Sero-Prevalence Of Contagious Caprine Pleuropneumonia (Ccpp) In Selected Regions Of Khyber Pakhtunkhwa Pakistan

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Abstract
Contagious Caprine Pleuropneumonia (CCPP) is a severe respiratory disease caused by *Mycoplasma capricolum subsp. capripneumoniae* (*Mccp*) that affects goats and other small ruminants worldwide. CCPP is characterized by fever, coughing, dyspnea, pleurisy, and high mortality rates. CCPP is transmitted through direct or indirect contact with infected animals or aerosols. CCPP causes significant economic losses due to reduced productivity, increased mortality, and increased veterinary costs. The aim of this study was to assess the sero-prevalence of CCPP in selected regions of Khyber Pakhtunkhwa (KP), Pakistan, and explore associated risk factors. We hypothesized that, CCPP is prevalent in the studied population and that it varies by district, age, and gender. A cross-sectional survey was conducted from January to March 2023, involving the collection of serum samples from 1230 small ruminants (900 goats and 330 sheep) from 15 districts within KP. A convenience sampling method was used to select 82 herds with an average herd size of 15 animals. A competitive enzyme-linked immunosorbent assay (cELISA) was employed to detect anti-*Mccp* antibodies. Samples were categorized as positive if the percentage of inhibition (SPI) exceeded 55%. Out of 1230 serum samples tested, 92 samples (7.47%) were found positive for anti-*Mccp* antibodies, indicating the presence of CCPP in the studied population. District-wise analysis revealed varying sero-prevalence rates, ranging from 3.66% in Karak to 13.41% in Abbottabad. However, these differences were not statistically significant (Chi-square test, $p=0.15$). The study further categorized animals by age (<6 months, 6 months-1 year, >1 year) and gender (male, female). Age-related patterns showed no significant differences in sero-prevalence across age groups in both goats (ANOVA test, $p=0.21$) and sheep (ANOVA test, $p=0.34$). However, gender-based analysis revealed a significantly higher sero-prevalence in female goats (11.8%) compared to males (4.6%) (t-test, $p<0.05$), while in sheep, a similar trend was observed, with higher sero-prevalence in female sheep (5.6%) compared to rams (2.6%) (t-test, $p<0.05$).

Keywords: Contagious Caprine Pleuropneumonia, CCPP, Sero-Prevalence, cELISA, Sheep, Goat, Small Ruminants, Khyber Pakhtunkhwa, KP, Pakistan.

Introduction

Contagious caprine pleuropneumonia (CCPP) is a highly infectious economically important disease of goats endemic in Africa and some countries of Asia caused by *Mycoplasma capricolum subsp. capripneumoniae* (*Mccp*) known in the past as F38 mycoplasma strain (Gelagay et al., 2007) This is the smallest (300 nm size) fastidious bacteria which possesses triple layer membrane but lacks cell wall. *Mccp* causes lesions specifically in thoracic cavity and is an important member of mycoides cluster (Peyraud et al 2014).Transmission of CCPP from diseased animals to susceptible animals occurs through aerosol droplets produced during coughing when animals are in close contact (Zahur et al., 1994).The disease is characterized by pyrexia (41-43 ^{0}C), painful and labored respiration sometime with snoring and grunting, violent and productive coughing, anorexia, copious nasal discharges, abortion with high morbidity and mortality (Nascimento et al.,1986). Macroscopically the CCPP lesions are observed in acute disease only and confined to thoracic cavity mainly includes fibrinous unilateral pleuropneumonia with accumulation of straw color fluid in the pleural cavity but still the differential diagnosis from other respiratory diseases is difficult (Ejaz et al., 2015). The disease was confirmed in Pishin district of Baluchistan, Pakistan. for the first time through Polymerase Chain Reaction PCR in 2009 by (Awan), the method of choice for *Mccp* detection (Nicholas et al., 2002). Mostly for seroprevalence studies so far, complement fixation test (CFT) was the only available approved test. CFT utilized crude antigen and is therefore complicated by cross reactivity problem with other member species of "mycoides cluster", a great obstacle in determination of exact prevalence of CCPP. A newly formatted monoclonal antibody based cELISA kit was developed that specifically detect *Mccp* directed antibodies in goat serum and therefore can be applied to determine the exact seroprevalence of CCPP in regions or countries with no vaccination programs (Woubit et al., 2004). The newly formatted monoclonal antibody based cELISA kit was utilized 1st time for the Sero-survey of CCPP in different districts of KP, Pakistan.

