



Seroprevalence of contagious caprine pleuropneumonia in small ruminants in Karnataka, India

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ABSTRACT

The objective of this study was to investigate the prevalence of *Contagious caprine pleuropneumonia* (CCPP) in small ruminants in Hassan district, Karnataka, India and to evaluate the accuracy of Slide Agglutination Test (SAT) over competitive ELISA (cELISA) diagnostic methods. A total of 227 serum samples from apparently healthy goat and sheep were screened for CCPP antibodies by SAT and cELISA. The study revealed no significant difference in seroprevalence of CCPP with respect to species (Goat vs Sheep), sex (Male vs Female) and farms (Organised vs Unorganized), where as significant difference was noticed in age groups by both SAT and cELISA diagnostic methods. The prevalence of CCPP by SAT and cELISA in sheep were 31.81% and 41.55% and goat 30.14% and 38.35% respectively. Higher seroprevalence rate was observed in older animals of more than five years compared to less than five years age groups. The sensitivity, specificity and accuracy of SAT as compared with cELISA in CCPP diagnosis was 78.41%, 98.56% and 97.75% respectively. The results of this study indicated the endemicity of the CCPP infection in and around Hassan region of Karnataka state and further showed that SAT being economical with good sensitivity and accuracy can be effectively used in the absence of cELISA kits.

Keywords: Competitive ELISA, Contagious caprine pleuropneumonia, Hassan, Mycoplasma, Serosurveillance, Small ruminants

Contagious caprine pleuropneumonia (CCPP) is a highly contagious infectious respiratory disease caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) with symptoms of fever, anorexia, coughing, nasal discharge, dyspnoea, polypnoea, exudative pleurisy and fibrinous pleuropneumonia (Radostitis *et al.* 2006). Although the significance and prevalence reports of CCPP is well documented in many parts of India, only one study has been reported from northern region of the Karnataka state (Awati and Chavhan 2013) and none from southern region of the state. The livestock in Hassan district (13.012°N, 76.068°E, Southern dry agro-climatic zone) in southern Karnataka have a high probability of picking up the infection due to interstate migration of livestock especially small ruminants. Keeping the above facts in mind and paucity of information about the prevalence of CCPP in sheep and goats, the present study was undertaken to determine the seroprevalence of CCPP in sheep and goats in Hassan region of Karnataka state and to compare the efficacy of the slide agglutination test (SAT) with competitive enzyme linked immune sorbent assay (cELISA) in diagnosing CCPP.

MATERIALS AND METHODS

Samples: In this study sheep and goat farms in Hassan region were screened for Mycoplasmosis (CCPP). The

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animals in organized farms were stall fed whereas in unorganized farms (Farmers herd) animals were left for free grazing in morning and were kept in close pen with supplementary feeding at night. A total of 227 serum samples were collected randomly from apparently healthy sheep and goat of different sex and age group (<1 year, 1 year to 5 year, >5 year) from different farms in and around Hassan during January to March 2019. All the samples were screened for Mccp antibodies by slide agglutination test using colored CCPP antigen and by monoclonal antibody based competitive ELISA kit.

Slide agglutination test: The coloured antigen of *Mycoplasma capricolum* subsp. *capripneumoniae* obtained from the Bacteriology and Mycology division of Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh was used for SAT. The slide agglutination test was performed as per the method described by Gupta *et al.* (2016).

Competitive enzyme linked immuno sorbent assay: ELISA kit (Monoclonal antibody 4.52 based Competitive ELISA by CIRAD/Institute Pourquier (Montpellier, France) was procured from Idexx (IDEXX Laboratories, Inc., Westbrook, ME). Competitive ELISA was carried out as per the manufacturers instructions and results were recorded.

Statistical analysis: Prevalence was calculated as proportion of number of animals positive for test to total

number of tested animals expressed in per cent. Chisquare/Fishers exact test was used to find the association of prevalence with factors such as species, sex, age group and farm. Confidence intervals (CI) were calculated using the formula proposed by Thrushfield (2005). The P value less than 0.05 at 95% confidence interval was considered for significance.

RESULTS AND DISCUSSION

Mycoplasma capricolum subsp. *capripneumoniae* (Mccp) causes severe and contagious disease in small ruminants, with significant economic impact (OIE 2017). Slide agglutination test using coloured antigen is simple, fast, easy to interpret the results and low cost with ease of application in field conditions (Gupta *et al.* 2016). This study revealed the CCPP prevalence rate of 31.27% (71/227) by SAT (Table 2). In India many seroprevalence studies have been carried out in small ruminants based on SAT with the prevalence rate ranging from 4.97% (Srivastava and Singh 2000) to 97% (Rana *et al.* 2009). Based on indirect ELISA, Singh *et al.* (2001), Singh *et al.* (1999) and Rana *et al.* (2009) in goats reported seroprevalence of 6.67%, 7.72% and 78% respectively. Specific detection of the CCPP can be done by using monoclonal antibody based competitive ELISA (cELISA) which overcomes the cross reactions with mycoplasma mycoides cluster. In the present study along with SAT, for the first time in India we used cELISA for detection of Mccp antibodies in small ruminants and found the prevalence rate of 40.52% (92/227) (Table 2). Sensitivity, specificity and accuracy of SAT considering cELISA as a standard reference test were shown in the Table 1.

Species wise prevalence: The species wise prevalence is presented in Table 2. No statistically significant difference in prevalence was observed between sheep and goats. The studies of Ramdev *et al.* (2008) and Suryawanshi *et al.* (2015) based on SAT showed the prevalence rate of 19.4%, 4.44%, 16.05% in sheep and 16.6%, 5.02%, 20.24% in goat, respectively which were lower compared with our results.

