



RESEARCH ARTICLE

Seroprevalence and Associated Risk Factors of Contagious Caprine Pleuropneumonia in the Small Ruminants of Oman

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is an economically important and potentially fatal disease of small ruminants caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*). We designed this cross-sectional study to investigate the seroepidemiology of CCPP in the small ruminants of Oman. For this purpose, we sampled a total of 4015 small ruminants (2119 goats and 1896 sheep) from 510 flocks belonging to different governorates of Oman. A commercial competitive enzyme-linked immune-sorbent assay (cELISA) was used to test the samples. Prevalence (%) along with 95% confidence intervals (CI) was calculated, and a univariable analysis was conducted to screen different risk factors. Furthermore, a binary logistic regression model was built at the animal and flock-level (Table 3). In total, 147 (28.8%, CI 24.9, 33.0) flocks tested positive for the CCPP and the seroprevalence ranged from 10.0 to 53.8% in various governorates ($p = 0.001$). The prevalence was significantly ($P < 0.001$) high in goats (28%, CI 23.8, 32.5) as compared to sheep (13.1%, CI 24.9, 33.0). At flock level; the open herds (OR 2.08, 1.33, 3.27), having a location in the coastal regions (OR 1.70, 1.14, 2.53) and flock size of more than 100 animals (OR 2.45, 1.54, 3.91) were the significant risk factors for CCPP in Oman. At the animal level; goats (OR 2.87, CI 2.17, 3.81), and small ruminants above the age of one year (OR 2.23, CI 1.38-3.59) were found more likely to acquire CCPP. We suggest that a control program based on the changes in the management system to minimize the risk factors and a possible mass vaccination should be devised to check CCPP in Oman.

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INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) affects small ruminants, mainly goats. The causative agent of CCPP was isolated in 1976 (MacOwan and Minette, 1976) and named as *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) in 1993 (Leach, Erno and Macowan, 1993). The clinical presentations of CCPP in goats include; fever, anorexia, and respiratory symptoms (cough, nasal discharge and dyspnea). The disease can

manifest itself in; the acute or subacute form with pleuropneumonia (OIE, 2008).

Contrary to the hypothesis that CCPP is only found in the Middle East and Africa, *Mccp* strains have been isolated from China (Chu *et al.*, 2011) and PCR confirms genetic evidence of their presence in Pakistan (Awan *et al.*, 2010). Numerous epidemiological studies have shown that the *Mccp* strains mainly circulating in Asia belonged to a specific clade as supported by significant bootstrap values (Manso-Silvan *et al.*, 2011), indicating that the presence of

the disease in Asia is not a new phenomenon. The condition like CCPP was reported to be found in India as early as 1914 (Walker, 1914). The presence of CCPP is also acknowledged in the European part of Turkey, which is a potential risk to small ruminants in the Balkan countries of the European Union. The CCPP has been shown to infect wildlife species held in captivity for conservation purposes in Qatar (Arif *et al.*, 2007) and it has also been reported in free-ranging wildlife population in Tibet (Yu *et al.*, 2013).

Due to the extremely fastidious nature of *Mccp* and antibiotic treatment of animals, laboratory diagnosis of CCPP was very difficult until the availability of specific nucleic acid-based assays (Bascañana *et al.*, 1994). For the serodiagnosis, many serological tests including latex agglutination test (LAT), complement fixation test (CFT), indirect hemagglutination test and slide agglutination test were developed. However, the elucidation of the results of these tests requires caution due to cross-reactivity with *M. capripneumoniae* and other members of *M. mycoides* cluster (OIE, 2008). Thiaucourt *et al.* (1994) developed a blocking enzyme-linked immunosorbent assay (ELISA) by using the monoclonal antibodies (mAb), which was used to detect the antibodies in naturally infected and vaccinated animals. The test was later modified to a competitive ELISA and used to detect the exposure in the goat flocks distributed across two continents (Asia and Africa) with diagnostic specificity close to 100%. The cELISA could detect both IgG and IgM that makes it an ideal test for epidemiological surveillance (Peyraud *et al.*, 2014). This test was later used to investigate the seroprevalence in goats of Kenya (Kipronoh *et al.*, 2016), and Ethiopia (Teshome *et al.*, 2019).

