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Seroepidemiological Analysis of Contagious Caprine Pleuropneumonia through cELISA in Selected Districts of Khyber Pakhtunkhwa-Pakistan

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Abstract

Contagious caprine pleuropneumonia (CCPP) is a fatal disease of goats caused by Mycoplasma capricolum subsp. capripneumoniae (Mccp).This disease has been causing huge economic losses to goat rearing farmers in Khyber Pakhtunkhwa. Seroepidemiological study of this disease was conducted for the first time in selected districts of Khyber Pakhtunkhwa namely Swat, Peshawar, Kohat and Dera Ismail Khan. Total 384 serum samples were collected randomly from goats having different ages and both sexes showing respiratory signs belonging to flocks with no vaccination history against CCPP. The serum samples were examined for Mccp directed antibodies using monoclonal antibody based cELISA. Out of total 384 samples 15 samples were detected positive on cELISA with 3.91% overall seroprevalence in the selected districts. The highest CCPP seroprevalence was recorded in district Swat (8.33%) followed by district Kohat and D.I Khan (3.13% in each district) and the lowest seroprevalence was observed in district Peshawar (1.04%). Age based CCPP seroprevalence was found highest (6.73%) in the goat kids of age 1 to 180 days followed by the (3.85%) young goat of 181 to 365 days while the lowest was found in the adult goats (2%) with age more than one year.

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Sex based seroprevalence was found more in female goats (4.01%) as compare to male goats (3.33%). This study determined the load of CCPP using highly specific monoclonal antibody based cELISA kit in the selected districts of Khyber Pakhtunkhwa for the first time. Further study on CCPP is needed with increased sample size, that cover wider region of Khyber Pakhtunkhwa as necessary for limiting this disease through effective control measures.

Keywords: Mycoplasma; Mccp; cELISA; CCPP.

1. 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 [9]. 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 [7].Transmission of CCPP from diseased animals to susceptible animals occurs through aerosol droplets produced during coughing when animals are in close contact [13]. 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 [1]. 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 [2]. The disease was confirmed in Pishin district of Balochistan, Pakistan for the first time through PCR in 2009 by [6], the method of choice for *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) detection [8]. 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 [2]. The newly formatted monoclonal antibody based cELISA kit was utilized 1st time for the Sero-survey of CCPP in different districts of Khyber Pakhtunkhwa, Pakistan.

2. Material and Methods

2.1. Sampling area

The seroprevalence study of Contagious Caprine Pleuropneumonia (CCPP) was conducted in selected districts namely Peshawar (34° 0' 28" North, 71° 34' 24" East), D.I Khan (31° 49' 58" North, 70° 54' 9" East), Swat (35° 22' 42" North, 72° 10' 47" East) and Kohat (33° 35' 13" North, 71° 26' 32" East) of Khyber Pakhtunkhwa, Pakistan.

2.2. Sample Size

Total 384 caprine serum samples were collected from both sexes and different age goats. The sampling was

done from goats with respiratory distress in flocks with no vaccination history against Contagious Caprine Pleuropneumonia (CCPP). Equal number of samples were obtained in each district from each flock.

2.3. Sample Shipping and Storage

The samples were transported in ice packs fitted box to Pathology and Bacteriology Lab, Veterinary Research Institute (V.R.I), Peshawar, Pakistan where samples were stored at -20^{0}C for analysis in future.

2.4. Sample Analysis

The serum samples were tested for *Mycoplasma capricolum* subsp. *capripneumoniae* (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 Khyber Pakhtunkhwa" by V.R.I, Peshawar, Pakistan for this 1st time sero-survey of CCPP in the province Khyber Pakhtunkhwa, Pakistan. The serum samples were processed according to CCPP cELISA kit procedure adopted by [2]. The collected data was analyzed using Chi square statistical test.

