 421 serum sample were examined from six peasant association of Bambasi district and the overall sero-prevalence of CBPP was (42.51%) ($p<0.05$). This finding was lower than the finding of previous report from other area of Ethiopia (Dejene, 1996) who reported a sero-prevalence of (56%) in North Omo, western Ethiopia. More over the finding was lower than the sero-prevalence observed in Banja district (66.3%) in Western Gojam (Gashaw, 1998), Qabribeyah (75%) and in Afdem (71%) in Mieso district (Gedlu, 2004).

The overall sero-prevalence of CBPP in the current study was higher than that of (Ahmed Ibrahim, 2004) reported 9.4% in Borena, (Sehnier *et al,* 2006*)* reported 9.7% in South Western Kenya, (Matua - Alumira *et al*, 2006) reported 16% in kajiado district Kenya and 4% in and around Adama (Dawit *et al*, 2013) was reported.

The variation in the prevalence of CBPP reported from different part of Ethiopia in particular and other country in general could be due to difference in agro-ecological system, animal management production system, population density and the type of test used to evaluate the sero-prevalence.

The difference in the sero-prevalence result was significant in Shobora peasant association (66.34%) than in Mender 52 (52%), Mutsa (43.90%), Mender 48 (39.58%), Keshmando number 2 (28.57%) and Mender 45 (24.67%). The higher prevalence reported in Shobora peasant association could be due to large grazing land so they have an access to contact with an infected animal from outbreak case in the area of the last 3 years reported from the peasant association and the cased animal remain carrier and transmitted to the uninfected animal. It also due to higher number of animal examined as compared to other peasant association.

The current study, prevalence of disease based on their sex was (42.14%) male, and (47%) female. The occurrence the disease across sex factors showed that there was no significant statistical difference ($p>0.05$). This result was agreed with (Eyob, 2015). But the current finding is in agreed with (Schnier, 2006) who reported female gender was significant.

The sero-prevalence result of (50.66%) and (32.77%) were recorded in adult and young age categories respectively which was statistical significant ($p<0.05$). This result was close agreement with the previous report by (Emanuel, 2013) and (Matua-Alumira *et al.,* 2006) in which sero - prevalence in adult would be higher as compared to the young. The low prevalence of infection was due to the decrease contact between the other animal because young animals do not move long distance but this result contradicted with the report of (Masiga *et al.,*1995) who reported young animal are susceptible to aricular form of CBPP than adult cattle.

In the current study there was no significant difference among body condition ($P>0.05$) in the sero-prevalence status of the animal. This result in the line with the report of (Biruhtsfa *et al*., 2015) in Bishoftu abattoir and export oriented feed lots around Adama town. The present study there was no significant difference among vaccinated and non-vaccinated animal ($p>0.05$) in the sero status of the animal.

In the current study retrospective data of the animal health post record the disease is highly prevalent in (4.16%), (5.48%), (8.64%) Shobora peasant association in the three consecutive year (2015, 2016 and 2017) respectively. This indicated that the current study which is confirmed by c-ELISA is similar which is higher in Shobora (66.34%). Because of the first out break or introduction of the disease is seen in that peasant association of relatively susceptible population. Similarly there is a case in other peasant association in different year. This finding is the same observation was made by (Almaw *et al*., 2012).

The serological test used in this study, competitive ELISA, is more sensitive for detecting chronically infected cattle than other test thus it is likely that individual animal at the early stage of infection can be missed by the test (Muuka *et al*., 2011; Schubert *et al*., 2011).

6. Conclusion And Recommendation

The current study indicated that contagious bovine pleuropneumonia is the prevalent disease in Bambasi district, suggesting the disease could be cause considerable economic losses through morbidity and mortality. The presence of statistically significant difference in the prevalence of contagious pleuropneumonia amongst the potential risk factors like peasant association which indicated that variation in management factors favours the occurrence and spread of the disease.

Therefore, based on the result of this study the following recommendation were suggested,

- Isolation of infected animal and strict ring vaccination with treatment should be implemented.
- Community based awareness creation about the economic impact of the disease.
- In the long term, regular annual vaccination should be given in endemic area.

- Improve animal husbandry and management practices.
- Control of animal movement, in order to mitigate the risk of transmission of the disease.
- Improvement of the public and private veterinary service delivery system so as to reduce the risk of CBPP.

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