2. Material and Methods

2.1. Sampling area

The seroprevalence study of CCPP was conducted in selected districts namely Abbottabad: (35° 18' 20" North, 73° 14' 58" East) Buner: (35° 26' 21" North, 72° 13' 20" East) Kohat: (33° 35' 13" North, 71° 26' 32" East) Bannu: (32° 59' 32" North, 70° 36' 51" East) (Karak: 32° 59' 18" North, 71° 04' 13" East) Dera Ismail Khan (D.I. Khan): (31° 49' 31" North, 70° 54' 47" East) Charsadda: (34° 08' 53" North, 71° 44' 37" East) Mardan: (34° 12' 15" North, 72° 01' 12" East) Nowshera: (33° 59' 30" North, 71° 57' 31" East) Peshawar: 34° 00' 48" North, 71° 31' 25" East) Bajaur: (34° 40' 40" North, 71° 30' 53" East) South Waziristan, (33° 01' 00" North, 70° 14' 00" East) North Waziristan, 33° 01' 00" North, 70° 14' 00" East Orakzai: 33° 41' 00" North, 71° 28' 00" East)) and Kurram (33° 44' 00" North, 70° 56' 00" East) of KP, Pakistan.

2.2. Sample Size

Total 1230 caprine serum samples were collected from both sexes and different age sheep and goats. The sampling was done from goats with respiratory distress in flocks with no vaccination history against CCPP. Equal number of samples were obtained in each district from each flock.

Sample Shipping and Storage.

The samples were transported in ice packs fitted box to Pathology and Bacteriology Lab, Veterinary Research Institute (VRI), Peshawar, Pakistan where samples were stored at -200 C for analysis in future.

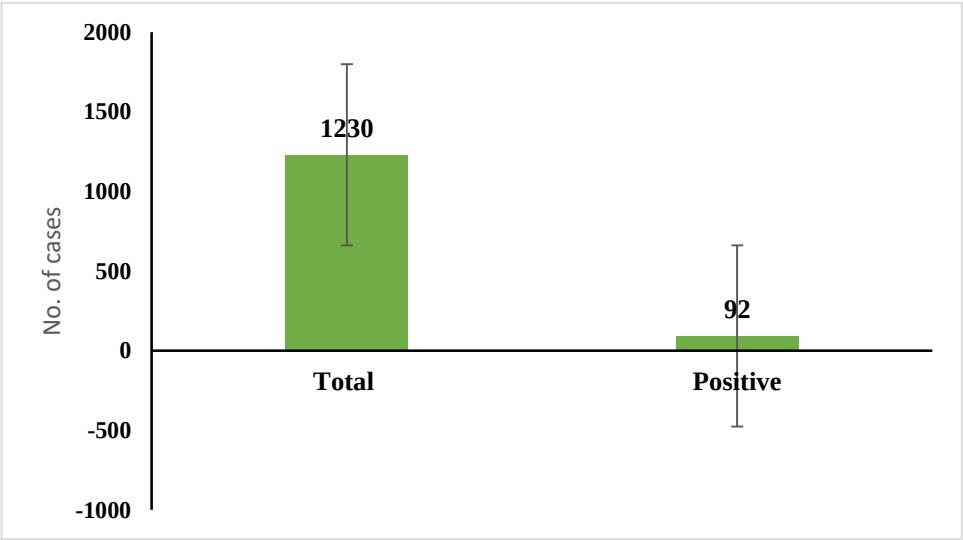
2.4. Sample Analysis

The serum samples were tested for Mccp directed antibodies using monoclonal antibody based competitive cELISA technique utilizing cELISA Test Kit (IDEXX CCPP, 06- 56231-01). The 1st batch of cELISA Kit developed at CCPP reference lab CIRAD-Montpellier, France was purchased under the research project "Use of Molecular Techniques in Livestock Research at KP" by VRI, Peshawar Pakistan for this 1st time sero-survey of CCPP in the province KP, Pakistan The serum samples were processed according to CCPP cELISA kit procedure adopted by (Peyraud et al 2014). The collected data was analyzed using Chi square statistical test.