3. Results

In the present study 384 caprine serum samples were collected from the selected districts of province Khyber Pakhtunkhwa, Pakistan. All the samples were analyzed for Mccp directed antibodies using cELISA test. Overall sero-prevalence of Contagious Caprine Pleuropneumonia (CCPP) in the study area was found 3.91 (Figure 1).

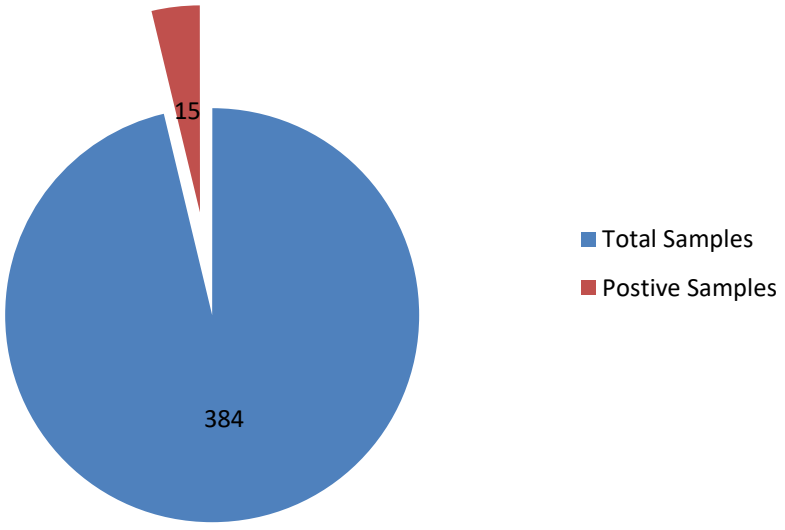

Figure 1: Over all sero-prevalence of CCPP by cELISA in selected districts of Khyber Pakhtunkhwa

3.1. District wise seroprevalence of CCPP through cELISA in different districts of Khyber Pakhtunkhwa

The prevalence of CCPP was recorded 1.04% in district Peshawar, 8.33% in district Swat and 3.13% each in districts Kohat and D.I Khan. A statistically non-significant difference (P>0.05) was observed in sero-prevalence of CCPP by cELISA among the selected districts (Figure 2).

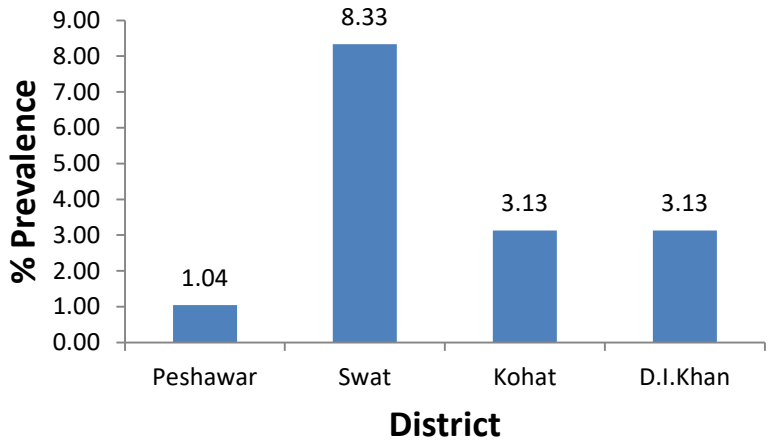

Figure 2: District wise seroprevalence of CCPP through cELISA

3.2. Seroprevalence of CCPP in different age groups of goats through cELISA

Sero-prevalence of CCPP in goats by cELISA was recorded 6.73% in 1 to 180 days age group, 3.85% in 181 to 365 days age group and 2% in >1 year age group. A statistically non-significant relation (P>0.05) was observed among the different age groups of goats in selected districts of Khyber Pakhtunkhwa (Figure 3).

