

## SERO-EPIDEMIOLOGICAL ANALYSIS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN GOATS

M. A. Chandio<sup>1,2</sup>, D. H. Kalhoro<sup>1\*</sup>, S. H. Abro<sup>1</sup>, M. S. Kalhoro<sup>1</sup>, A. Kaka<sup>1</sup>, G. M. Lochi<sup>1</sup>, A. A. Soomro<sup>3</sup>, M. A. Nizamani<sup>1</sup> and A. A. Chandio<sup>1</sup>

<sup>1</sup>Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-70060, Pakistan

<sup>2</sup>Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan

<sup>3</sup>Central Veterinary Research and Diagnostic Laboratory Tando Jam

\*Corresponding author's email: drdildarkalhoro@gmail.com

### ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is an important disease of small ruminants. An investigation on the prevalence of contagious caprine pleuropneumonia was carried out in randomly selected goats of Hyderabad and Tando Allahyar districts. The data regarding risk factors, such as; age, sex and breed associated to CCPP infections were obtained. A total of 200 blood samples were collected from CCPP suspected goats from studied area. The samples were screened using competitive Enzyme Linked Immuno-Sorbent Assay (C-ELISA) and Capri-Latex Agglutination Test (LAT). An overall prevalence of 24% and 20% was recorded among goats using Capri-LAT, while 20% and 17% of CCPP infections by C-ELISA in goat population of districts Hyderabad and Tando Allahyar, respectively. The non-significant difference ( $P>0.05$ ) was found for both districts. Whereas, significant difference ( $P\leq 0.05$ ) was observed for age and breed wise in both districts, breed wise prevalence was found higher for Tapri 47.05% and 47.05% at district Hyderabad while, Pateri shows higher prevalence of 37.93% and 34.48% at Tando Allahyar district on both diagnostic techniques. Major gross pathological lesions were observed on the mucosal surface with moderate haemorrhages and purulent exudates accumulated in lumen of the trachea. Multifocal and diffuse type lesions and accumulation of serous fluid in pleural cavity were prominent in lungs. Epithelial linings of bronchi and alveoli were disrupted, thickened interlobular septa with extensive infiltration of polymorphic nucleated cells. Histopathological lesions were prominent in trachea as compared to lungs.

**Keywords:** Contagious caprine pleuropneumonia, Enzyme Linked Immuno-Sorbent Assay, Capri-Latex Agglutination Test, Goat, Sindh, Pakistan.

### INTRODUCTION

The small ruminant faces serious constraints in form of inadequate nutrition, low genetic potential, intense harsh climatic conditions, poor management and various diseases. Contagious Caprine Pleuropneumonia (CCPP) is a devastating threat to the small ruminants with major economic importance (Awan *et al.*, 2009). CCPP is a lethal respiratory disease having serious impact in reduction of small ruminants' production, as the fastidious, intracellular bacteria infect domestic as well as wild breeds of small ruminants with high morbidity (100%) and mortality rates may reach up to (60-70%) (Ostrowski *et al.*, 2011). All age groups and both sexes were susceptible but high mortality was recorded in young kids as compared to adults (Arif *et al.*, 2007). The CCPP is caused by *Mycoplasma capricolum sub-specie capripneumoniae (Mccp)* (OIE 2012). It belongs to the genus *Mycoplasma* and has a very short incubation period of 3-5 days (Thiaucourt and Bölske 1996). The typical clinical signs of CCPP are hyperpyrexia (41-43 °C), after an onset of high fever, respiratory symptoms become more pronounced with

increased nasal discharge and lacrimation (OIE, 2009). Other clinical signs such as lameness, diarrhea, inability to move, unappropriated gait with stiff neck and prostration on ground with lateral recumbency can be observed in advance stages of the disease (OIE, 2008).

Analyzing the seroprevalence provides a scientific knowledge about the occurrence of disease in a particular area, to better understand about its risk factors such as age, sex, breed, specie and its seasonally outbreaks, as it also provides a baseline data for the progressive development of future strategies for diagnosis and control of certain highly contagious diseases. Highlighting the studies on seroprevalence of CCPP in different areas with variation in results such as, overall seroprevalence of 18.61% was observed in southern Ethiopia (Mekuria and Asmare 2009). Higher seroprevalence of 32.5% was recorded in Beetal goat breed in Pakistan (Hussain *et al.*, 2012) and 37.5% was documented in East Turkey (Cetinkaya *et al.*, 2009). On the contrary, Mekuria *et al.*, (2008) reported a lower seroprevalence of 15.5% in a participatory investigation of CCPP in the Benna-Tsemay and Hammer districts of southern Ethiopia.

Gross pathological lesions of CCPP are limited exclusively to lung and pleura. Affected lungs shows necrotic multi focal and sometime diffused lesions having a port wine color (Thiaucourt and Bölske 1996). Swelling of Bronchial and mediastinal lymph nodes with plural adhesions on the wall of chest cavity are common features of the disease. Hemorrhagic lesions and hypertrophy can also be visualized on liver and kidneys (Gelagay *et al.*, 2007; Mondal *et al.*, 2004). Hemorrhages can be easily seen in tracheal; with sloughing of the epithelial layers of the lung tissues may show acute serofibrinous condition to the chronic fibrino-necrotic pleuropneumonia (Hernandez *et al.*, 2006).

There are different techniques used for diagnostic purpose during CCPP outbreaks. The ELISA (Enzyme Linked Immunosorbent Assay) and the latex agglutination test (LAT) play an important role in identifying animals suffering from contagious caprine pleuropneumonia (OIE, 2009). Properties like low cost, easy to use and sensitivity increases its worth in detection of disease in outbreaks. The competitive ELISA and LAT are more sensitive than biochemical tests (Thiaucourt *et al.*, 1994).

In spite of aforementioned prevailing situation with an existence of the numerous problems due to the CCPP, there is lack of well documented scientific information on the occurrence of CCPP among goats in Sindh, especially in district Hyderabad and Tando Allahyar. Therefore, this study was designed with the aim to determine the seroprevalence of CCPP in Hyderabad and Tando Allahyar districts of Sindh province, and to assess the potential risk factors for the occurrence of disease.

## MATERIALS AND METHODS

**Study Area:** For the present study, a project was designed to record Sero-epidemiological status and pathogenesis of contagious caprine pleuropneumonia in District Hyderabad and Tando Allahyar. The study area was divided into Rural and Urban sectors of Hyderabad and Tando Allahyar, involving randomly selection of Government and private farms. To address the stated objectives, semi-structured questionnaires was managed to select individual farms in study areas to gather information of risk factors such as age (1, 2, 3 and 4 years), sex and Tapri, Kamori, Tedi and Pateri breeds for CCPP occurrence in the area.

