Sero-surveillance of Contagious Caprine Pleuropneumonia (CCPP) in Six Selected Districts of East and West Hararghe Zones of Oromia Regional State, Ethiopia

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Abstract: A cross-sectional study was conducted from November 2018 to February 2019 to estimate the seroprevalence of contagious caprine pleuropneumonia (CCPP) and to evaluate the exact picture and distribution of CCPP in selected districts of East and West Hararghe Zones in Ethiopia. Multistage sampling method was implemented to select districts, peasant associations (villages), and households. The peasant associations (PAs) within the selected six districts were the primary sampling unit and the flock per household was used as a secondary sampling unit, meanwhile the individual goats with no history of CCPP vaccination and aged above six months were the third sampling unit. A total of 960 blood samples were collected from goats and the resultant sera were screened for the presence of antibodies against *Mycoplasma capricolum* subspecies *capripneumoniae* using a competitive enzyme-linked immunosorbent assay (cELISA). The result showed that 93 (9.7%: 95% CI= 7.9% - 11.7%) goat sera were positive for CCPP. There was a statistically significant difference in the prevalence of CCPP between the study districts ($\chi^2 = 59.3758$; $p < 0.001$). The highest prevalence (22.95%) was observed in Gumbi-Bordede district, followed by Midhaga-Tola (13.85%), Anchar (8.12%), Chinaksen (5.4%), Gemechis (5.63%), and Chiro (1.25%). There was also a significant difference in the seroprevalence of CCPP between different age groups with ($\chi^2 = 4.8459$; $p = 0.028$), in that adult goats had a higher prevalence (10.84%) compared to young goats (5.86%). Similarly, the seroprevalence of CCPP was significantly different between agro-ecological zones ($\chi^2 = 33.2990$; $p < 0.001$), in that goats in the lowlands (16.4%) had higher seroprevalence than those in the midland (5.2%) agro-ecology. Numerically, higher seroprevalence was recorded in female goats (10.1%; 95% CI = 8.1-12.4) than in males (7.7%; 95% CI = 4.06-13.13), but no statistically significant difference was observed among the sex groups ($\chi^2 = 0.7997$; $p = 0.371$). Hence, agro-ecology based appropriate control measures including regular investigation and vaccination should be implemented to alleviate the situation.

Keywords: *C-ELISA, CCPP, Ethiopia, Goat, Hararghe, Sero-prevalence*

Introduction

Goats, being an important component of the livestock subsector, play a significant role in the socio-economy of developing countries because of their better adaptation to the unfavorable arid environment and suitability for resource-poor farmers (FAO, 1994). Peste des petitis ruminants (PPR), contagious caprine pleuropneumonia (CCPP), pasteurellosis, sheep and goat pox diseases cause substantial losses through high morbidity and mortality (Teklaye *et al*., 1992).

Contagious caprine pleuropneumonia is an infectious disease that clinically affects only goats and it is one of those rampant and highly contagious animal diseases with a potential for rapid spread irrespective of national borders (Rurangirwa, 1996). *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) is the causal agent of CCPP. It is originally known as the F38 biotype and was first isolated in Sudan, Tunisia, Oman, Turkey, Chad, Uganda, Ethiopia, Niger, Tanzania, and the United Arab Emirates (Rurangirwa *et al*., 1987). CCPP was first reported in the mainland of Europe in 2004, when outbreaks were confirmed in Thrace, Turkey, with losses of up to 25% of kids and adults in some herds (OIE, 2008). Several tests may be used for serological diagnoses such as complement fixation test (CFT), passive haemagglutination test (Muthomi *et al*., 1983), latex agglutination (Robust test) (Rurangirwa, 1996; Rurangirwa *et al*., 1987) and indirect ELISA. CFT remains the most widely used serological test for CCPP and it is more specific though less sensitive than the indirect haemagglutination test. Moreover, it is the official test recommended for international trade (OIE, 2008).

In Ethiopia, goats play a unique role in the livelihood of communities, because they provide milk and dairy products, which in turn serve as a source of income to cover school fees for children and other family expenses. The current epidemiology of CCPP is not well documented in the eastern part of Oromia Regional State, specifically, areas neighboring Afar and Somali Regional States of Ethiopia. Hence, reliable epidemiological information is needed to design

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effective control measures. Therefore, the objectives of this study were to estimate the seroprevalence of contagious caprine pleuropneumonia (CCPP) and to evaluate the exact picture and distribution of CCPP in selected districts of the East and West Hararghe Zones in Ethiopia.