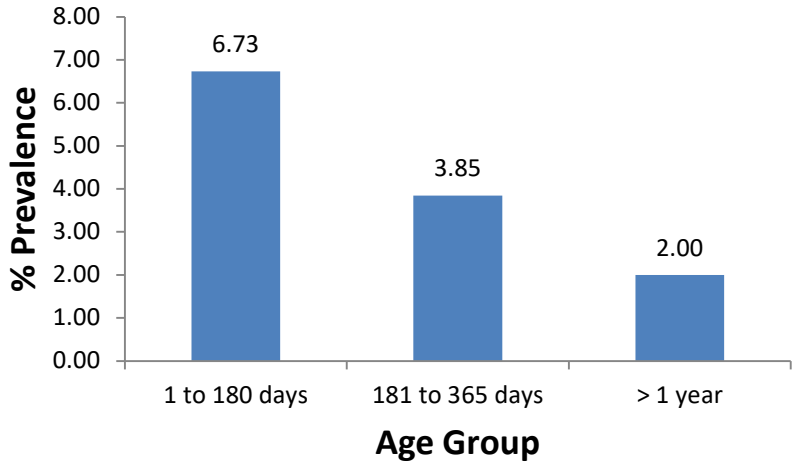

Figure 3: Over all seroprevalence of CCPP in different age groups of goats by cELISA

3.3. Sex based seroprevalence of CCPP through cELISA among goats of selected districts

Overall sex based sero-prevalence of CCPP by cELISA in selected districts of Khyber Pakhtunkhwa was found 3.33% and 4.01% in male and female goats respectively. A statistically non-significant difference (P>0.05) was found in sex based prevalence implying that both sexes are equally affected by CCPP in the selected districts of Khyber Pakhtunkhwa (Figure 3).