**Collection of Samples:** For the determination of prevalence rate, a total of 200 blood samples were collected during October 2016-March 2017 from goats showing clinical signs of pneumonia, with the suspected CCPP infection.

**Collection of blood Samples:** About 3 ml of blood was aseptically collected from goats in a clot activator

vacutainer. Samples were transported in a cold chain to the Central Veterinary Diagnostic Laboratory Tandojam for the further investigation. Once the serum was separated by centrifugation, it was either immediately used or stored in deep freezer at -20°C till screened.

**Processing of serum samples:** Serum samples were thawed at the room temperature before analyzed by Latex Agglutination Test kit (Capri-LAT) and C-ELISA kit (Competitive Enzyme Linked Immunosorbent Assay) using three replicates for confirmation of disease as prescribed by (OIE, 2009).

**Latex Agglutination Test:** Capri-LAT prepared reagent was brought from the Animal and Plant Health Agency-Weybridge, United Kingdom, donated by Central Veterinary Diagnostic Laboratory Tandojam under the project of ‘Strengthening of Laboratories in identification and eradication of CCPP from Sindh’. The test procedure was followed as prescribed by manufacturer and adopted by (Shahzad *et al.*, 2012).

**Competitive Enzyme Linked Immunosorbent Assay:** The samples were also analysed for the detection of *Mycoplasma capricolum subsp. capripneumoniae (Mccp)* using monoclonal antibody based competitive C-ELISA Test Kit (IDEXX CCPP, 0656231-01). The 1st batch of C-ELISA Kit was developed at CCPP reference laboratory CIRAD-Montpellier France, purchased under the research project ‘‘Strengthening of Laboratories in identification and eradication of CCPP from Sindh, at Central Veterinary Diagnostic Laboratory Tandojam. The serum samples were processed according to CCPP C-ELISA kit.

**Sampling for isolation:** For the isolation of *Mycoplasma capricolum sub-specie capripneumoniae*, sampling from pleural fluids and lung tissues are required as prescribed by OIE (2008), for this purpose postmortem was performed on dead animals during disease outbreaks. Tissue samples were taken in sterile container containing 10 % formalin with proper labelling. Pleural swabs were aseptically taken and inserted in test tube containing modified hay flicked medium, after proper sampling each tube was labelled and transported under proper refrigeration (4°C) to CVDL Tandojam.

**Growth Medium for *Mycoplasma*:** Modified hay flick culture medium is well known to be used for cultivation of *Mycoplasma*. The standards were followed as mentioned by (OIE, 2008). Both agar and broth mediums were prepared as suggested by manufacturers. Samples were inoculated and streaked according to standard operating procedure (OIE, 2009).

**Tissue Sampling:** Tissue samples collected from the lungs and trachea were fixed in 10% formalin. The standard protocol of H&E technique was followed by tissue sectioning, and staining for detailed

histopathological examination as suggested by Wesonga *et al.*, (2004).

## RESULTS

During current sero-epidemiological study 200 serum samples were collected from different areas of both districts and examined by C-ELISA and Capri-LAT tests. To accomplish parameters of risk factor analysis, data regarding age, sex and breed were also collected. On postmortem examination of field isolated dead animals, gross lesions were recorded from lungs and trachea. Samples were also processed for bacterial isolation and histopathological examination.

**Prevalence of CCPP among goats in districts Hyderabad and Tando Allahyar using Capri-LAT and C-ELISA:** Table-1 shows the overall comparative results between two tests used in the current study. The data revealed that 44 samples (22%) were found positive by Capri-LAT test and 37 (18.50%) samples were found positive on C-ELISA. However, individually 24 (24%) samples were detected positive on Capri-LAT, and 20 (20%) through C-ELISA in Hyderabad district. Whereas, 20 (20%) on Capri-LAT, and 17 (17%) through C-ELISA was recorded in Tando Allahyar district. Results of both techniques were non-significant ( $P > 0.05$ ). However, the differences between the positive percentages of both districts on both techniques were also found non-significant ( $P > 0.05$ ).

**Table 1. The overall seroprevalence of CCPP among goats in districts Hyderabad and Tando Allahyar analysed by different techniques.**

Tests	Districts		Overall prevalence No. (%)	P-Value
	Hyderabad Prevalence No. (%)	Tando Allahyar Prevalence No. (%)		
Capri-LAT	24 (24.00)	20 (20.00)	44 (22.00)	0.495
C-ELISA	20 (20.00)	17 (17.00)	37 (18.50)	0.585
P-Value	0.495	0.585	0.313	-

### Prevalence of CCPP in relation to Sex by Capri-LAT:

Among 100 samples from each district, 11 and 89 samples were collected from male and female at district Hyderabad, while for district Tando Allahyar, 09 and 91 samples were collected from male and females respectively. The results are summarized in Table-2. A total of 2 (18.18 %) male and 22 (24.71%) females in Hyderabad district and 01 (11.11%) male and 19 (20.87%) females were found positive on Capri-LAT. However, statistically there was no gender wise difference ( $P > 0.05$ ) at both districts.

### Prevalence of CCPP in relation to Age by Capri-LAT:

During present study, Age was considered as an important risk factor that may have association with the prevalence of disease among goats in districts Hyderabad and Tando Allahyar. To achieve that goal, a total of 100 random samples for both districts were divided among 4 different age groups i.e., <1 year, 1-2 years, 2-3 years and >3 years. Samples were analyzed by Capri-LAT and are summarized in Table-2. The result shows that the prevalence in the age group of > 3 years was found higher 16 (44.44%) and 12 (41.37%) in districts Hyderabad and Tando Allahyar. Whereas, the lowest positive samples 03 (13.04%) were recorded in age group of 1-2 years at district Hyderabad, and none of animal found positive in <1-year age group in district Tando Allahyar respectively. Statistical analysis of different age

groups shows significant difference ( $P < 0.05$ ) for both districts.

### Prevalence of CCPP in relation to breed by Capri-LAT:

During present study breed is another risk factor that was considered and challenged by Capri-LAT. Results are summarized in Table 2. In Hyderabad district 08 (47.05%) Tapri, 09 (28.12%) Tedi, 05 (18.51) Pateri, and 02 (8.33%) Kamori breeds were recorded positive through Capri-LAT. While, in Tando Allahyar the highest 11 (37.93%) samples were recorded for Pateri breed with lowest of 03 (12.00%) for Kamori breed respectively. The data shows statistically significant difference ( $P < 0.05$ ) in Age and breed groups in districts Hyderabad and Tando Allahyar.