Saponin adjuvanted inactivated *Mccp* antigen-based vaccines are used to control CCPP in the endemic flocks (OIE, 2008). Currently, a few commercial vaccines are available against CCPP. Commercially available vaccines are either live (Pulmovac[®], Vital Veterinary Vaccines Production Co., Turkey and Capridoll[®], Dollvet, Turkey) or killed (Caprivax[®], Kenya Veterinary Vaccines Production Institute, Kenya).

In South Asia, serological studies recorded the prevalence ranging from; 32.5 to 45.7% in goats of Pakistan (Awan *et al.*, 2010; Shahzad *et al.*, 2012), and 9.9 to 33.7% in small ruminants of India (Suryawanshi *et al.*, 2015; Parray *et al.*, 2019). In Africa, the seroprevalence ranging from 18.6 to 52.1% was recorded in Ethiopia (Asmare *et al.*, 2016; Teshome *et al.*, 2019), Kenya (Kipronoh *et al.*, 2016), and Tanzania (Mbyuzi *et al.*, 2014). Common risk factors linked with higher seroprevalence of *Mccp*, as reported by various studies are; species (Mbyuzi *et al.*, 2014; Teshome *et al.*, 2019), age (Mbyuzi *et al.*, 2014; Parray *et al.*, 2019; Teshome *et al.*, 2019), location (Mbyuzi *et al.*, 2014; Kipronoh *et al.*, 2016b; Teshome *et al.*, 2019), sedentary farming system (Asmare *et al.*, 2016), introduction of new animals from markets (Mbyuzi *et al.*, 2014), season (Parray *et al.*, 2019), and absence of therapeutic intervention (Parray *et al.*, 2019).

The outbreaks of CCPP were observed in the small ruminants of Oman, and the country regularly imports live goats and sheep from the CCPP endemic countries of South Asia, Africa and the Middle East. In the year 2016, the recorded live animal trade of goats and sheep was 698,817 and 406785, respectively (FAO, 2018). Furthermore, the isolates collected from Oman had shown the sequences

comparable to those of North or East African strains (Lorenzon *et al.*, 2002). This study was conducted to investigate the seroprevalence of CCPP in the small ruminants of Oman.

MATERIALS AND METHODS

Study location and settings: The Sultanate of Oman lies between 21.4735° N latitude and 55.9754° E longitude and consists of 11 Governorates and 61 districts (wilayats). The terrain consists of desert, Al Hajar mountain range and a long coastline (3165 km) on the Persian (Arabian) Gulf, the Arabian Sea and the Gulf of Oman. The small ruminant population consisted of around 2.6 million heads with 20,85206 goats and 5,48231 sheep (MAF, 2013). The nomadic, transhumance, backyard and sedentary livestock production systems are practiced in Oman (Shaath and Al-Habsi, 2016). The small ruminant vaccination program of the Ministry of Agriculture and Fisheries (MAF) did not include the vaccination against CCPP.

Sampling design and collection: There was no previous report on the prevalence of CCPP in Oman, therefore, the sample size was calculated for a disease with the expected prevalence of 50% at 95% confidence interval and 5% desired absolute precision (Thrusfield *et al.*, 2018). The estimated number was increased by 1.5% in each governorate to compensate for any losses during transportation (Table 1). In a wilayat, random villages were selected through the Survey Toolbox software (Cameron, 1999). In a flock, 4 to 5 samples were randomly collected from each available species (goats or sheep) at 50% expected diseased percentage with the probability of finding at least one positive case (Thrusfield *et al.*, 2018).

