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Seroprevalence and Associated Risk Factors of Contagious Caprine Pleuropneumonia in Selected Districts of Borana Pastoral Area, Southern Oromia, Ethiopia

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ABSTRACT

CCPP is considered one of the most severe and highly infectious diseases in goats. It results in heavy economic losses for countries involved in goat farming, especially in Africa, Asia, and the Middle East. The main mode of infection is by inhaling infectious droplets from active or carrier animals into healthy animals. The morbidity and mortality rates are quite high. Infection of goats with pleuropneumonia is limited to the alveolar tissue of infected goats, although available data suggest that sheep may also be infected. A cross-sectional study was carried out in a selected Borena zone of (Dubuluk and Gomole) districts between April and December 2020 G.C. The main goals of this study were to determine the sero-prevalence of contagious caprine pleuropneumonia in goats, examine the connection between risk factors, and find antibody in sheep against Mycoplasma capricolum subsp. Capripneumoniae. A multistage random sample approach was employed to select districts and pastoral groupings, or villages. A total of 452 serum samples from non-vaccinated animals (126 sheep and 326 goats) were collected. A competitive enzyme-linked immunosorbent assay was employed for antibody detection. The overall seroprevalence of CCPP in goats and sheep was 33.6% (152/452) at the individual animal levels recorded. In goats, 39.9% (130/326) and 17.5% (22/126) in sheep were recorded, with a statistically significant variation (p value = 0.000). A multivariable logistic regression analysis revealed that districts, species, age, and flock size were also significantly associated with sero-positivity, with odd ratios of (OR = 3.07, CI 95% 1.38–6.75, OR = 4.0, CI 95% 2.16-7.44, OR =.33, CI 95%.14–.76, OR = 1.16, CI 95%.59–2.29), respectively. Also, the study offers proof that sheep may have played a part in the disease's persistence. It is important to use caution when bringing new animals into a flock in endemic areas. The spread of the disease is controlled by on-site quarantine, flock testing, and positive animals being culled from the flock.

Keywords: CCPP, cELISA, Endemic, Infection, Ruminant.

INTRODUCTION

Mycoplasma capricolum subsp. capripneumoniae (Mccp) is the cause of contagious caprine pleuropneumonia (CCPP), one of the most severe and contagious respiratory diseases that affect small ruminants, mainly goats

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[1,2]. The contagious caprine pleuropneumonia (CCPP), a typical transboundary animal disease, is included in the list of notifiable diseases of the World Organization for Animal Health's (OIE) list and needs to be reported [3,4]. OIE reports that CCPP has been detected in approximately 40 nations, particularly in Africa and the Middle East, which are home to the majority of the world's goat population [5-7].

Contagious caprine pleuropneumonia (CCPP) is a fatal respiratory infection that results in high morbidity (100%) and mortality (80–100%) [8]. Furthermore, the disease has a devastating financial impact on the goat farming sector in underdeveloped nations [9,10]. Although sheep have been documented with subclinical cases of the disease, goats are typically affected [11]. In contagious caprine pleuropneumonia (CCPP) outbreaks, sheep may also become infected in mixed goat and sheep herds. Mycoplasma capricolum subsp. capripneumoniae (Mccp) is isolated and an antibody is detected in clinically affected and healthy sheep, acting as a reservoir for the disease [12-14]. Recently, CCPP was confirmed in wild ruminants kept in a wildlife preserve [15].

Contagious caprine pleuropneumonia (CCPP) is characterized by fibrinous pleuro-pneumonia with increased straw-colored pleural fluid in the diseased lung, and the lesions often occur in the thoracic cavity only [16,17]. The usual clinical symptoms of CCPP include hyperpyrexia (41-43 °C), significant morbidity and mortality rates in vulnerable herds regardless of age or sex, and respiratory manifestations such as grunting and snoring, persistent nasal discharges, and anorexia [18,19]. Moreover, abortion and high mortality have been reported in some cases [4,16].