### Prevalence of CCPP in relation to sex by C-ELISA:

Sex wise prevalence was also recorded through C-ELISA during present study and summarized in Table-3. A total of 100 samples were analyzed in Hyderabad, and divided in two groups for male and female. Out of 11 males and 89 female samples, 01 (09.09%) male and 19 (21.34%) females were found positive for CCPP. Similarly, 09 males and 91 females samples were analyzed in district Tando Allahyar, the prevalence for male and females was 01 (11.11%) and 16 (17.58%) respectively. The statistical analysis did not reveal any significant difference ( $P > 0.05$ ) between male and females of both districts.

**Table 2. Prevalence of CCPP among goats in Districts Hyderabad and Tando Allahyar in relation to the various risk factors analyzed by Capri-LAT.**

Risk Factors		Districts					
		Hyderabad		P-Value	Tando Allahyar		
		Total Samples	Prevalence No. (%)		Total Samples	Prevalence No. (%)	P-Value
Sex	Male	11	02 (18.18)	0.632	09	01 (11.11)	0.485
	Female	89	22 (24.71)		91	19 (20.87)	
Age	< 1 Yr	11	01 (09.09)	0.019	11	00 (00.00)	0.005
	1-2 Yrs	23	03 (13.04)		27	02 (07.40)	
	2-3 Yrs	30	05 (16.66)		33	06 (18.18)	
	> 3 Yrs	36	15 (41.66)		29	12 (41.37)	
Breed	Tapri	17	08 (47.05)	0.030	13	02 (15.38)	0.041
	Kamori	24	02 (08.33)		25	03 (12.00)	
	Tedi	32	09 (28.12)		33	04 (12.12)	
	Pateri	27	05 (18.51)		29	11 (37.93)	

**Prevalence of CCPP in relation to Age by C-ELISA:**

100 samples from both districts were divided among 4 groups of age i.e., <1 year, 1-2 years, 2-3 years and >3 years and were challenged by C-ELISA (Table-3). The data revealed that among all groups the highest prevalence of 13 (36.11%) and 10 (32.25%) was recorded in age group of >3 years at district Hyderabad and Tando Allahyar. While, the lowest 01 (09.09%) and 00 (00%) prevalence was detected in young animals of age group <1 year at district Hyderabad and Tando Allahyar. The data was analyzed statistically and showed significant difference ( $P \leq 0.05$ ) between age groups in district Hyderabad as well as Tando Allahyar.

**Prevalence of CCPP in relation to Breed by C-ELISA:**

Random sampling from Tapri, Kamori, Tedi and Pateri breeds was done as most important breeds of goats. Results are summarized in Table-3. The highest seroprevalence rate was found in Tapri breed with positive number of 08 (47.05%) with lowest 01 (04.16%) from Kamori breed at Hyderabad district. Contradicted results were found in district Tando Allahyar with highest positive number of 10 (34.48%) from Pateri breed with lowest of 03 (09.09%) from Tedi breed. Statistically, data revealed that there is a

significant difference between goat breed in Hyderabad and Tando Allahyar districts ( $P \leq 0.05$ ).

**Isolation of *Mycoplasma*:** The isolation of *Mycoplasma* was carried out from pleural fluid and lung tissues of dead animals in modified hay flick medium. During present study a total of 4 suspected animals were found dead. *Mycoplasma* was successfully isolated from only 2 goats for very first time in district Hyderabad. Typically, nipple or fried egg-shaped colonies of *Mycoplasma capricolum sub-specie capripneumonie* were developed on 8<sup>th</sup> day of post inoculation in modified hay flick medium. The maximum numbers of colonies were recorded on 14<sup>th</sup> day of post inoculation using stereomicroscope.

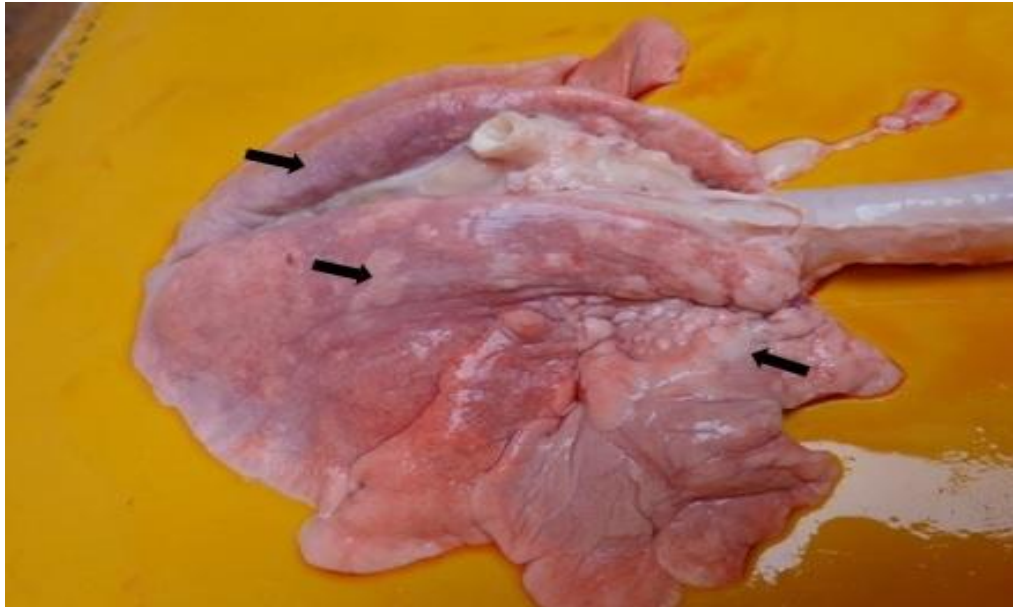
**Gross pathology:** The gross pathology lesions were observed to draw baseline for confirmed diagnosis of the disease and pathological changes in lungs and trachea.

**Lungs:** Unilateral and bilateral involvement of lungs with the pulmonary consolidation and marbled appearance of lungs was observed as primary characteristic lesions of CCPP. Multifocal lesions of necrosis and abscess were prominent on lungs surface. Hepatisation colours of lungs varying from deep blue to reddish in colour were also observed (Fig-1-3).