Results

Sero prevalence of contagious caprine pleuropneumonia (CCPP)

cELISA kit was used for the determination of anti-*Mccp* antibodies in the serum samples collected from small ruminants. The absorbance of each test and control well were determined and the percentage of inhibition (SPI) was estimated with the help of formulas. The sample was declared positive for having SPI greater than 55% and negative for SPI less than 55%. Out of a total of 1230 serum samples, 92 samples (7.47%) tested positive for anti-*Mccp* antibodies. (**Error! Reference source not found.**).



Error! Reference source not found.**)** Overall sero-prevalence of CCPP by cELISA in selected districts of KP

District wise seroprevalence of CCPP through cELISA in different districts of KP

The district-wise seroprevalence of *Mccp* was 13.41% in Abbottabad, 6.1% each in Bajaur and Bannu, 10.98% in Buner, 6.1 % in Charsadda, 4.88% in D.I. Khan, 3.66% in Karak, 7.32% each in Kohat, Kurram and Mardan, 6.1% each in Orakzai and Nowshera, 9.76% in Peshawar, 4.88% in South Waziristan, and 12.2% in North Waziristan. (**Error! Reference source not found.**). Although there was a slight numerical variations in the seroprevalence of *Mccp* in different districts, however, the difference was non-significant. (**Error! Reference source not found.**1).

Table: 1 District-wise seroprevalence of CCPP in small ruminants in KP

Districts	Positive (n=92)	Positive (%)	Total (n=1230)	P value
Abbottabad	11	13.41	82	
Bajaur	5	6.10	82	
Bannu	5	6.10	82	
Buner	9	10.98	82	
Charsadda	5	6.10	82	
DI Khan	4	4.88	82	
Karak	3	3.66	82	
Kohat	6	7.32	82	0.23
Kurram	6	7.32	82	
Mardan	6	7.32	82	
Orakzai	5	6.10	82	
Nowshera	5	6.10	82	
Peshawar	8	9.76	82	
South Waziristan	4	4.88	82	
North Waziristan	10	12.20	82	