Sex wise prevalence: The prevalence in females was

Table 1. Sensitivity and specificity of slide agglutination test compared with cELISA

Particular	Computed values	95% Confidence interval
True positives (A)	69 (30.39%)	16.95% to 43.82%
False positives (B)	02 (0.88%)	0.0% to 3.6%
False negatives (C)	19 (8.37%)	0.27% to 16.46%
True negatives (D)	137 (60.35%)	46.05% to 74.64%
Sensitivity	78.41%	68.35% to 86.47%
Specificity	98.56%	94.90% to 99.83%
Positive likelihood ratio	54.49	13.71 to 216.67
Negative likelihood ratio	0.22	0.15 to 0.33
Positive predictive value	97.18%	89.67% to 99.28%
Negative predictive value	87.82%	82.88% to 91.48%
Accuracy	97.75%	86.21% to 94.18%

more than that of males by SAT and cELISA methods (Table 3). However, the difference was not statistically significant indicating that the disease affects equally in both sexes. Similar, no significant reports were documented by Lakew *et al.* (2014). Contrary to the above studies, Suryawanshi *et al.* (2015) recorded higher prevalence of CCPP in males (35.71%) than in females (17.14%).

Age wise prevalence: Age wise prevalence of CCPP in small ruminants both by SAT and cELISA are presented in Table 4. The prevalence was significantly higher among young (< 1 year) and aged (> 5 years) compared with adults (1–5 years). Kipronoh *et al.* (2015) observed that there was no significant difference in CCPP seropositivity of goats in the various age categories indicating absence of age factor in CCPP epidemiology. However, similar to our results Lakew *et al.* (2014) recorded higher prevalence in animals more than five years of age. Suryawanshi *et al.* (2015) and Gupta *et al.* (2016) showed the highest prevalence in younger animals less than five years of age. This higher rate of prevalence in old animals compared to young and adult could be due to reduction in immunity.

Farm wise prevalence: The prevalence of the CCPP by SAT and cELISA was not significantly different in organized and unorganized farms (Table.5) Dinesh *et al.*

Table 2. Species wise prevalence of CCPP

Species	Prevalence of CCPP by SAT			P value	Prevalence of CCPP by cELISA			P value
	No. positive	Prevalence	Confidence interval		No. Positive	Prevalence	Confidence interval	
Sheep	49/154	31.81	24.98–39.54	0.462	64/154	41.55	34.07–49.46	0.377
Goat	22/73	30.14	20.83–41.44		28/73	38.35	28.05–49.83	
Total	71/227	31.28	26.21–38.38		92/227	40.52	34.35–47.02	

Table 3. Sex wise prevalence of CCPP

Species	Prevalence of CCPP by SAT			P value	Prevalence of CCPP by cELISA			P value
	No. positive	Prevalence	Confidence interval		No. Positive	Prevalence	Confidence interval	
Male	8/33	24.24	12.83–41.02	0.2325	10/33	30.3	17.37–47.34	0.1346
Female	63/194	32.47	26.28–39.34		82/194	42.26	35.54–49.31	
Total	71/227	31.28	26.21–38.38		92/227	40.52	34.35–47.02	

Table 4. Age wise prevalence of CCPP

Species	Prevalence of CCPP by SAT			P value	Prevalence of CCPP by cELISA			P value
	No. positive	Prevalence	Confidence interval		No. Positive	Prevalence	Confidence interval	
<1 Year	19/52	36.54	24.80–50.13	0.045	25/52	48.07	35.11–61.32	0.0083
1 to 5 Years	29/119	24.37	17.54–32.81		37/119	31.09	23.47–39.38	
>5 Years	23/56	41.07	29.17–54.12		30/56	53.6	40.70–65.98	
Total	71/227	31.28	26.21–38.38		92/227	40.52	34.35–47.02	

Table 5. Farm wise prevalence of CCPP

Species	Prevalence of CCPP by SAT			P value	Prevalence of CCPP by cELISA			P value
	No. positive	Prevalence	Confidence interval		No. Positive	Prevalence	Confidence interval	
Organized	24/91	26.37	18.41–36.25	0.123	32/91	35.16	26.13–45.39	0.1132
Unorganized	47/136	34.56	27.09–42.88		60/136	44.11	36.05–52.51	
Total	71/227	31.28	26.21–38.38		92/227	40.52	34.35–47.02	

2013 showed higher occurrence of disease in unorganized farms (43.90%, 38%) in comparison with organized farms (15.97%, 20%) which is in line with our results. The probable reason for higher prevalence in unorganized farms could be due to the extensive method of rearing which increases the consequent chance for contacting infection from infected and/or carrier animals. In addition, stress factors due to exposure to cold and wet conditions, malnutrition and movement over long distances could predispose the animal to disease (Vihan 2010). Whereas in contrast to our results, Ravishankar *et al.* (2011) and Gupta *et al.* (2016) reported the occurrence of the disease is more in organized sector with prevalence of 62.5% and 13.54% respectively.

The present study was carried out to know the seroprevalance of Contagious caprine pleuropneumonia in and around Hassan region of Karnataka state and to compare the efficiency of slide agglutination test with cELISA. The monoclonal antibody based competitive ELISA, which detects of *Mycoplasma capricolum* subsp. *capripneumoniae* antibodies in the serum sample without any cross reactions, is a sensitive and specific serological technique for detection of Contagious caprine pleuropneumonia. However, slide agglutination test is having good sensitivity and accuracy and being economical, can be effectively used in the absence of competitive ELISA kits.

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