Goats may fall dead in acute cases within one to three days with minor clinical indications [19]. Through an aerogenic route of contaminated droplets, MccP is directly transmitted [20]. When lesions affect only one lung, are confined to the respiratory tract, and cause a noticeable pleurisy with a profuse effusion of pleural fluid, CCPP may be suspected. However, in the field, differential diagnosis may be difficult because goats can have multiple mycoplasma species infections that cause similar symptoms. Pasteurellosis, or peste des petits ruminants, may also be mistaken for CCPP [7].

Classical methods of isolation and identification of the mycoplasma are laborious and time-consuming because of their meticulous nature [21]. For successful disease control and monitoring, diagnosis is essential. However, the extent and impact of CCPP remain largely unknown because veterinary services are underfunded, there is no infrastructure for the quick transport of samples, and there are no specialized diagnostic tests that can be used in the field [22].The paucity of immunizations, the scarcity of medications, the high cost of treatment, and the lack of veterinary services exacerbated the situation in the most distant areas [23,24]. However, to identify antibodies against Mccp, serological methods such as the complement fixation test, indirect hem agglutination, and latex agglutination were commonly used [3,25]. Recently developed and now in widespread use is a highly specific cELISA for CCPP [26-28].

The disease was first discovered in Algeria in 1873 [29]. Since 1983, there have been reports about the existence of the CCPP in Ethiopia. Mycoplasma capricolum subsp. Capripneumoniae (Mccp) was isolated from the disease outbreak in goats in Ogaden, Eastern Ethiopia, and confirmed in 1990 [30] and 70% of the goat population is found in the pastoral area of the country [31].Furthermore, in pastoral areas, goats and sheep are significant suppliers of milk, meat, and income [32, 33]. Since it is endemic, diverse seroprevalence in sheep and goats have been reported in various publications in almost every part of the country [11, 34]. In a meta-analysis, CCPP was found to be 25.7%; samples collected at the field level accounted for 22.4%, and samples gathered at abattoirs for 39.2% [11]. The data on CCPP in pastoral areas, its dynamics, and its dispersion across borders are poorly documented. Thus, the objectives of the study were to ascertain the presence of Mccp antibodies in sheep, the seroprevalence of CCPP in goats, and the association between potential risk factors and seropositivity.

CCPP is considered as one of the most severe and highly infectious disease of goats. It results in heavy economic losses to countries involved in goat farming especially in Africa, Asia and the Middle East (Jones and Wood 1988; Wesonga et al. 2004; OIE 2 Contagious caprine pleuropneumonia firstly reported in Algeria in 1873 and distributed all over the world (Martin et al. 1980). The mycoplasmas belonging to the "mycoides cluster" are classified under class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae, and the genus Mycoplasma. Mycoplasma mycoides cluster is made up of 6 species, subspecies or group of strains that are pathogenic for ruminants (Younis et al., 2015). It includes Mycoplasma mycoides subspecies mycoides (MmmSC), Mycoplasma mycoides subspecies mycoides (MmmLC), Bovine sero-group 7, Mycoplasma mycoides subspecies Capri (Mmc), Mycoplasma capricolum subspecies capripneumoniae (Mccp) and Mycoplasma capricolum subspecies capricolum (Mcc) (Santos, et al., 2013; Fischer et al., 2015). Most of the members of the Mycoplasma mycoides cluster are significant pathogens of small ruminants including Mccp, Mmc and Mcc all of which can infect lungs of small ruminant inducing respiratory disease (Ejaz et al., 2015). The disease is a classical transboundary animal disease, included in the list of notifiable diseases of the World Organization for Animal Health (OIE)(Yatoo.,et al 201).