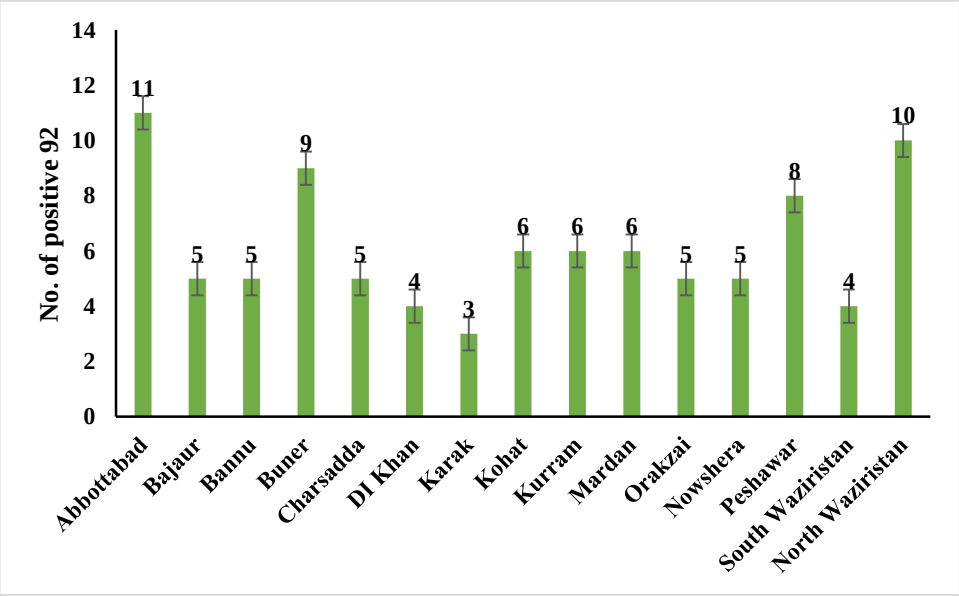


Figure 2: District wise seroprevalence of CCPP through cELISA

Seroprevalence of CCPP in different age groups of sheep and goats through cELISA

The sheep and goats in the current study were categorized into three different age groups i.e., <6 months, 6 months to 1 Year, and >1 year age group. The seroprevalence of *Mccp* was 10.1% in <6 months kids, 9.7% in 6 months to 1 year age group, and 10.5% in >1 year age group goats. There was a slight variation in the prevalence of *Mccp* in different age groups of goat but the difference was non-significant (P>0.05). The association of age to *Mccp* in goats as per the univariate logistic regression analysis was; OR=0.842, CI=0.643-1.102, p=0. 21. (**Error! Reference source not found.**).

Table Error! No text of specified style in document.**:** Age-wise seroprevalence of *Mccp* in goats in KP

Age categories	Positive (n=62)	Total (615)	Percent positivity	p-value
<6months	17	168	10.1	
6m-1Y	26	267	9.7	0.218
>1Y	19	180	10.5	

Similar age groups i.e., <6 months, 6 months- 1 year, and > 1 year, were also constituted in sheep. The seroprevalence of *Mccp* was 6.17% in <6 months lambs, 6.6% in 6 months to 1 year age group, and 6.5% in >1 year age group sheep. The seroprevalence among different age groups in sheep was non-significant and the association as per the logistic regression was, OR=0.724, CI=0.535-0.993, and P=0.34. (**Error! Reference source not found.**).

Table 3 Age-wise seroprevalence of CCPP in sheep in KP

Age categories	Positive (n=30)	Total (615)	Percent positivity	p-value
<6months	11	178	6.17	
6m-1Y	17	255	6.6	0.34
>1Y	12	182	6.5	

Sex based seroprevalence of CCPP through cELISA among sheep and goats of selected districts

The seroprevalence of CCPP in goats was significantly ($p<0.05$) higher in females (11.8%) as compared to males (4.6%). The association between the dependent variable (sex of animal) and independent variable (*Mccp* prevalence) according to univariate logistic regression analysis was; OR=1.02, CI=0.847-1.65, p=0.01. (**Error! Reference source not found.**).

Table Error! No text of specified style in document.**.1** Sexwise seroprevalence of *Mccp* in goats in KP

Gender	Positive (n=62)	Total (n=615)	Percent positivity	p-value
Female	55	465	11.8	0.01
Male	7	150	4.6	

Sexwise seroprevalence of CCPP in sheep was 5.6% in female sheep, as compared to 2.6% in ram. The difference in the Sexwise seroprevalence of *Mccp* in sheep was significant and the association as per the univariate logistic regression was; OR=0.85, CI=0.673-1.04, and p=0.04. (**Error! Reference source not found.**).

Table Error! No text of specified style in document.**.2** Sex wise seroprevalence of *Mccp* in sheep in KP

Gender	Positive (n=30)	Total (n=615)	Percent positivity	p-value
Female	26	465	5.6	0.04
Male	4	150	2.6	

Discussion

In this study, we investigated the seroprevalence and risk factors of CCPP in goats and sheep in selected districts of KP, Pakistan, using the cELISA kit. We found that the overall seroprevalence of CCPP was 7.47%, with significant variation among districts and between sexes, but not among age groups. We also found that female goats and sheep were more susceptible to CCPP infection than males.

