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# Seroprevalence of contagious bovine pleuropneumonia in cattle of Punjab, Pakistan and assessment of risk factors

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## ABSTRACT

Contagious bovine pleuropneumonia (CBPP) is an important infectious disease of respiratory system of cattle, caused by *Mycoplasma mycoides* subspecies mycoides. A cross sectional study was conducted in Lahore, Kasur and Jhang districts of Punjab to determine the prevalence of CBPP. A total of 560 serum samples were examined for the specific antibodies against *M. mycoides* subspecies mycoides, using a sandwich enzyme-linked immunosorbent assay. Associated risk factors that were evaluated include age, gender and breed. In this study, the overall seroprevalence was 8.75%. The seroprevalence of CBPP in Lahore, Kasur and Jhang were 8.26, 8.20 and 10.06%, respectively, but no significance difference (P<0.05) was observed. Among the assessed potential predisposing factors, gender and breed were not significantly associated with the serological status of the animal (P>0.05), in which the age affected significantly the positivity to CBPP (P<0.05). The highest seropositivity was observed in animals of more than 7 years of age (32.08%) followed by in 4-7 years of age (8.16%) and lowest in animals of less than 4 years old (0.79%). This study confirms the presence of CBPP in the state which could be a threat to livestock export market and warrants the implementation of control measures to mitigate the economic losses associated with the disease.

**Key words:** Contagious bovine pleuropneumonia, Enzyme-linked immunosorbent assay, *Mycoplasma*, Prevalence, Risk factor.

## INTRODUCTION

Contagious bovine pleuropneumonia (CBPP), an infectious disease of respiratory system of ruminants caused by *Mycoplasma mycoides* subsp. *mycoides* small colony biotype. Currently, CBPP is endemic in sub-Saharan African countries where most of the outbreaks of the disease have been reported from 20 different countries, with the highest number of cases reported in Ethiopia, Cameroon, Angola, and Nigeria (Wade *et al.*, 2015; Abdela and Yune, 2017; Ankeli *et al.*, 2017; Daniel *et al.*, 2017). According to the Office International des Epizooties, the CBPP- economic losses of more than $2 billion per annum has been reported, due to mortality, production losses and increased investments to control the disease (Onono *et al.*, 2017).

At herd level, the disease can spread from one animal to another through respiratory aerosols, saliva, urine, fetal membranes and uterine discharges (Mariner *et al.*, 2006; Alhaji and Babalobi, 2015). CBPP is manifested by anorexia, fever, dyspnea, polypnea, cough and nasal discharges. Most infections are limited to the respiratory tract, although arthritis occurs in calves usually less than 6 months of age (Tardy *et al.*, 2011; Nehra *et al.*, 2018).

Clinical diagnosis of CBPP is difficult, as early signs may be slight or non-existent and are indistinguishable from any other severe pneumonia with pleuritis. Necropsy findings include marbling, thickening of interlobular septa, sequestra formation and consolidation of lungs of CBPP- infected animals. Lesions are often unilateral with cranio-ventral pattern. Sometimes, straw colored fluid is also seen in thoracic cavity (Massimo, 2018). Confirmatory diagnosis of CBPP involves combination of tests including typical pathological lesions, isolation of the etiologic agent from pneumonic lungs and serological investigations (Giovannini *et al.*, 2000). Serological tests, including the complement fixation test (CFT) and the enzyme linked-immunosorbent test (ELISA), are being used at herd level for screening of CBPP positive animals, but none of the two tests can be considered as gold standard for confirmation, due to their nature as presumptive tests (Amanfu *et al.*, 2000; Muuka *et al.*, 2011). To our knowledge, there was no documented report regarding the prevalence of CBPP in Punjab,

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Pakistan. This seroprevalence data will provide a preliminary indication of the occurrence of CBPP in targeted animals reared within this geographical area.

## MATERIALS AND METHODS

### Geographical area of this study

This study was conducted from December 2016 to November 2018 in Lahore, Kasur and Jhang districts of Punjab. Lahore is located between 31°32' 59' N and 74°20' 37" E of Punjab, with altitude of 217 m above sea level. Absolute location of Kasur in Punjab province is 31° 7' 4.315" N and 74° 26' 27.018" E, with altitude of 218 m above sea level. Jhang of Punjab province is located between 31° 16' 41.046" N and 72° 19' 54.033" E, with altitude of 164 m above sea level.

### Sample collection and processing

Before slaughtering in abattoirs, a total of 560 blood samples were collected aseptically in clot activator vacutainer from individual animals showing respiratory signs. Vacutainers were labelled and transported to molecular-pathology lab, Department of Pathology, University of Veterinary and Animal Sciences, Lahore-Pakistan. Serum was separated from each sample as described by (Geetha *et al.*, 2015) and analyzed by qualitative bovine contagious pleuropneumonia sandwich enzyme-linked immunosorbent assay kit (MyBioSource, California, United States) to detect the antibodies specific to coated antigens of CBPP by using Bio rad PR 4100 Microplate Reader (Hercules, California, United States). The interpretation of positives versus negatives was as per (Muuka *et al.*, 2011).

### Associated risk factors

Data on age, breed and gender of sampled animals were also collected. Animals were categorized into less than 4 years, 4-7 years and more than 7 years of age. On the basis of breed the cattle were categorized into local breeds and cross breeds (Francis *et al.*, 2018).

### Statistical analysis

Pearson chi-square ($\chi^2$) test was used to analyze the differences in frequencies of measured prevalence. Significant differences in frequencies were reported at value of $P<0.05$.