MATERIALS AND METHODS

Study area

This study was carried out in Dubuluk and Gomole districts of Borana zone, Oromia, Ethiopia, which is mostly home to the Borana community. The geographical location is 4 0 2"56".58N and 380 13"35".39E south of Oromia Region in the south direction. The altitude ranges between 943 and 2,400 meters above sea level. The average annual rainfall ranges between 350 and 900 mm, with considerable variability in quantity and distribution. The zone has bimodal rainfall; the long rainy season extends from March to May, whereas the short rainy season occurs from mid-September to mid-November. The annual temperature varies between 19°C and 42°C [3]. The pastoralists usually move with their animals depending on the availability of forages and water across national and international boundaries. Borena Zone is an international boundary in the south with Kenya, in the west by the Southern Nations, Nationalities, and People Region (SNNPR), in the north by Gugi, and in the east share boundary with the neighboring regional states of Somali. According to [36], the climate in the Borana rangeland ranges from semiarid to arid. The main source of income for the Borana people is extensive pastoralism [31].

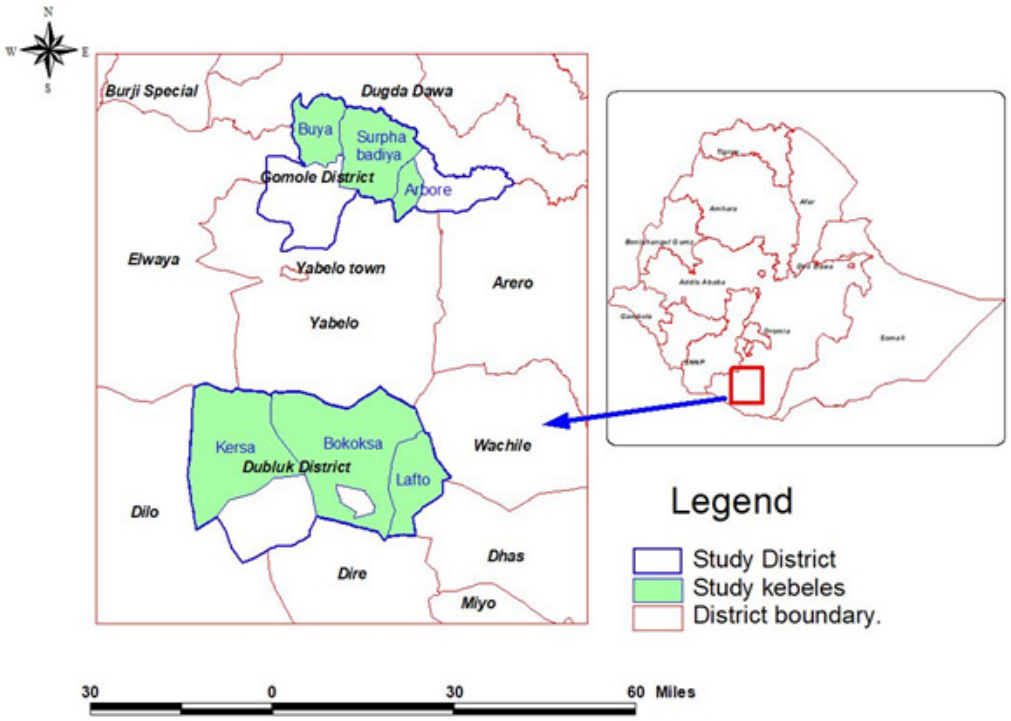

Figure 1. Map of Ethiopia showing Study areas

Study population

The long-eared Somali goats and native blackhead Somali sheep reared in pastoralist areas of the Borena Zone are included in the study. The Borana Zone has a documented livestock population of 1,416,180 cattle, 1,262,782 goats, 776,870 sheep, 237,205 camels, 102,767 donkeys, 1,841 horses, and 4,433 mules, according to a report from the Borena Zone Pastoral Development Office [35]. The study comprised sheep and goats who were older than 6 months and had never received a vaccination against CCPP, particularly goats. Age, species, sex group, PAs, and flock size were noted. According to [37], animals were divided into young (less than one year old) and adult (greater than one year old) categories based on their sexual development.