Materials and Methods

Study Areas

The study was conducted in six selected districts. Among these districts, four (Anchar, Chiro, Gemechis, and Gumbi-Bordede) were from West Hararghe Zone, while two (Chinaksen and Midhega Tola) were from East Hararghe Zone (Figure 1). West Hararghe is subdivided into 11 *woredas,* the majority of which are lowland areas (East and West Hararghe Zone Bureaus of Agriculture, (2006), unpublished report). The zone is located at latitude of 7°50'–9°50' N and longitude of 40°00'–41°25' E in the altitude range 1200–3060 m above sea level (asl). Their annual average rainfall ranges between 500-1300 mm and the average temperature ranges between 25 ^{O}C to 30 ^{O}C.

East Hararghe is located at the altitude of 7°30'–9°45' N and longitude of 41°10'–42°50' E in the altitude range 500–3400 m asl. East Hararghe Zone has 18 *woredas* (district equivalent) of which four are in the lowlands (< 1500 m), and the remaining ones are located at higher altitudinal ranges (> 2000 m).

The Zones have two rainy seasons, the short rainy season and the main rainy season, with a mean annual rainfall ranging from below 700 mm in the lowlands to nearly 1200 mm at higher altitudes. Most of the people living in Hararghe lowlands are nomadic agro-pastoralists who move their livestock seasonally, following grazing opportunities and water availability (Guinand, 2000).

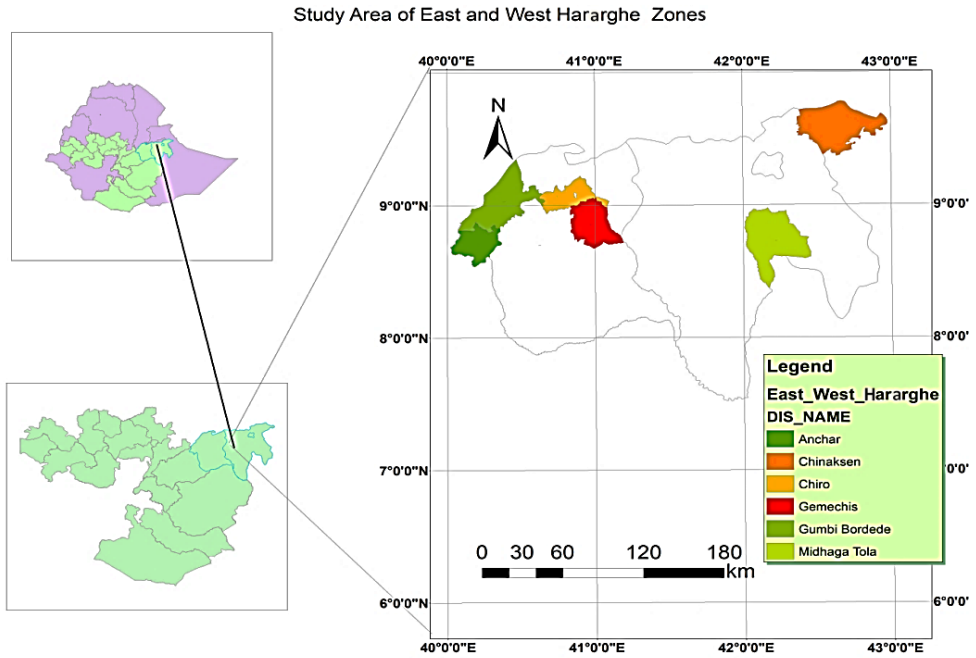

Figure 1. Map of the study areas.

Study Animals

The study was carried out on extensively reared goats. The study animals were apparently healthy goats and not involved in vaccination against CCPP for at least 2 years prior to the sample collection. The livelihood of animal owners depends on a sedentary mixed crop-livestock farming system. The major crops grown are sorghum, maize, chat, coffee, sweet potato, potato, and onions.

Study Design and Sampling Method

Multistage random sampling was applied, in which PAs within the selected districts were considered as the primary sampling unit and the flock per household was used as secondary sampling unit, whereas the individual goats within the flock at the household with no CCPP vaccination history and above six months of age were the third sampling units. Due to the difference in population size of goats within the selected districts and even within PAs in the same district, the sample size was also allocated proportionally based on the existing goat population per district. Hence 16.6% (n=160) of the sample was drawn from each district of Anchar, Gemechis, and Chiro, respectively and 19.06% (n=183), 13.54% (n=130), 17.39% (n=167) samples were allocated to Boardede, Midhaga-Tola, and Chinaksen, respectively.