During the sampling (2015-2017), a total of 510 flocks of small ruminants (unvaccinated against *Mccp*) were visited, and a total of 2119 goats (429 flocks) and 1896 sheep (398 flocks) were randomly sampled. The epidemiological information along with geographical coordinates of a flock was collected through a predesigned and pretested questionnaire by using the Epicollect software.

Approximately 9 mL of blood was collected through the jugular vein of the selected animals. Samples were labelled and transported in a cool box to the Animal Health Research Center (AHRC) for further processing. Upon arrival, serum was extracted by centrifugation at 1500 rpm for 10 minutes. Each sample was given a unique identification number and stored at -20°C, until further processing.

Serodiagnosis of CCPP: Sera were tested through a commercial competitive ELISA (cELISA) kit by following the manufacturer's instructions (IDEXX Montpellier SAS, France). The optical density (OD) value of each sample and controls was recorded at 450 nm using ELISA reader. The percentage of inhibition (PI) was calculated, and the samples with PI more than or equal to 55% were considered positive for the CCPP.

Statistical analysis: Prevalence along with 95% binomial exact confidence interval (CI) was calculated. The differences recorded for the seroprevalence among various

variables were assessed by Chi-square (χ^2) or Fisher's exact test, and a p -value of less than 0.05 was considered significant. Univariate analysis was conducted, and the odds ratio (OR) and respective 95% CI were calculated to find any association. The variables with a p -value less than 0.20 at the flock and animal level were used to construct a backward stepwise binary logistic regression model (Hussain *et al.*, 2014). The analysis was performed by using the SPSS software (IBM SPSS Statistics for Windows, Version 20.0).

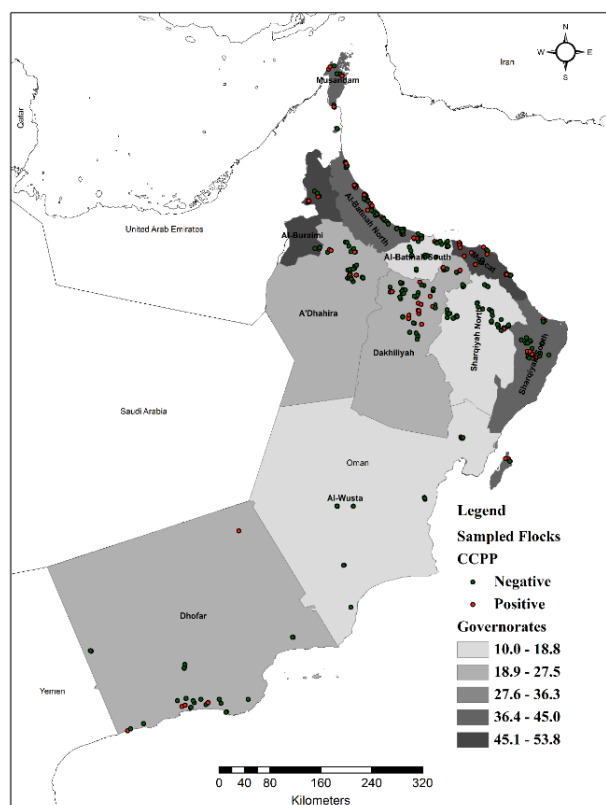
RESULTS

We sampled 510 small ruminant flocks all over Oman for this study, and a total of 4015 serum samples from 2119 goats (429 flocks), and 1896 sheep (398 flocks) were randomly collected (Table 1). Out of the total, 3229 (80.4%) were females, and 786 (19.6%) were males. Local breeds (73.9%) constituted most of the sampled population followed by imported (15.3%) and crossbred (10.7%) animals. The samples were further categorized in three different age groups; less than equal to 1 year ($n = 574$), 1.1 to 3 year ($n = 2521$) and above 3 year ($n = 920$). The age, breed and sex-related distribution of the samples is presented in Table 2.