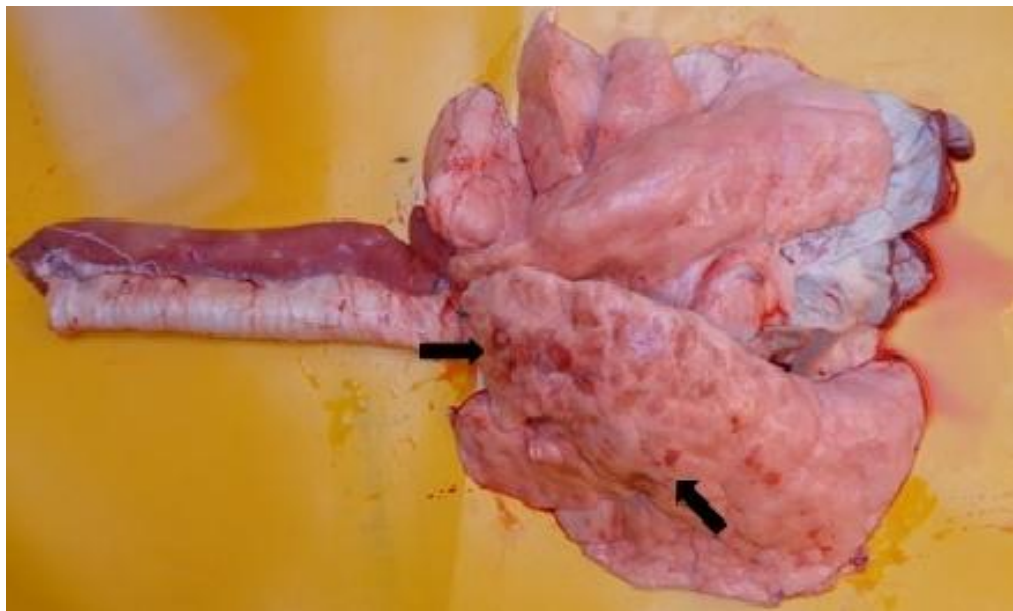
**Table 3. Prevalence of CCPP among goats in Districts Hyderabad and Tando Allahyar in relation to the various risk factors analyzed by C-ELISA:**

RISK FACTORS		DISTRICTS					
		Hyderabad		P-Value	Tando Allahyar		
		Total Samples	Prevalence No. (%)		Total Samples	Prevalence No. (%)	P-Value
Sex	Male	11	01 (09.09)	0.338	09	01 (11.11)	0.622
	Female	89	19 (21.34)		91	16 (17.58)	
Age	< 1 Yr	11	01 (09.09)	0.025	10	00 (00.00)	0.041
	1-2 Yrs	23	02 (08.69)		26	03 (11.53)	

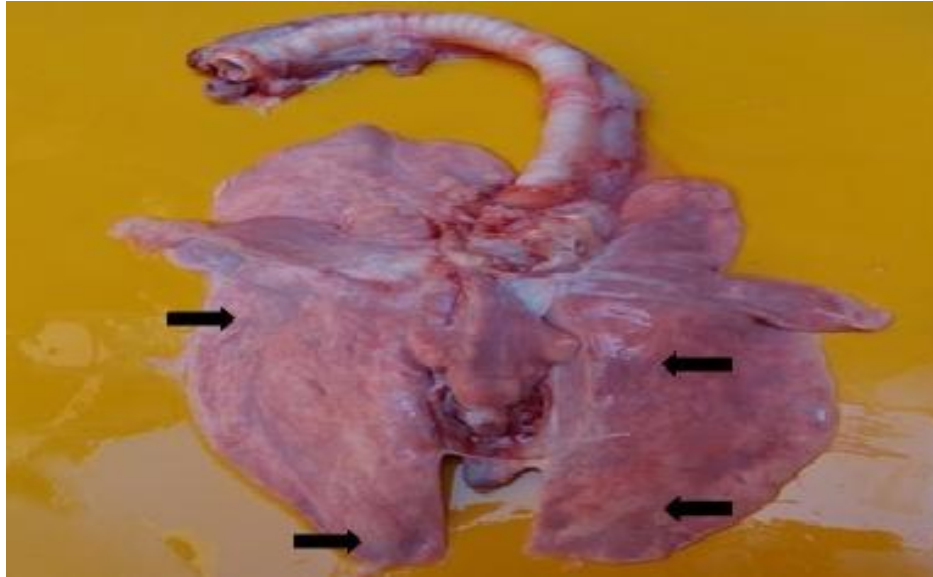
	2-3 Yrs	30	04 (13.33)		33	04 (12.12)	
	> 3 Yrs	36	13 (36.11)		31	10 (32.25)	
<b>Breed</b>	Tapri	17	08 (47.05)	0.004	13	01 (07.69)	0.029
	Kamori	24	01 (04.16)		25	03 (12.00)	
	Tedi	32	08 (25.00)		33	03 (09.09)	
	Pateri	27	03 (11.11)		29	10 (34.48)	



**Plate I. Lungs showing discoloration and necrotic lesions**



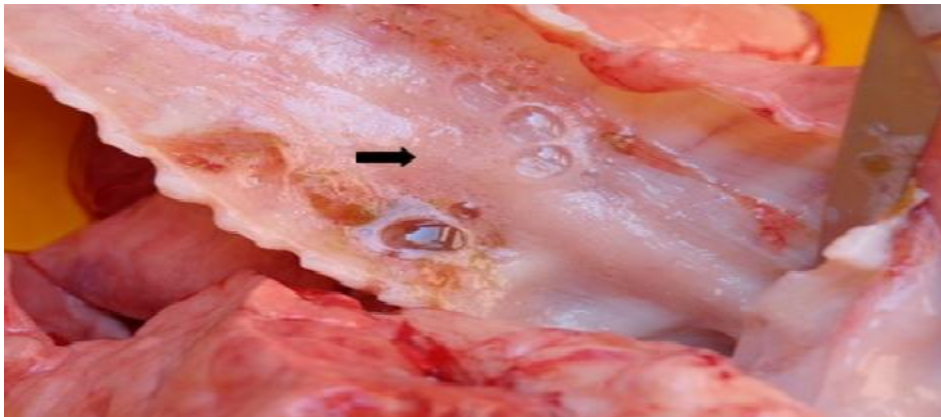
**Plate II. Unilateral, multifocal severe necrotic lesions of lungs**



**Plate III. Bilateral consolidation with multifocal necrotic and hepatised lesions.**

**Trachea:** The Numerous streaks of hemorrhages were found in lumen of trachea with various degrees. Most severe changes like hemorrhages were recorded in inner

lining of trachea. Purulent exudate as characteristic lesion of CCPP was also detected in trachea (Fig-4-5).