## RESULTS AND DISCUSSION

The analysis of 560 samples of sera collected from cattle revealed a presence of 49 (8.75%) positives for CBPP. Non-significantly the highest prevalence was recorded in Jhang (10.25%), followed by Lahore (8.26%) and the lowest (8.20%) in Kasur district (Table 1). Results of this study were in agreement with serological survey conducted in commercial market of cattle in Nigeria by (Olabode *et al.*, 2013), revealing a prevalence of 10.65%, using the serological procedure of ELISA. In contrast to our findings, high seroprevalence (32%) was observed by (Aliyu *et al.*, 2003) who conducted abattoir survey for CBPP surveillance in Nigeria, this can be attributed to health status of animals, handling of samples, vaccination program and management of farms and herds.

In Lahore district, the highest CBPP prevalence was recorded in animals of the age group > 7 years (38.46%), followed by age group 4-7 years (7.64%) and the age group < 4 years (2.08%). These results showed significant relationship between the age of and occurrence of CBPP in the surveyed animals ($P<0.05$). A high prevalence of CBPP was found in female animals (9.92%) as compared to male animals (6.19%) ($P>0.05$). In addition, a higher seropositivity was found in cross breed animals (9.39%) compared to the local breed (2.70%) ($P>0.05$) as presented in Table 2.

In Kasur district, the highest CBPP prevalence was detected in the age group of > 7 years (23.81%), followed

**Table 1:** Prevalence of contagious bovine pleuropneumonia in selected districts of Punjab.

| District | No. of samples tested | Positive samples | Prevalence (%) | $\chi^2$ |
|---|---|---|---|---|
| Lahore | 218 | 18 | 8.26 | 0.49 |
| Kasur | 183 | 15 | 8.20 | P-value 0.78 |
| Jhang | 159 | 16 | 10.06 | |
| Overall | 560 | 49 | 8.75 | |

**Table 2:** Comparison of CBPP-serological prevalence in relation to risk factors at selected districts.

| Factors | Classification | % Seroprevalence (No. positive /No. examined ) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Lahore | Kasur | Jhang | Overall | $\chi^2$ | Odd-ratio | P-value |
| Age | <4 years | 2.08 (1/48) | 0.00 (0/37) | 0.00 (0/42) | 0.79 (1/127) | 46.37 | - | 0.00 |
| | 4-7 years | 7.64 (12/157) | 8.00 (10/125) | 9.18 (9/98) | 8.16 (31/380) | | | |
| | >7 years | 38.46 (5/13) | 23.81 (5/21) | 36.84 (7/19) | 32.08 (17/53) | | | |
| Gender | Male | 6.19 (6/97) | 4.60 (4/87) | 6.74 (6/89) | 5.86 (16/273) | 5.57 | 2.09 | 0.018 |
| | Female | 9.92 (12/121) | 11.46 (11/96) | 14.29 (10/70) | 11.50 (33/287) | | | |
| Breed | Local breed | 2.70 (1/37) | 6.25 (1/16) | 4.76 (1/21) | 4.05 (3/74) | 2.35 | 2.47 | 0.12 |
| | Cross breed | 9.39 (17/181) | 8.98 (15/167) | 10.14 (14/138) | 9.47 (46/486) | | | |

by the age group 4-7 years (8.00%) (P<0.05); A higher prevalence of CBPP was found in female animals (11.46%) compared to the male (4.60%) (P>0.05). Moreover, seropositive cases were more in cross breed animals (8.98%) compared to that in local breed (6.25%) (P>0.05) as shown in Table 2.

In Jhang district, a higher CBPP prevalence was recorded in animals of the age group > 7 years (36.84%), followed by animals of age group 4-7 years (9.18%) (P<0.05). A higher prevalence of CBPP was found in female (14.29%) compared to male animals (6.74%) (P>0.05). In addition, higher seropositive cases were observed in cross breed (10.14%) compared to local breed animals (4.76%) (P>0.05) as described in Table 2.

Among the three age categories, significantly (P<0.05) the highest seropositivity was found in age group of >7 years followed by the age group of 4-7 years, while the and lowest frequency was obtained in the age group of < 4 years (Table 2). The results were in agreement with those obtained by Kassaye and Molla (2012), who reported that CBPP is highly prevalent in animals of more than 7 years of age. In addition, Andrews and Windsor (2004) reported that CBPP is prevalent in older animals. This might be due to the chance by age for longer time of exposure to pathogens, low level of immunity at older age, and the persistence of sequestrum in older animals as compared to younger ones who may benefit from the maternal immunity to some extent (Séry *et al.*, 2015; Dar *et al.*, 2018). In contrast to our findings, Gizaw (2004) reported no significance difference (P>0.05) regarding the prevalence of CBPP among cattle, in relation to their age.

It is worth mentioning that a high seropositivity was observed in female (11.50%) rather than male animals (5.86%) and odd ratio was 2.09 (Table 2), which depicted that female animals were 2.09 times more prone to be infected with CBPP rather than male animals. Moreover, highest occurrence of CBPP was observed in cross breed (9.47%) as compared to the local breed (4.05%) of animals and 2.47 odd ratio was (Table 2) which revealed that cros-bred animals were 2.47 times more prone to be infected with CBPP rather than local breed of animals. Results of the current study also revealed that the prevalence of CBPP was higher in non-descriptive animals, results that were in agreement with previously published data of Séry *et al.* (2015). This might be explained by the fact that the male animals were sold at younger age for income purposes, while females were usually retained for breeding.

## CONCLUSION

In conclusion, although the preliminary data of this study suggest the prevalence of CBPP in targeted animals included in this research, however this work had its limitations. The main limitation in this study is the inability of the ELISA to detect the low antibody levels in animals at the early stages of CBPP. It is recommended to include more confirmative approaches in the future investigations, including clinical findings, National serological surveillance, post-mortem examination at abattoir, and culture of the etiologic agent of CBPP, followed by its molecular characterization.

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