Seroprevalence of Mccp in goat and sheep flocks: Small ruminant flocks with at least one positive animal were considered positive for the Mccp. The antibodies against Mccp were detected in the 147 (28.8%, CI 24.9, 33.0) of the sampled flocks. The prevalence varied among different governorates, and the highest seroprevalence was recorded in Muscat (53.8%, CI 33.9, 72.5) and the lowest in Al-Wusta (10%, CI 1.2, 31.7), $\chi^2 = 31.360$, $p = 0.001$ (Table 1 & Map 1).

The antibodies against MccP were detected in 120 goat flocks (28%, CI 23.8, 32.5), and 52 sheep flocks (13.1%, CI 24.9, 33.0), $\chi^2 = 27.80$, $P < 0.001$. Similarly, the animal's level seroprevalence in goats (9.8%, CI 8.6, 11.2) was significantly higher than sheep (3.6%, CI 2.8, 4.5), $\chi^2 = 60.66$, $P < 0.001$ (Table 2). In goat flocks, the highest prevalence was recorded in Al-Buraimi (72.7%, CI 39.0, 94.0) and the lowest in Al-Wusta (11.8) governorate, $\chi^2 = 43.056$, $P < 0.001$. In sheep, the seroprevalence ranged from 4.3% (CI 0.5, 14.5) in Sharqiyah North to 26.9% (CI 11.6, 47.8) in Muscat governorate, $\chi^2 = 23.063$, $P = 0.011$. The seroprevalence was significantly ($\chi^2 = 7.96$, $P = 0.005$) higher in the coastal areas of Oman (35.3%, CI 29.0, 42.0) as compared to the drier interior regions (23.9%, CI 19.1, 29.2).

The seroprevalence increased with age of small ruminants, and the lowest was recorded in animals below or equal to age of 1 year (3.3%, CI 2.0, 5.1) preceded by those between 1.1 to 3 years of age (7.4%, CI 6.5, 8.4) and above 3 years old (8.6%, CI 6.9, 10.6), $P < 0.001$. Higher prevalence was detected in imported (7.4%, CI 5.1, 10.3) and local breeds (7.4%, CI 6.5, 8.4) as compared to crossbred (4.1%, CI 2.6, 5.9) animals ($P = 0.011$). The difference observed in the seroprevalence regarding female (7.0%, CI 6.1, 7.9) and male (6.5%, CI 4.9, 8.4) sex was not significant ($P = 0.634$).



Map 1: Location of sampled flocks and flock-level prevalence of the antibodies against Mccp in different governorates of Oman.

Univariable analysis: The univariate analysis (Table 2) at flock level indicated; location of flocks in the coastal regions (OR 1.74, CI 1.18, 2.56), flock size of more than 100 animals (OR 2.16, CI 1.39, 3.34), open replacement system (OR 1.62, CI 1.06, 2.47), mixing of imported breeds with local and crossbred animals (OR 1.81, CI 1.23, 2.68), and confined housing (OR 1.31, CI 0.84, 2.06) were the factors significantly ($P < 0.05$) associated with higher CCPP seroprevalence.

At the animal level, the analysis indicated that goats (OR 2.93, CI 2.21, 3.88), animals above three years (OR 2.74, CI 1.64, 4.58) and between 1.1 to 3 years of age (OR 2.22, CI 1.37, 3.59), belonging to imported (OR 1.90, CI 1.11, 3.25) and local breeds (OR 1.88, CI 1.23, 2.88) were found significantly more likely to test seropositive.

Multivariable analysis: At flock level, the significant ($P < 0.05$) risk factors were; flocks that frequently introduced new stock (OR 2.08, 1.33, 3.27), having the location in the coastal regions (OR 1.70, 1.14, 2.53) and flocks with more than 100 animals (OR 2.45, 1.54, 3.91). The Hosmer and Lemeshow Test ($\chi^2 = 6.309$, $P = 0.277$) and Nagelkerke R Square (0.077) values indicated that it was an equitable model for prediction of seroprevalence of CCPP at flock level.