Study design

Contagious Caprine Pleuropneumonia (CCPP) seropositivity was assessed using a cross-sectional study design from April to December 2020 G.C. The study animals were chosen using multistage random sampling, and the sampling frame included a list of all districts in the zone as well as pastoral associations (PAs) or villages. Three PAs from each of the two randomly chosen districts were included. Gomole district (Arbore, Buya, and Badiya Supha PAs) and Dubuluk (Kersa, Bokossa, and Lafto) were chosen for the study.

Sampling method

To get the necessary sampling units, a multistage sampling procedure was adopted, with the pastoralist association

(PAs) as a primary unit, the flock as a secondary unit, and each individual animal as a tertiary unit. Based on the population of sheep and goats in each PA, the number of samples used in the study was proportionally allocated among them.

Sample size determination

To estimate the sample size for an epidemiological analysis involving a serological assay the [38] method was used. This method considered a 95% confidence level, 5% absolute precision, and an estimated prevalence of 7.1% for sheep [31] and 31.2% for goats [6]. Thus, for the purposes of this study, 452 samples from sheep and goats were gathered.

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

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Blood Sample collection and transportation

5-7 mL of blood samples were taken from the jugular veins of sheep and goats that were apparently healthy and had not previously had CCPP vaccinations for at least a year. Blood was collected using sterile vacutainer tubes and needles. Drawn blood was kept at room temperature over night to separate the serum from the clot. Each sample was labeled with the date of collection, sex, age, and PAs. The serum samples were collected into sterile cryogenic vials, maintained in a cold chain, and shipped to the Animal Health Institute (AHI), Sebeta, Ethiopia, for examination.

Serological Examination

The antibodies against Mccp were serologically detected using a commercial cELISA kit (ID Vet, France). The optical density (OD) was measured using a Biotech ELx800 ELISA reader at 450nm. The results were interpreted according to the manufacturer's instructions: percent of inhibition (PI) = (OD of monoclonal antibodies (Mab) minus OD of test serum) divided by (OD Mab - OD conjugate) multiplied by one hundred. The results are considered positive when PI% is greater than or equal to 55 [39].

Data management and analysis

The primary data was gathered during the study period. Microsoft Excel was used to code and enter pertinent data. Organized data were further analyzed using IBM-SPSS version 20. To ascertain the prevalence rate of CCPP in goats and sheep, descriptive statistics were employed. Using Chi-square ($\chi 2$), the relationship between risk factors and disease positivity was evaluated. To calculate the degree of correlation between risk factors and the illness, bivariate logistic regression was used. Using a 95% confidence level (CI) and a p-value less than 0.05, multivariate logistic regression analysis was used to further examine risk factors that showed a significant association with the condition.

RESULTS

Serological test

A total of 452 goats and sheep were tested from the two districts (Dubuluk and Gomole) of Borena Zone, of which 326 were goats and 126 were sheep. The overall seroprevalence of CCPP was 33.6% (152/452) at individual animal levels recorded in goats and sheep (Table 1). In goats, 39.9% (130/326) and 17.5% (22/126) were in sheep, which was a statistically significant variation between goats and sheep (chi-square ($\chi 2$) = **20.4605**, p-value = 0.000). The analysis of the seroprevalence rate in two districts indicated that the highest sero-prevalence rate was recorded in Dubuluk (42.9%) (91/212), followed by Gomole (25.4%) (61/240), and there was significant statistical variation (Chi-square ($\chi 2$) = **15.4592,** p-value = 0.000) in CCPP seroprevalence between the two districts.

Among pastoral associations (PAs), the CCPP was detected in all sites, and statistically significant variation (chi-square ($\chi 2$) = **16.4826,** p-value = 0.006) was observed. Based on age group, 15.7% (8/51) were young and 35.9% (144/401) were adults, which is a statistically significant variation (chi-square ($\chi 2$) = **8.2913**, p-value = 0.004) (Table 1). Analysis of the seroprevalence of CCPP with respect to males and females showed no significant variation (P value = 0.591). In small, medium, and large flock sizes, the sero-prevalence of CCPP was 22.6%, 28.7%, and 44.3%, respectively. There was significant statistical variation in sero prevalence among various flock sizes (chi-square ($\chi 2$) =**19.3834**, p-value =0.000).