**Plate IV. Purulent exudate in lumen of trachea.**



**Plate V. Haemorrhages in inner lining of trachea.**

**Histopathological Examination:** Histopathological examination of trachea and lungs was carried out to check microscopic lesions.

**Lungs:** Lungs were seriously affected and showed prominent lesions of haemorrhage with leukocytic infiltration especially neutrophils. Thickening of alveolar membrane with atelectasis and emphysema were also detected in all field isolates (Fig-6-7).

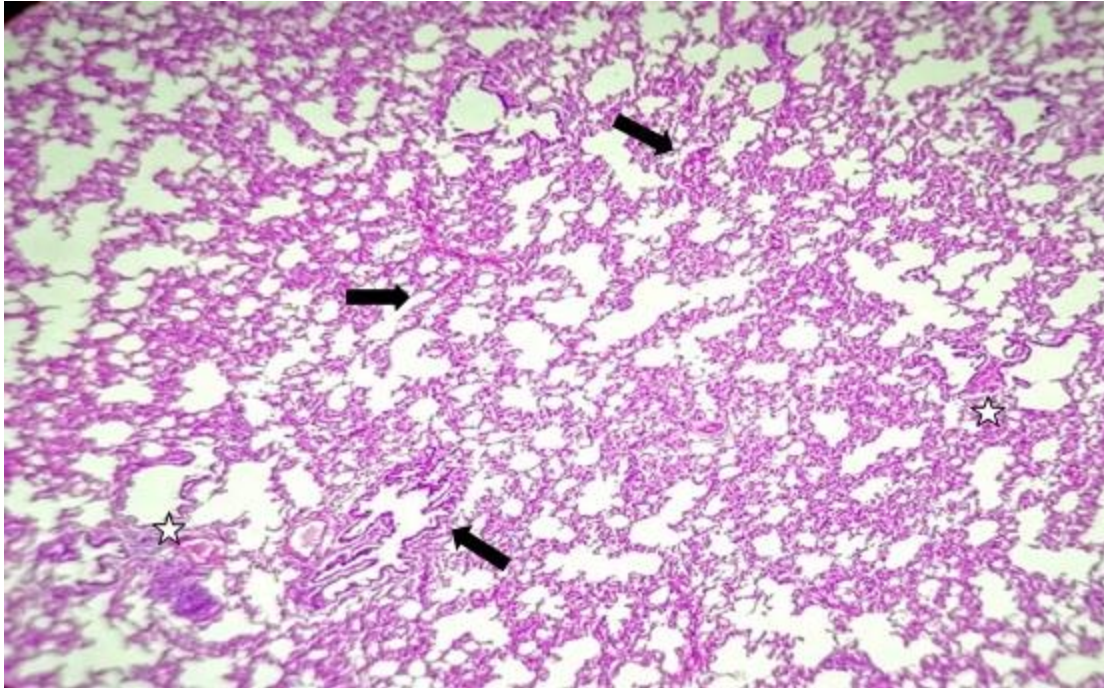


Plate VI. Lungs on 10x showing hemorrhage with star and arrow showing thickening of epithelial lining

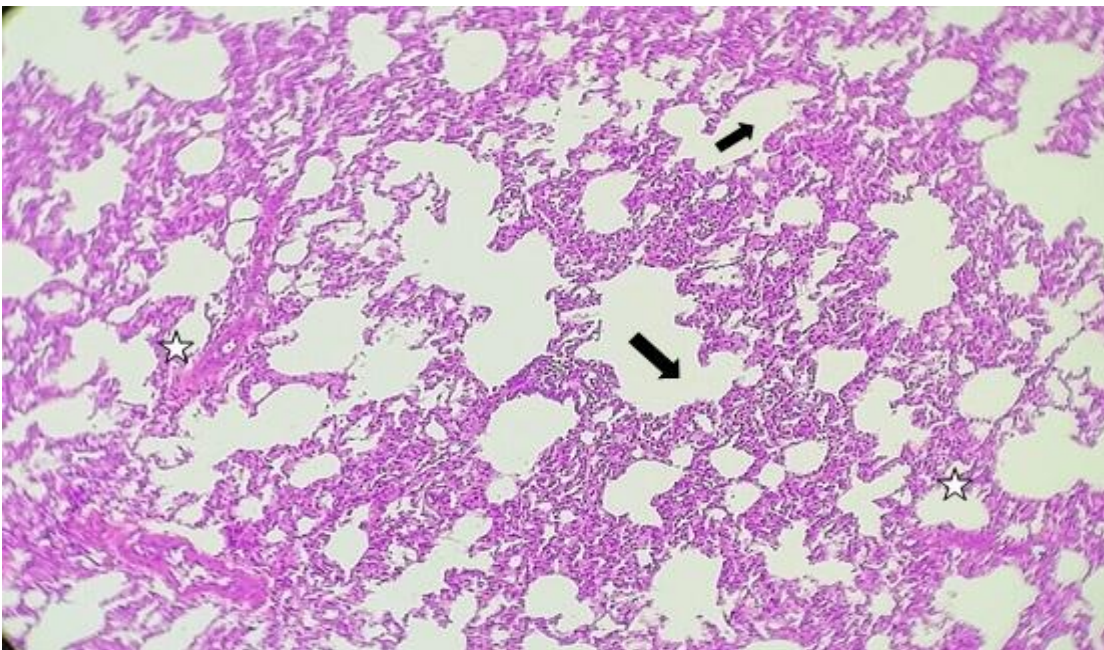


Plate VII Lungs on 10x showing emphysema with arrow and hemorrhage and leukocyte infiltration with star.

**Trachea:** Histopathological lesions were observed in epithelial lining of trachea. Ciliated epithelial layer shows

the sloughing and leukocyte infiltration accompanied by haemorrhagic lesions (Fig-8-9).