The model at the animal level indicated that goats (OR 2.88, CI 2.17, 3.81) and small ruminants above the age of 1 year (OR 2.23, CI 1.38-3.59) were found more likely to acquire the infection. The Hosmer and Lemeshow Test ($\chi^2 = 1.825$, $P = 0.402$) and Nagelkerke R Square (0.048) indicated that it was a fair model at the animal level.

Table 1: Flock-level seroprevalence of antibodies against *Mccp* in goats and sheep of Oman

Governorate	Goats		Sheep		Overall	
	Pos. / Tested	Prev. % (95% CI)	Pos. / Tested	Prev. % (95% CI)	Pos. / Tested	Prev. % (95% CI)
Muscat	15/22	68.2 (45.1-86.1)	7/26	26.9 (11.6-47.8)	15/28	53.8 (33.9,72.5)
Al-Buraimi	8/11	72.7 (39-94)	1/14	7.1 (0.2-33.9)	8/17	47.1 (23,72.2)
Musandam	4/13	30.8 (9.1-61.4)	1/9	11.1 (0.3-48.2)	5/13	38.5 (13.9,68.4)
Al-Batinah North	24/82	29.3 (19.7-40.4)	17/82	20.7 (12.6-31.1)	34/91	37.4 (27.4,48.1)
Sharqiyah South	21/59	35.6 (23.6-49.1)	12/57	21.1 (11.4-33.9)	26/70	37.1 (25.9,49.5)
Dakhiliyah	16/56	28.8 (17.3-42.2)	4/59	6.8 (1.9-16.5)	19/74	25.7 (16.2,37.2)
Dhofar	10/39	25.6 (13-42.1)	0/12	0 (0-26.5)	10/42	23.8 (12.1,39.5)
A'Dhahira	7/41	17.1 (7.2-32.1)	6/44	13.6 (5.2-27.4)	11/48	22.9 (12,37.3)
Al-Batinah South	6/40	15 (5.7-29.8)	2/33	6.1 (0.7-20.2)	8/44	18.2 (8.2,32.7)
Sharqiyah North	7/49	14.3 (5.9-27.2)	2/47	4.3 (0.5-14.5)	9/63	14.3 (6.7,25.4)
Al-Wusta	2/17	11.8 (1.5-36.4)	0/15	0 (0-21.8)	2/20	10 (1.2,31.7)
Total	120/429	28 (23.8-32.5)	52/398	13.1 (9.9-16.8)	147/510	28.8 (24.9-33)

Flock-level prevalence was significantly different for goat flocks of various governorates ($\chi^2 = 43.056$, $df = 10$, $P < 0.001$): Flock-level prevalence was significantly different for sheep flocks of different governorates ($F = 2.381$, $df = 10$, $p = 0.010$): Overall prevalence was significantly different among governorates ($\chi^2 = 31.360$, $df = 10$, $p = 0.001$): Seroprevalence was significantly different between goats and sheep ($\chi^2 = 27.80$, $df = 1$, $P < 0.001$).

Table 2: Univariable analysis of the risk factors for the seroprevalence of CCPP in the sampled goats and sheep flocks of Oman