A multivariable logistic regression analysis revealed that among the risk factors districts, species, age, and flock size were also significantly associated with sero-positivity (P< 0.05), with odd ratios of (OR = 3.07, CI 95% 1.38–6.75, OR = 4.0, CI 95% 2.16-7.44, OR =.33, CI 95%.14–.76, OR = 1.16, CI 95%.59–2.29), respectively. However, the difference between sex and seroprevalence has no statistically significant effect. p=0.592).

Table 1. Univariable logistic regression analysis of risk factors associated with contagious caprine pleuropneumonia sero prevalence in sheep and goats in two districts

Risk Factors	Category	Sample examined	Positive for c-ELISA	Prevalence rate (%)	p-Value
District	Dubuluk	212	91	42.9	0
	Gomole	240	61	25.4	
Species	Caprine	326	130	39.9	0
	Ovine	126	22	17.5	
Gender	male	26	10	38.5	0.674
	female	426	142	33.3	
Age (years)	Young	51	8	15.7	0.009
	Adult	401	144	35.9	
Flock size	Small	150	34	22.6	0
	Medium	101	29	28.7	
	Large	201	89	44.3	

Table 2. Multivariable logistic regression analysis of risk factors associated with contagious caprine pleuropneumonia sero prevalence in sheep and goats

Risk factor	Odds ratio	Std. Err.	z	p > |z|	95% confidence interval
District	**2.206883**	**0.4481304**	**3.9**	**0**	**1.482293 3.285674**
Species	3.135435	0.8168049	4.39	0	1.881712 5.224471
Gender	0.8	3328071	-0.54	0.592	3539832 1.807996
Age	3320413	0.1324388	-2.76	**0.006**	.1519427 .7256122
Flock size	.3688494 .	889814	-4.13	**0**	.2298819 .591825

DISCUSSIONS

More than 40 nations globally, especially in Africa, Asia, and the Middle East, have been affected by the extremely contagious and devastating respiratory disease known as CCPP in sheep and goats (39). As previously demonstrated, Oromia was the source of multiple CCPP outbreaks reported in the nation, with Borana accounting for the majority of these cases [40]. The results of the current investigation showed that sheep and goats in the Borena zone had a prevalence rate of 33.6% for CCPP.This was higher than the national prevalence estimated from pooled sero-prevalence (25.7%) through a systematic review [11] and the result is in line with Previous reported in the Somali region 32.6% [41], in SNNPR 32.9% [33], in Borena pastoral area 31.6% [34] and in the Nagpur district Vidarbha region of Pakistan 33.67% [42].

In contrast to our findings, a lower prevalence rate of CCPP has been reported earlier from different parts of Ethiopia [43-46] and in Pakistan [3,16]. On the other hand, a higher sero-prevalence of 44.5%, 47.3%, 51.8%. and 43'9% were reported from the Dire Dawa, Afar, Oromia and Tigray regions of Ethiopia, respectively [47,48]. The observed variation in seroprevalence reported by several researchers could be attributed to variations in immunization histories, agro-ecological location, husbandry practices, sample size and test method used.

The prevalence rates in sheep and goats were found to be 17.5% and 39.9%, respectively. In this investigation, we found a significant difference in the seroprevalence of CCPP between sheep and goats. This result is consistent with previous research [12,49,50]. It was revealed in this investigation that sheep reared with goats and in contact with infected CCPP goats became seropositive for CCPP.