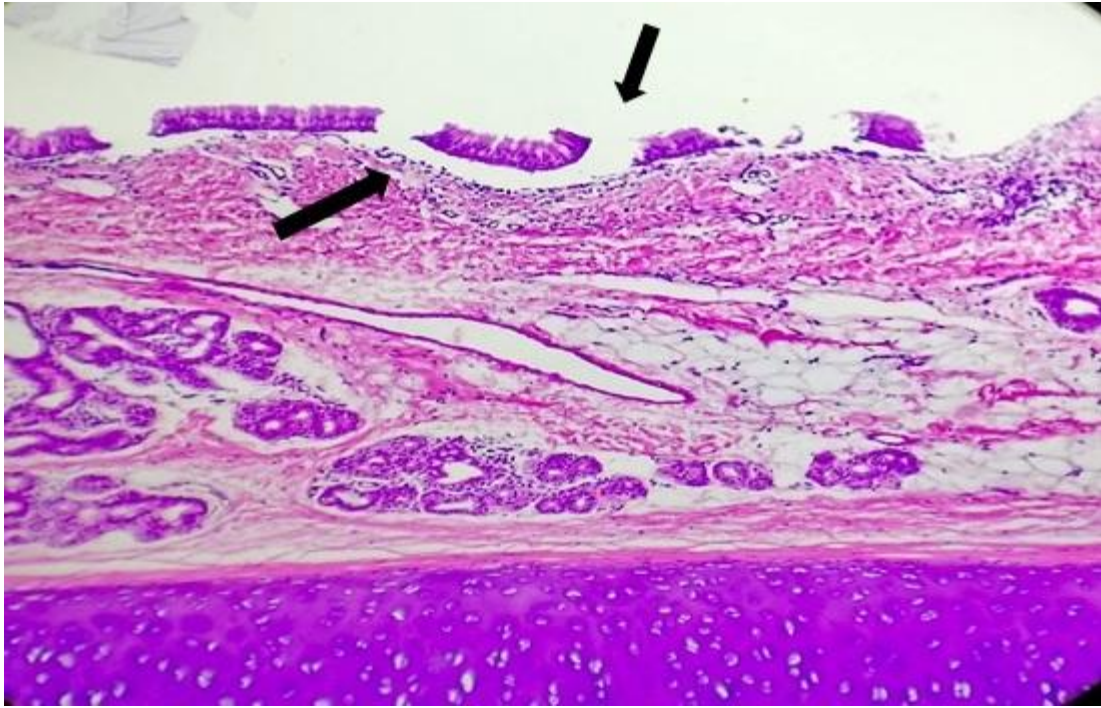


Plate VIII. Trachea on 10x showing sloughing of epithelium with short arrow, and neutrophil infiltration with hemorrhagic lesions with long arrow.

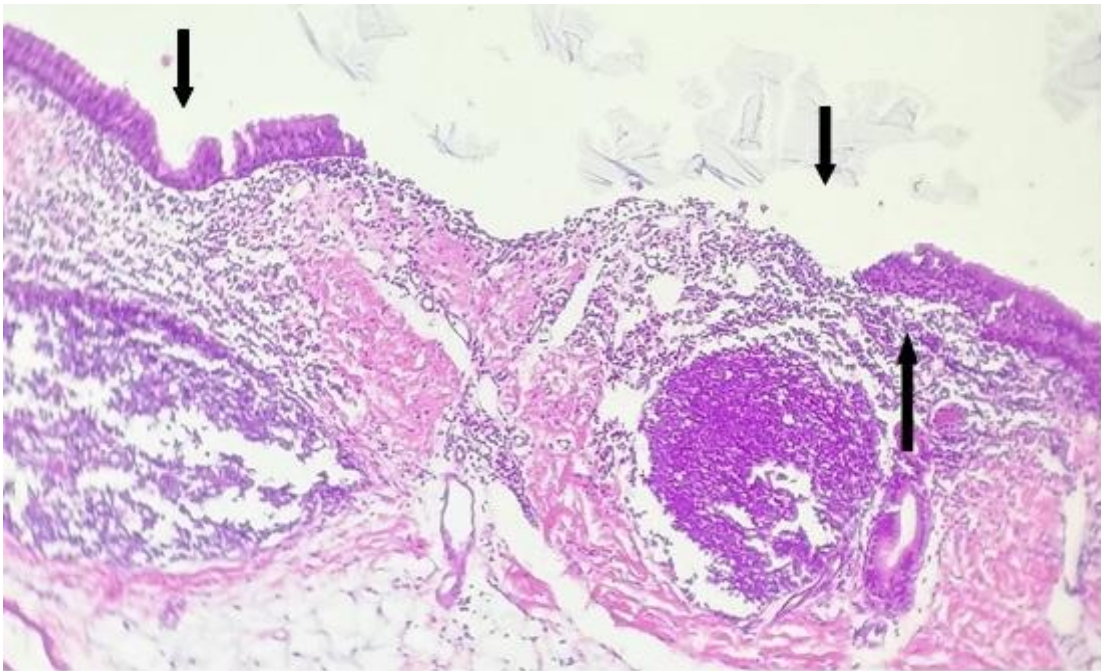


Plate IX. Trachea on 10x showing sloughing of epithelium with short arrow, and neutrophil infiltration with long arrow.

## DISCUSSION

Contagious Caprine Pleuropneumonia (CCPP) is one of the major constraints in the development of small ruminant's population and exerting severe negative

effects on farmer's economy. Due to its transboundary nature, CCPP is regarded as List "B" disease by Office of international des Epizooties (OIE, 2018). CCPP affects small ruminants globally directly and indirectly, as small ruminant's commodities larger portion of world



population as source of meat, milk, and skin the direct loss in production is exerted due to huge mortality and morbidity, reduced milk and meat yield, cost of treatment, surveillance and disease diagnosis, whereas, indirect losses are in terms of trade restrictions (FAO, 2005). Pakistan is sustaining 92.3 million heads of small ruminants which have worth of around 71 billion rupees. These animals are susceptible population to losses due to Contagious Caprine Pleuropneumonia.

In the present sero-epidemiological study a total of 200 samples were examined by C-ELISA and Capri-LAT to record the seroprevalence of CCPP in goats in Hyderabad and Tando Allahyar districts, Sindh. Out of 200 sera samples 44 (22%) were found positive on Capri-LAT and 37 (18.50%) on C-ELISA. The variation in results between tests can be due to sensitivity, as Capri-LAT is found more sensitive than C-ELISA. The findings of the present study are in line with the reports of (Eshete, 2006; Gelagay *et al.*, 2007; Mekuria and Asmare 2009) who reported 19.9% seroprevalence in Afar Pastoral area, 20.12% in Borona Pastoral Area, 22.49% in Afar region, and 18.61% in South Omo respectively. However, the overall seroprevalence of CCPP in present serological study was lower than the findings of (Eshete, 2006; Hadush *et al.*, 2009; Mohammed 2008; Sherif *et al.*, 2012) who reported 31% prevalence in an extoir abattoir, 32.63% in selected districts of Jiga, 32% in Eastern Ethiopia, 32.68% in Tigray and 29% in Wollo showing irrespective nature of disease to national borders.