Our findings are consistent with some previous studies that reported a low to moderate seroprevalence of CCPP in different regions of Pakistan using the same diagnostic test. However, our study showed a lower seroprevalence than some other studies that reported a high seroprevalence of CCPP in Pakistan using different diagnostic tests such as PCR or LAMP. This could be due to the differences in the sensitivity and specificity of the tests, as well as the sample sizes, sampling methods, geographical areas, or epidemiological situations.

We also compared our results with some international studies on CCPP that used the cELISA kit. We found that our seroprevalence was similar to some studies that reported (Peyraud et al., 2015) a low seroprevalence of CCPP in Egypt, Ethiopia , Mauritius , Afghanistan , and Tajikistan , but lower than some studies that reported a high seroprevalence of CCPP in Kenya , Sudan , Iran , Turkey , and China . These variations could be attributed to the differences in the prevalence and distribution of *Mccp* strains, as well as the climatic conditions, animal husbandry practices, vaccination programs, and disease control measures in different countries and regions.

One of the risk factors that we identified in our study was sex. We found that female goats and sheep were more likely to be infected with CCPP than males. This is in agreement with some studies that (Selim et al., 2015) reported a higher seroprevalence of CCPP in females than males, but not with others that reported no significant difference between sexes. The higher susceptibility of females could be explained by their lower immunity due to pregnancy or lactation, or by their higher exposure to *Mccp* due to their frequent contact with other animals or humans.

Another risk factor (Kandeel et al., 2018) that we investigated was age. We found that there was no significant difference in the seroprevalence of CCPP among different age groups in both goats and sheep. This is contrary to some studies that reported a higher seroprevalence of CCPP in older animals than younger ones. The lack of association between age and CCPP infection could be due to the low sample size or the low prevalence of *Mccp* in our study area. Alternatively, it could be due to the high mortality rate of young animals infected with CCPP, which reduces their chance of being sampled or tested.

(Khan et al 2018) Some other risk factors that we did not examine in our study but could be important for future research are breed, flock size, season, management system, and co-infection with other pathogens. Some studies have reported that these factors could influence the seroprevalence and transmission of CCPP in small ruminants. Therefore, it would be useful to collect more data on these factors and include them in the statistical analysis to better understand the epidemiology and risk factors of CCPP in Pakistan.

The main implications of our study are that CCPP is a prevalent disease affecting small ruminants in KP. Pakistan. and that it poses a serious threat to the livelihoods and food security of the farmers and the rural communities. Therefore, there is a need for more awareness and education on the clinical signs, diagnosis, treatment, prevention, and control of CCPP among the stakeholders. There is also a need for more surveillance and monitoring of CCPP outbreaks and Mccp strains in different regions of Pakistan, as well as for more research on the development and evaluation of effective vaccines and diagnostic tests for CCPP.

The main limitations of our study are that we used a cross-sectional design, which does not allow us to establish a causal relationship between CCPP infection and risk factors, or to measure the incidence or prevalence of CCPP over time. We also used a convenience sampling method, which may introduce selection bias or reduce the representativeness of our sample. We also used only one diagnostic test, which may have some limitations in terms of sensitivity, specificity, or

accuracy. Moreover, we did not collect any data on the clinical signs, history, or treatment of the animals, which could provide more information on the disease status and outcome of the animals.

Some directions for future research are to use a longitudinal or cohort design, which can provide more reliable and valid data on the risk factors and transmission dynamics of CCPP. We also suggest using a random or stratified sampling method, which can improve the generalizability and comparability of our results. We also recommend using more than one diagnostic test, such as PCR or LAMP, which can confirm or complement the results of cELISA. Furthermore, we advise collecting more data on the clinical signs, history, or treatment of the animals, which can enhance our understanding of the disease impact and response of the animals.

Conclusion

CCPP in goats and association of *Mccp* antibodies with sex and age of goats has been investigated to a large extent in the selected districts of KP, Pakistan. This study concluded that *Mccp*, the causal agent of classical lethal CCPP is the prevalent specie in the studied areas. However, further study is needed to explore this fatal disease through increased sample size over a wide geographical area of KP, Pakistan.

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