Sampling Frame and Sample Size Determination

A total of 960 goats from six selected districts were sampled. The selected goats were identified and necessary information on household and animal attributes were recorded before sampling. The ages of the study animals were determined based on their

dental eruption patterns and categorized in to young (1-3 years old) and adult (greater than 4 year) according to Gatenby (1991) and Steel (1996).

The sample size was determined using the formula given by Thrusfield (2005) considering 95% confidence level, expected prevalence of 50% and 5% absolute precision

$$n = \frac{1.962\ Pexp\ (1 - Pexp)}{d^2}$$

Where: n = required sample size; P_{exp} = expected prevalence; and d^2 = desired absolute precision.

Accordingly, the minimum sample size was 384 goats. However, to account for intra-class correlation at herd, village, and district levels, a design effect of 2 was considered, resulting in a minimum sample size of 768 (Thrusfield, 2005). Due to the availability of CCPP kits, 192 extra samples were added, giving a final sample size of 960.

Blood Sample Collection

Approximately 5-7ml of blood was collected from the jugular vein of sampled animals using sterile plain vacationer tubes and allowed to clot overnight in slant position for 24 hours at room temperature. The serum was decanted carefully from clotted blood into a sterile cryogenic vial, labeled with a code and then transported using an icebox to Hirna Regional Veterinary Laboratory Serology Department. Serum samples were stored at −20 °C until subjected for analysis. Meanwhile, corresponding to each sera sample code, age, sex, and necessary animal's information was recorded on a separate sheet.

Laboratory Analysis of Serum Samples

The serum samples were examined for the presence of specific antibodies against Mccp by using a commercial cELISA (IDEXX, Montpellier, France), according to the instructions of the manufacturer. The test was characterized by specificity of 99.9%. Competitive enzyme-linked immunosorbent assay (cELISA) based on competition between specific monoclonal antibody '4.52' against *Mycoplasma* sp. Type F38 and antibody developed due to infection in suspected animals for binding to a specific antigen coated on a microtiter plate (Thiaucourt *et al*., 1994; Peyraud *et al*., 2014). Results were calculated by percent inhibition (PI) = (OD Mab - OD S)/ (OD Mab –OD CC) x 100; where OD stands for optical density at 450 nm, Mab for monoclonal antibody, S for sample, and CC for conjugate. At the end of the reactions, ELISA plates were read at 450 nm using an ELISA reader (Biotech, ELx800 ELISA) to determine the optical density (OD) and the percentage of inhibition was calculated. Samples with a percentage of inhibition greater than or equal to 55% were considered positive (Peyraud *et al*., 2014).

Data Analysis

Data collected from the field surveillance and laboratory assays were entered and stored in a Microsoft Excel spreadsheet, screened for proper coding and errors, and analyzed. For all the risk factors considered, disease prevalence differences among variable categories were compared using a Chi-square test along with 95% confidence interval of the point prevalence estimates using STATA 13.0. A p-value less than 0.05 was considered significant.

Results

Sero-positivity was detected in all localities surveyed as shown in Table 1. Among the total 960 goat sera examined, 93 (9.69%) were positive for anti-Mccp antibodies. The highest prevalence (22.95%) was observed in Gumbi-Bordede district, followed by Midhaga-Tola (13.85%), Anchar (8.12%), Gemechis (5.63%), Chinaksen (5.4%), and Chiro (1.25%).

Table 1. Results of analysis to identify risk factors of seroprevalence of CCPP in goats

Risk factors	Category	No. examined	No. positive	% prevalence (95% CI)	χ^2	P-value
Districts						
	Anchar	160	13	8.13 (4.4-13.50)		
	Gemechis	160	9	5.63 (2.6-10.40)		
	Chiro	160	2	1.25 (0.2-4.40)	59.37	<0.001
	Gumbi-Bordede	183	42	22.95 (17.1-29.70)		
	Chinaksen	167	9	5.40 (2.50-10.00)		
	Midhaga-Tola	130	18	13.85 (8.40-21.00)		
Sex						
	Male	155	12	7.74 (4.06-13.13)	0.79	0.371
	Female	805	81	10.06 (8.07-12.35)		
Age						
	Young	222	13	5.86 (3.15-9.80)	4.84	0.028
	Adult	738	80	10.84 (8.68-13.30)		
Agro-ecology						
	Midland	577	30	5.20 (3.54-7.34)	33.29	<0.001
	Lowland	383	63	16.45(12.87-20.99)		
Overall		**960**	**93**	**9.69**		