Present study was designed to detect *Mycoplasma capricolum sub-specie capripneumonie* in goats. During the current study, the highest prevalence of CCPP (24%) and (20%) was observed in district Hyderabad on Capri-LAT and C-ELISA as compared to (20%) and (17%) at district Tando Allahyar. Representing similarity in results of Shahzad, *et al.*, (2016) who reported definite variation in prevalence percentage among tested districts of Punjab with highest of 33.33% and 14.89% in Faisalabad and Layyah district with lowest of 2%, 3.70%, 5.15% and 6.45% in Lahore, Pakpattan, Bahawalpur and Okara respectively. Whereas, Suryawanshi *et al.*, (2015) also detected higher seroprevalence of 20.24% in goats.

Sex wise seroprevalence in goats was also carried out during current study, 18.18% sero-positivity was observed among males and 24.71% in females challenged by Capri-LAT in district Hyderabad. Similarly, on C-ELISA seroprevalence of 09.09% and 21.34% was recorded for male and females in district Hyderabad. This data showed that sex was not associated in seropositivity of CCPP, this result is in accordance with (Bekele *et al.*, 2011; Eshete, 2006; Yousuf *et al.*, 2012) who reported that CCPP is highly contagious disease of small ruminants with irrespective of sex variation (OIE, 2018).

Older animals showed higher prevalence as compared to young animals. The data indicates that the highest seropositivity of 36.11% was recorded in age >3 years group in goats, while lower in <1year group 09.09% on Capri-LAT. Identical results were observed for seroprevalence of CCPP in goats in districts Hyderabad and Tando Allahyar on both techniques used in the study. Similarly, significant varying in age groups was also reported by (Bekele *et al.*, 2011; Eshete, 2006; Yousuf *et al.*, 2012). In these studies, it was reported that humoral immunity might be the reason for low seropositivity in young ones. Sherif *et al.*, (2012) also reported enormous difference between age groups. The seroprevalence in old age as compared to young age might be explained by the fact that as with the increase in age, small ruminants are repeatedly exposed to various stress conditions due to (adverse weather, movement on long distance and malnutrition) which can predispose animal to disease.

Seroprevalence of CCPP among different breeds of goats were also examined during current study, the data showed the difference among seropositivity in different breeds of goats on Capri-LAT as well as on C-ELISA. During current study seroprevalence of 47.05%, 08.33%, 28.12 and 18.51% on Capri-LAT was observed in Tapri, Kamori, Tedi and Pateri in district Hyderabad showing significant difference ( $P \leq 0.05$ ). Variation of breed wise seroprevalence was in line with findings of Shahzad *et al.*, (2016) who reported prevalence of 18.18% and 8.1% in Okara district with epidemiological variation of 42.85%, 11.76% and 8.69% in Beetal, Nachi and Angora breeds at district Layyah. The difference in seropositivity may be due to variation in number of samples and time of sampling during outbreaks.

On post-mortem examination dead animals, lesions were extensively observed in lungs and trachea. Gross pathological lesions showed unilateral and bilateral involvement of lungs showing focal and multifocal lesions of necrosis, with abscess and excessive pleural fluid. Trachea showed remarkable changes in terms of haemorrhages in lumen and excessive plural fluid. Histopathological examination indicates clear cellular changes in trachea and lungs such as sloughing of epithelial layer in trachea with leukocytic infiltration, while in lungs haemorrhagic lesions were duly observed with thickening of alveolar walls and disruption of cells in highly infected animals. Our results are in agreement with authors who reported congested trachea with the presence of frothy exudates and chronic tracheitis Mondal *et al.*, (2004) and pleural adhesions (Wesonga *et al.*, 2004). CCPP affected animals showed granular with copious straw-colored exudates; yellow pea-sized nodules that are surrounded by areas of congestion with varying degrees of lung consolidation and enlargement of bronchial lymph nodes are frequently found (OIE, 2009). Whereas, Rodríguez *et al.*, (2010) observed

fibrinopurulent and necrotic pleuropneumonia with acute pyogenic bronchopneumonia and fibrinous pericarditis in goats. Tracheal lesions were characterized with erosion of the epithelium lining with infiltration of polymorph nucleated cells. Hemorrhagic lesions were also observed in the muscular layer of trachea with hyperactive mucous-secreting cells (Mondal *et al.*, 2004), oedema mixed with fibrin fluid and inflammatory cells, such as lymphocytes and polymorphonuclear neutrophils in the alveolar spaces and interstitial septae (Adehan *et al.*, 2006).

**Conclusion:** Based on findings of present study it is concluded that higher prevalence of CCPP was observed in Hyderabad comparison to Tando Allahyar. CCPP cases were recorded higher in female as compared to male animals. The older animals with age group of >3 years have shown higher rate of CCPP infection as compared to young and adult animals. The prominent pathological changes (emphysema and sloughing) were observed in trachea and lungs of affected animals.