In sheep, different seroprevalence rates were documented by different authors: 12.9% and 7.14% in the Borena pastoral area [6,31], 47.6% in Afar, and 40.7% in the Tigray regions [48]. In Tanzania, sheep serum showed 36.7% and 22.9% seroprevalence rates were recorded, respectively [49]. The pooled CCPP prevalence ranged from 15.74% to 50.0% in sheep, depending on the country [2]. Interestingly,

our results are in line with the pooled prevalence of CCPP in sheep across Africa, Asia, and Southeast Europe, which was found to be 23.2% [2]. Sheep housed alongside goats demonstrated CCPP seropositivity in the majority of the investigated locations. Consequentially, this study lends acceptance to the concept that sheep play a significant part in the epidemiology of CCPP [6].

Moreover, studies describing the isolation of Mccp from sheep with respiratory diseases [51], from healthy sheep in Kenya in contact with goat herds [14], sick sheep mixed with goats in Uganda [13], and from sheep lung and nasal swabs [52] have all been published. This raises concerns about sheep's role as a reservoir and their contribution to the continuation of the Mccp. To precisely understand the role sheep play in maintaining and passing on Mccp to goats, more research is needed. To the best of our knowledge, a large-scale investigation is exploring the CCPP seroprevalence among sheep and goats and the related risk factors. Consequently, this study's findings significantly advance our knowledge of the dynamics of CCPP in the Borena pastoral region of Oromia and the significance of sheep in its epidemiology. They also aid in the selection and development of successful preventative initiatives.

The adult age category has shown significantly higher seroprevalence as compared to young animals 35.9% and 19.5% were recorded, respectively. There was significant statistical variation with age groups (P-value <0.000). Our findings also agree with the reports of [6,34,41,44,53,54], who observed the presence of significant variation among age groups. However, the findings of this study contradict the works of [34,47,48], who observed the absence of an association between age group and the occurrence of seropositivity. Adults are often frequently exposed to different stress conditions, which can predispose the animal to the disease and make them more susceptible to infection. Furthermore, they also tend to be infected repeatedly. Therefore, the probability of being seropositive in adulthood for CCPP would be high as compared to young animals.

In this study, there was no relationship between the animal's sex and seropositivity. This result was in line with studies conducted in different areas by [34,41,53]. Furthermore, susceptible goats of both sexes have been shown to be at risk for the fatal and extremely contagious CCPP disease [19].

In this study, the percentages of small flock sizes, medium flock sizes, and large flock sizes observed were 22.6%, 28.7%, and 44.3%, respectively. There was a significant variation in seroprevalence between the three flock size categories p value=0.000. Numerous past investigations agree with these findings [39,55]. Conversely, a number of studies [6,56] found no discernible relationship between flock size and the frequency of CCPP. The greater persistence of Mccp is the cause of the higher prevalence of CCPP in large flock sizes as compared to lower flock sizes and the mixing of afflicted and unafflicted animals [56]. The primary method of spreading CCPP through aerosols, droplets, or nasal discharge is direct contact [4]. Furthermore, because intensive management in large flocks is more challenging than in small flocks, pastoralism in large flocks permits the mixing of animals from other flocks, which spreads the disease. Goats and sheep flocks tend together during feeding, grazing, and rest periods, which would facilitate the infection's transmission across the flock.

CONCLUSION AND RECOMMANDATIONS

The current study's findings demonstrate that CCPP is circulated among sheep and goats in the area. The study also showed that the overall seroprevalence rate of CCPP in goats and sheep was 33.6% (152/452) at the individual animal levels recorded. In goats, 39.9% (130/326) and 17.5% (22/126) in sheep were recorded. This calls into question sheep's function as a reservoir and their involvement in sustaining Mccp transmission. More research is required to determine the precise role that sheep play in the upkeep and transmission of Mccp to goats. It is evident from our research as well as earlier studies that CCPP is a priority for goat farming and that more concerted efforts are required to both prevent and lessen the disease's effects. Additionally, studies concentrated on the isolation and molecular characterization of the circulating strain for both sheep and goats, as well as the economic impact of the disease on production performance.

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CONFLICT OF INTERESTS

The author(s) have not declared any conflict of interests.

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