The difference in seroprevalence between districts was statistically significant ($p < 0.001$). There was also a significant difference in the seroprevalence of CCPP between different age groups ($p = 0.028$). Numerically, adult goats (10.84%) tested positive more than young goats (5.86%). Similarly, the seroprevalence of CCPP was 5.20% and 16.45% in midland and lowland agro-ecological zones, respectively. Numerically, relatively higher seroprevalence was recorded in female goats (10.06%) than in males (5.86%), even though the difference was not statistically significant ($p>0.05$) (Table 1).

Discussion

The present study revealed that CCPP was a major health constraint of goats in the study areas. All samples were tested for the presence of specific antibodies against CCPP infection using cELISA revealed that Mccp has been established and was circulating in the area. The seroprevalence of CCPP in the present study (9.7%) is lower than the national prevalence estimated from pooled seroprevalence (25.7%) by Asmare *et al.* (2016). In contrast to our findings, higher seroprevalences were reported from areas in Dire Dawa (44.5%), Afar (47.3%), and Oromia Region (51.8%) of Ethiopia (Daniel *et al.*, 2009). Similarly, Birhanu *et al.* (2009) has also reported higher prevalence of 38.6% and 43.9% from Afar and Tigray Regions of Ethiopia, respectively. In other parts of the world, such as Tanzania, Kenya, Uganda, higher prevalences were reported compared to our finding (Nyanja *et al.*, 2013; Mbyuzi *et al.*, 2014; Atim *et al.*, 2016; Kipronoh *et al.*, 2016).

An international collaborative study conducted by Peyraud *et al.* (2014) also reported seroprevalence of 6 to 90%; 14.6%; 8%; 10.1%; 0%; and 2.7% to 44.2% from Kenya; Afar Region of Ethiopia; Mauritius; Tajikistan; Afghanistan; and Pakistan, respectively, using monoclonal antibody-based cELISA. The observed variation in seroprevalence reported from different studies may be due to differences in the husbandry practices, agro-ecology, vaccination history, sampling methods applied, and sample size used.

Our finding is similar to that reported recently by Parray *et al.* (2019) in Himalayan Pashmina goats (9.93% (95%CI=7.10–12.76) and also in Pakistan with reports of 3.9-8.52% prevalence (Shahzad *et al.,* 2016; Wazir *et al.*, 2016). Similar to our findings, a lower prevalence of CCPP has been reported earlier from different parts of Ethiopia (Mekuria *et al.*, 2008; Mekuria and Asmare, 2010; Regassa *et al.*, 2010; Tesfaye *et al.*, 2011; Yousuf *et al.*, 2012; Aklilu *et al.*, 2015).

In this study, the highest prevalence of CCPP among the study districts was found to be (22.95%) and (13.85%) in Gumbi-Bordede and Midhaga-Tola, respectively, whereas the lowest CCPP prevalence among the study site (1.25%) was at Chiro district. This could be due to the fact that Gumbi-Bordede is recently established district departed from the former Mieso district and has a proximity to the pastoral community of Afar Region, which is considered as area of high infection risk for CCPP (Peyraud *et al.*, 2014).

In the current study, higher seropositivity (10.84 %) was noted in older age groups than younger age groups (5.86%) and significant association between seropositivity and age reflects older age groups being exposed to infection for longer periods than the younger age groups. This is in agreement with the findings of Parray *et al.* (2019). A significant association between CCPP seropositivity and age has also been noted by Mekuria and Asmare (2010); Mohamed *et al.* (2012) and Elemo *et al.* (2017). This is because goats' humoral immunity in response to CCPP is influenced by age (Mohamed *et al.*, 2012).

The current study also found that the seropositivity to CCPP was not associated with sex, which is inline with the findings of Mohamed *et al.* (2012), who have stated that seropositivity in CCPP has no association with sex.

Conclusion

Although there were no official reports of outbreaks and clinical cases of CCPP during the study period in the study areas, the cELISA sero-surveillance had shown that CCPP is prevalent. The prevalence was relatively higher in low land compared to the mid land. Hence, agro-ecology based appropriate control measures including regular investigations and vaccinations should be implemented to alleviate the problem.

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Conflict of Interests

The authors declare that they have no competing interests.

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