Variable	Category	Pos. / Tested	Prev. % (95% CI)	Significance	OR (95% CI)	P-value
Animal level						
Species	Goats	208/2119	9.8 (8.6-11.2)	$\chi^2 = 60.66$ $p < 0.001$	2.93 (2.21, 3.88)	< 0.001
	Sheep	68/1896	3.6 (2.8-4.5)			
Age	<= 1 year	19/574	3.3 (2-5.1)	$\chi^2 = 15.74$ $p < 0.001$	1.0	0.001
	1.1 to 3 Year	178/2521	7.1 (6.1-8.1)			
	> 3 Year	79/920	8.6 (6.9-10.6)			
Breeds	Imported	32/431	7.4 (5.1-10.3)	$\chi^2 = 9.01$ $p = 0.011$	1.90 (1.11, 3.25)	0.013
	Local	219/2968	7.4 (6.5-8.4)			
	Cross	25/616	4.1 (2.6-5.9)			
Sex	Female	225/3229	7.0 (6.1-7.9)	$\chi^2 = 0.23$ $p = 0.634$	1.08 (0.79, 1.48)	0.634
	Male	51/786	6.5 (4.9-8.4)			
Flock-level						
Location	Coastal	78/221	35.3 (29.42)	$\chi^2 = 7.96$ $p = 0.005$	1.74 (1.18, 2.56)	0.005
	Dry	69/289	23.9 (19.1,29.2)			
Herd size	> 100	47/112	41.9 (32.7,51.7)	$\chi^2 = 12.08$ $p = 0.001$	2.16 (1.39, 3.34)	0.001
	<= 100	100/398	25.1 (20.9,29.7)			
Mixing of imported breeds	Yes	69/188	36.7 (29.8,44)	$\chi^2 = 9.01$ $p = 0.003$	1.81 (1.23, 2.68)	0.003
	No	78/322	24.2 (19.6,29.3)			
Housing	Confined	114/377	30.2 (25.6,35.1)	$\chi^2 = 1.41$ $p = 0.235$	1.31 (0.84, 2.06)	0.005
	Open	33/133	24.8 (17.7,33)			
Breeding strategy	Open	107/333	32.1 (27.1,37.4)	$\chi^2 = 5.12$ $p = 0.024$	1.62 (1.06, 2.47)	0.024
	Closed	40/177	22.6 (16.7,29.5)			

Table 3: Final binary logistic regression model to predict the seroprevalence of *Mccp* in the small ruminants at animal and flock level in Oman

Variable	Exposure	Comparison	OR	95%CI	P-value
Species	Goats	Sheep	2.87	2.17-3.81	< 0.001
Age	1.1 to 3 Year	Less than equal to 1 year	2.10	1.29-3.41	0.001
	Above 3 Year	Less than equal to 1 year	2.58	1.54-4.32	
Geographical location	Coastal regions	Dry regions	1.70	1.14-2.53	0.009
Flock size	Above 100	Less than 100	2.45	1.54, 3.91	< 0.001
Breeding strategy	Open	Closed	2.08	1.33, 3.27	0.001

DISCUSSION

The samples were tested through a commercial cELISA (IDEXX Montpellier SAS, France), and our results indicated that both goats and sheep across Oman are exposed to the *Mccp* infection. The multivariable analysis indicated that likelihood of testing positive was higher for goats (OR 2.87) compared to sheep. Although, goats are the primary hosts for CCPP, sheep could also become a secondary host especially in the mixed flocks. Studies conducted in Ethiopia (Hadush *et al.*, 2009; Teshome *et al.*, 2019) and Tanzania (Mbyuzi *et al.*, 2014) have reported the prevalence of *Mccp* in the sheep. This evidence of seroconversion in sheep is epidemiologically significant because it might perpetuate the CCPP infection in mixed flocks of the small ruminants in Oman.

The higher seroprevalence of CCPP found in our study, further endorses that the disease is endemic in the

flocks of the Middle East and Africa (Peyraud *et al.*, 2014). Clinical incidence of CCPP has been reported by authors in Qatar (Arif *et al.*, 2007), Saudi Arabia (El-Deeb *et al.*, 2017), Yemen and the United Arab Emirates (Rurangirwa *et al.*, 1987). The seroprevalence recorded in Oman was in accord with that reported in; Ethiopia (Hadush *et al.*, 2009; Teshome *et al.*, 2019), Kenya (Kipronoh *et al.*, 2016), some Central Asian countries (Peyraud *et al.*, 2014), Pakistan (Shahzad *et al.*, 2012; Peyraud *et al.*, 2014) and India (Suryawanshi *et al.*, 2015). We suspect that live animal import from the endemic countries of Africa and South Asia might have introduced the disease in Oman. However, isolation of the *Mccp* from the suspected cases and molecular epidemiological studies are required to establish it.