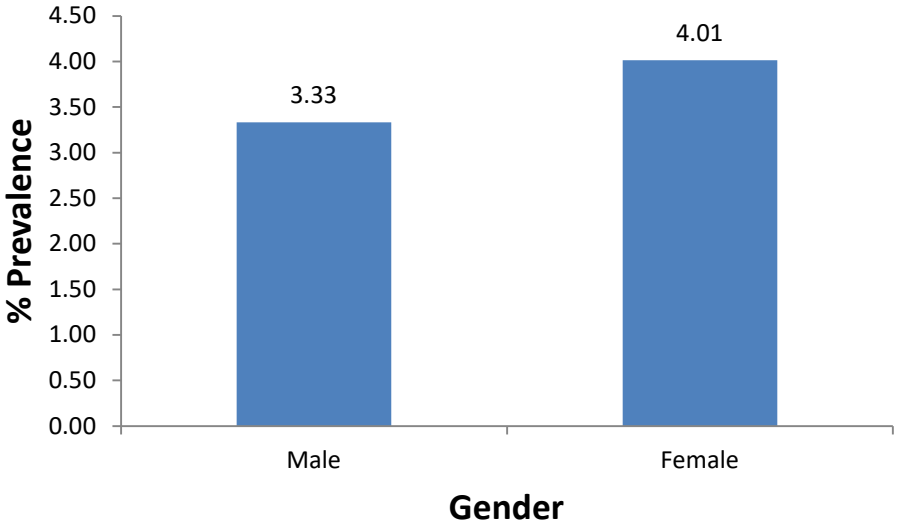

Figure 3: Overall **Sex based seroprevalence of CCPP through cELISA**

4. Discussion

In this study, the overall seroprevalence of CCPP by cELISA was recorded 3.91% in the study area. Reference [13] utilized the same cELISA kit, observing high (8.52%) Mccp seroprevalence in goats at different districts of Punjab, Pakistan. Similarly [9] reported high prevalence (16.6%) of CCPP in two districts (Otuke and Agago) of Northern Uganda. The previous research works are not consistent with the present research work because of difference in sample size, sampling period and sampling area. Reference [5] observed zero percent prevalence of Mccp conducting mycoplasmosis molecular prevalence study in small ruminants at Balochistan, Pakistan. The reason for different prevalence value with the present research study may be the use of different assays, based on different principles, used for sample analysis. The PCR employed directly on the field samples in the previous research specifically amplify the target genome as the organism of interest in field samples is always low as compare to cELISA technique employed in the present study detect anti-Mccp antibodies in the serum sample. **[2]** confirmed the seroprevalence of CCPP in an international collaborative study in Kenya (district Narok, seroprevalence among herds varies between 6 to 90%), Afar region of Ethiopia (14.6%), Mauritius (16%), Tajikistan (10.1%), Afghanistan (district Wa Khan 0%) and Pakistan (Gilgit 2.7%, Diamer 44.2%, districts of Gilgit-Baltistan) utilizing the same monoclonal antibody cELISA kit used in my studies. Seroprevalence values in this study, as opposed to high values recorded in the previous research may be due differences in the sample size, sampling from CCPP endemic countries and from animals under different

management systems. The high prevalence values in previous research were observed because sampling was not done randomly and from small geographical region, most of the sampling was done from infected flocks presented for CCPP vaccination. The exact prevalence may be estimated by performing sampling randomly over wide geographical region. [12] observed zero percent prevalence of Mccp in 2006-2009 by Latex Agglutination Test in different districts of Pakistan does not favor the present study. The variation may be due to sample size, huge time gape between the sample size and the different technique used in the previous research.

In this study, the high seroprevalence was recorded in district Swat (8.33%) followed by district Kohat and Dera Ismail Khan (3.13% each district) and lowest seroprevalence was observed in district Peshawar (1.04%). The difference in the prevalence values among selected districts may be a chance not real one. The results of this study are in agreement with the study results of [12; 3] who also observed maximum positive results in hilly and sub hilly areas. Moreover the little part of results in this study are supported by the previous research study by [12] in terms of the same prevalence recorded in district Kohat while other part of my research is not supported by the same previous research showed high prevalence value in district Peshawar (4.16%) in comparison to prevalence (1.04%) recorded in this study. The variation in the results is because in the previous research all Mycoplasma species pathogen to caprine specie were targeted as compare to this study which focused only on the Mccp directed antibodies in the serum using cELISA kit with almost cent percent specificity.

Seroprevalence in this study by monoclonal antibody-based cELISA was recorded highest (6.73%) in the goat kids with age 1to 180 days followed by young goats with age 181 to 365 days and the lowest (2%) seroprevalence was recorded in the adult goats with age more than one year. This variation might not be real one but chance. The research work by [13] is in partial agreement with the present study who observed high Mccp prevalence in young goats (9.89%) followed by goat kids (8.43%) and lowest in adult goats (7.89%), using the same cELISA kit at different districts of Punjab, Pakistan. The findings of this study is supported by [12] research work who found the same results with high seroprevalence in goat kids than adult goats. This study results are also in agreement with the results of [11] who observed more positive results in freely ranging young Spanish ibex. Similar results were found by [4] who observed 90% prevalence in goat kids.

Sex based seroprevalence of CCPP in goats was recorded 3.33% in male and 4.01% in female showing insignificant variation between the values implying that both sexes are equally affected by disease. This variation may be due to chance not real one. The findings of the present research work is supported by the findings of [13] who also observed high Mccp prevalence in female goats (10.24%) as compare to male goats (7.07%) at Punjab, Pakistan. This results are in agreement with the previous research work results by [12] who found more positive results in female goats as compare to male goats. The present study results can be correlated to the previous research work by [11] who observed more positive results in freely ranging female Spanish Ibex. Similarly [10] found the same results with high prevalence (16.1 %) in female relative to male (10.7%).

5. Conclusion

Contagious Caprine Pleuropneumonia (CCPP) in goats and association of *Mycoplasma capricolum* subsp.

capripneumoniae (Mccp) antibodies with sex and age of goats has been investigated to a large extent in the selected districts of Khyber Pakhtunkhwa, 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 Khyber Pakhtunkhwa, Pakistan.

Acknowledgments

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