**Competing interests:** The authors declare that they have no competing interests.

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## REFERENCES

- Adehan, R. K., A. T. P. Ajuwape, A. I. Adetosoye and O. O. Alaka (2006). Characterization of *Mycoplasmas* isolated from pneumonic lungs of sheep and goats. *Small Rumin. Res.* 63:44–49.
- Arif, A., J. Schulz, F. Thiaucourt, A. Taha and S. Hammer (2007). Contagious Caprine Pleuropneumonia Outbreak In Captive Wild Ungulates At Al Wabra Wildlife Preservation, State Of Qatar. *J. Zoo Wildl. Med.* 38:93-96.
- Awan, M. A., F. Abbas, M. Yasinzai, R. A. J. Nicholas, S. Babar, R. D. Ayling and Z. Ahmed (2009). Prevalence of *Mycoplasma capricolum* subspecies *capricolum* and *Mycoplasma putrefaciens* in goats in Pishin district of Balochistan. *Pakistan Vet. J.* 29:179–185.
- Bekele, T., Y. Asfaw and B. Abebe (2011). Seroprevalence of contagious caprine pleuropneumonia in Borana and Guji lowlands, Southern Ethiopia. *Ethiop. Vet. J.* 15:69-76.
- Cetinkaya, B., R. Kalin, M. Karahan, E. Atil, L. Manso-Silvan and F. Thiaucourt. (2009). Detection of contagious caprine pleuropneumonia in East Turkey. *Rev. Sci. Tech.* 28:1037–1044.
- Eshete, G. (2006). Serological and Participatory Epidemiological Survey of Contagious Caprine Pleuropneumonia in Afar Pastoral areas of North East Ethiopia. In: Abstracts of MSc Theses (1998-2006). Faculty of Veterinary Medicine, Addis Ababa University, Debre-zeit, Ethiopia.
- FAO, (2005). Contagious caprine pleuropneumonia in Animal Health Disease Cards. Agriculture Department, Animal Production and Health Division. Available at: <http://www.fao.org/ag/againfo/subjects/en/health/diseases-cards/ccpp.html>.
- Gelagay, A., S. Teshale, W. Amsalu and G. Esayas (2007). Prevalence of contagious caprine pleuropneumonia in the Borana pastoral areas of Ethiopia. *Small Rumin. Res.* 70:131–135.
- Hadush, B., L. Eshetu, W. Mengistu and M. Hailesilassie (2009). Seroprevalence of contagious caprine pleuropneumonia in Kefta Humera, Alamata (Tigray) and Aba-’ala (Afar), Northern Ethiopia. *Trop. Anim. Health Prod.* 41:803–806.
- Hernandez, L., J. Lopez, M. St-Jacques, L. Ontiveros, J. Acosta and K. Handel (2006). *Mycoplasma mycoides subsp. capri* associated with goat respiratory disease and high flock mortality. *Can. Vet. J.* 47:366-369.
- Hussain, R., M. Auon, A. Khan, M. Z. Khan, F. Mahmood and S. Ur-Rehman (2012). Contagious caprine pleuropneumonia in Beetal goats. *Trop. Anim. Health Prod.* 44:477–481.
- Mekuria, S and K. Asmare (2009). Cross-sectional study on Contagious Caprine Pleuro Pneumonia in selected districts of sedentary and pastoral production systems in Southern Ethiopia. *Trop. Anim. Health Prod.* 42:65–72.
- Mekuria, S., A. Zerihun, B. Gebre-Egziabher and M. Tibbo (2008). Participatory investigation of Contagious Caprine Pleuropneumonia (CCPP) in goats in the Hammer and Benna-Tsemay districts of southern Ethiopia. *Trop. Anim. Health Prod.* 40:571–582.
- Mohammed, H (2008). Sero-epidemiological study contagious caprine pleuropneumonia in Eastern Ethiopia. DVM thesis, Faculty of Veterinary Medicine, Haramaya University, Ethiopia.
- Mondal, D., A. K. Pramanik and D. K. Basak (2004). Clinico-haematology and pathology of caprine mycoplasmal pneumonia in rain fed tropics of West Bengal. *Small Rumin. Res.* 51:285–295.
- OIE, (2008). Manual of Standards for Diagnostic Tests and Vaccines-Bovine brucellosis. Manual of standards for diagnostic tests and vaccines. Office of International Epizootics, Paris. 1000-1012.
- OIE, (2009). Contagious caprine pleuropneumonia. Available at: <http://www.oie.int/fileadmin/>

- [Home/eng/Animal\\_Health\\_in\\_the\\_World/docs/pdf/Contagious\\_Caprine\\_Pleuro\\_Final.Pdf](#).
- OIE, (2012). Contagious caprine pleuropneumonia. OIE Terrestrial Manual. Chapter 2.7.6. Office International Des Epizootics, Paris, France. 995-1007.
- OIE, (2018). OIE-Listed diseases 2018: OIE - World Organisation for Animal Health. Available at: <http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2018/>.
- Ostrowski, S., F. Thiaucourt, M. Amirbekov, A. Mahmashoev, L. Manso-Silvan, V. Dupuy, D. Vahobov, O. Ziyoev and S. Michel (2011). Fatal outbreak of *Mycoplasma capricolum pneumonia* in endangered markhors. *Emerg. Infect. Dis.* 17:2338–2341.
- Rodriguez, S., B. Romero, J. Bezos, L. de Juan, J. lvarez, E. Castellanos, N. Moya, F. Lozano, S. Gonzalez, A. Mateos, L. Dominguez and A. Aranaz (2010). High spoligotype diversity within a *Mycobacterium bovis* population: Clues to understanding the demography of the pathogen in Europe. *Vet. Microbiol.* 141:89–95.
- Shahzad, W., T. Yaqoob, N. Mukhtar, R. Munir, R. Ahmad, M.S. Khan and A. Hussain (2016). Sero-prevalence of *Mycoplasma capricolum subsp. capripneumoniae* in goats through cELISA in different districts of punjab, Pakistan. *The J. Anim. Plant Sci.* 26:931–937.
- Shahzad, W., R. Munir, M. S. Khan, M. U. D. Ahmad, M. A. Khan, M. Ijaz, M. Shakil, M. Iqbal and R. Ahmad (2012). Characterization, molecular diagnosis and prevalence of caprine mycoplasmosis in different areas of Pakistan. *Pakistan J. Zool.* 44:559–568.
- Sherif, M., M. Addis and M. Tefera (2012). Contagious caprine pleuropneumonia: Serological survey in selected districts of Jijiga zone, Ethiopia. *Asian J. Anim. Sci.* 6:309–315.
- Suryawanshi, S.N., P. A. Tembhrne, S. Gohain, J.A. Kesharkar, U.M. Tumlam, A and V. C. Ingle (2015). Seroprevalence Of Contagious Caprine Pleuropneumonia in small ruminants in Maharashtra. *Indian J. Vet. Sci. Biotechnol.* 10:73-74.
- Thiaucourt, F.; G. Bolske, G. Libeau, C. Le Goff and C, L. (1994). The use of monoclonal antibodies in the diagnosis of contagious caprine pleuropneumonia (CCPP). *Vet. Microbiol.* 41:191–203.
- Thiaucourt, F and G. Bolske. (1996). Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. *Rev. Sci. Tech.* 15:1397–1414.
- Wesonga, H. O., Goran Bolske, T. Francois, C. Wanjohi and R. Lindberg. (2004). Experimental contagious caprine pleuropneumonia: A Long term Study on the Course of Infection and Pathology in a Flock of Goats Infected with *Mycoplasma capricolum subsp. capripneumoniae*. *Acta Vet. Scand.* 3:167–179.
- Yousuf, Eskindir, Bogale, A and Melaku and Basaznew (2012). Seroprevalence of contagious caprine pleuropneumonia in Dire Dawa provisional administrative council, Eastern Ethiopia. *J. Vet. Med. Anim. Heal.* 4:93–96.