The seroprevalence of antibodies against *Mccp* and odds for testing positive were higher in flocks located in the coastal regions as compared to the drier areas. This can be related to the differences in the population density,

management system, availability of the animal health services and climatic conditions in these areas. The practice of sedentary farming system is common in the coastal areas of Oman. In this system, animals are kept inside confined spaces, and the introduction of new stock from the local markets is quite common. This introduction of new animals and sedentary farming system are also reported as an important risk factor associated with the seroprevalence of *Mccp* in the endemic areas (Mbyuzi *et al.*, 2014; Asmare *et al.*, 2016; Kipronoh *et al.*, 2016).

We found that herd size above 100 and open herds (introduction of new animals from the local market) were significantly more likely to test positive for antibodies against *Mccp*. Close contact in case of large flocks due to the congestion around feed and water sources might have resulted in the spread of CCPP in these flocks. Furthermore, owners of larger flocks are more likely to respond to an outbreak with a therapeutic intervention, that might have improved; the survival rates and presence of seropositive animals in these flocks (Mbyuzi *et al.*, 2014). However, flock size was not found associated with the seroprevalence of CCPP in Ethiopia (Teshome *et al.*, 2019), Kenya (Kipronoh *et al.*, 2016) and India (Parray *et al.*, 2019). The variations in farming systems between Oman and these countries could be a possible reason for this difference.

Small ruminants between 1.1 to 3 years (OR 2.10) and above three years (OR 2.58) of age were more likely to test positive for antibodies against *Mccp*. This suggested that the exposure to *Mccp* is proportional to the age of animals. Various authors reported that in the endemic areas, the prevalence of CCPP is positively associated with the age of animals (Mbyuzi *et al.*, 2014; Parray *et al.*, 2019; Teshome *et al.*, 2019).

Although the seroprevalence was found associated with the imported and local breeds at univariable analysis, but this variable was knocked out in the multivariable analysis. This is in agreement with studies from Ethiopia (Hadush *et al.*, 2009) and India (Suryawanshi *et al.*, 2015), that reported that the prevalence of CCPP is not associated with the a breed. Moreover, the seroprevalence was not found associated with the gender of the small ruminants. It is reported that CCPP, irrespective of age and sex of the animal, remains highly contagious and fatal disease of susceptible goats (Asmare *et al.*, 2016; Kipronoh *et al.*, 2016).

Conclusions: Our results indicate a widespread prevalence of contagious caprine pleuropneumonia in the goat and sheep flocks of Oman. If a carefully chalked out control program based upon changes in the management and vaccination is not adopted, the *Mccp* will continue to cause considerable economic losses to the farmers. We suggest that outbreaks of the respiratory diseases in small ruminants should be subjected to microbiological and molecular investigations. Since mixed farming of small ruminants is a common practice in the endemic regions, the possible role of sheep as a potential reservoir of *Mccp* should be further investigated before contemplating a control programme.

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Authors contribution: MH Hussain supervised field work and performed data analysis. MN Asi, MK Mansoor and M Saqib conceived and designed the study and supervised the laboratory work. H Al-Tahir, AH Alrawahi, SS Al-Makhladi, MG Al-Maawali, AHA Al-Subhi, NYA Al-Senaidi collected sampled and epidemiological information. SSR Al-Uahmadi, RSN Al-Subhi, MKI Al-Beloushi, FSS Al-Sinani, BST Al-Riyami conducted laboratory investigations. All authors critically reviewed the manuscript and approved final version.

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