



# Pathomorphology of Concurrent *Peste des Petits Ruminants* (PPR) and Contagious Caprine Pleuropneumonia (CCPP) in a Cross Breed Goat Flock

P. Shanmugavadivu, P. Srinivasan, A. Arulmozhi, S. Sivaraman,  
G.A. Balasubramaniam, M. Sasikala, P. Nithya

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## ABSTRACT

**Background:** Respiratory diseases are very common in goats and cause severe economic loss to the rural poor farmers. Proper identification of the actual pathogen(s) responsible for the respiratory infections is critical for timely and proper management of those diseases. The present communication describes the spontaneous occurrence of concurrent Peste des petits ruminants (PPR) and contagious caprine pleuro-pneumonia (CCPP) in a goat flock.

**Methods:** An outbreak of respiratory disease in a goat flock consisting of 45 Tellicherry cross bred animals in varying age groups was investigated during the month of February 2019 in Namakkal district of Tamil Nadu. Information of disease outbreak and flock details were collected. Samples from live (nasal, ocular and rectal swabs) and dead (lung, spleen and mesenteric lymph nodes) were collected for detection of *Peste des petits ruminants virus* (PPRV) and *Mycoplasma capricolum* subsp. *capripneumoniae* (MCCP) antigen by polymerase chain reaction. Tissues from heart, lung, liver, kidney, intestine, spleen and lymph node were collected in 10% neutral buffered formalin for histopathological examination.

**Result:** Out of 45 goats at risk, 23 showed clinical signs and 17 died of disease conditions with morbidity, mortality and case fatality rate of 51.11%, 37.77% and 73.91% respectively. PPR was confirmed by RT-PCR with M gene specific primers (191bp) and CCPP was confirmed by PCR with 16S rRNA gene fragment (316 bp). Affected animals showed clinical signs of pyrexia, anorexia, marked depression, mild erosions in the oral mucosa, nasal and ocular discharge, coughing and diarrhoea. Necropsy examination revealed congested oral mucosa, extensive pleuritis with large fibrin clots on lung surface and greyish pink consolidation of cranial lobes and anterior parts of diaphragmatic lobes. On section, consolidated areas were granular in appearance. Histopathology of lung revealed thickening of pleura, interlobular septa and alveolar wall by fibrin deposition and infiltration of inflammatory cells. Pulmonary congestion, infiltration of neutrophils and mononuclear cells, syncytial cell formation with eosinophilic intracytoplasmic and intranuclear inclusions were also noticed. Visceral organs showed degenerative and necrotic changes.

**Key words:** CCPP, Goats, Polymerase chain reaction, PPR, Pathology.

## INTRODUCTION

Goats are important livestock contributing significantly to the economic status of the low income groups and hence it is regarded as poor man's cow. In spite of their contribution to rural economy and food security, the productivity of goats is very much constrained by various factors such as high rate of infectious diseases occurrence, poor nutrition and marketing systems. Among the diseases, peste des petits ruminants (PPR) and contagious caprine pleuro-pneumonia (CCPP) are the most serious ones (Bolske *et al.*, 1996; Singh, 2011). PPR is caused by the PPR virus, a member of the morbillivirus genus of the family *Paramyxoviridae*. The disease is enzootic in India and outbreaks occur regularly among small ruminants, incurring significant economic losses in terms of morbidity, mortality, loss of productivity and trade restriction (Singh *et al.*, 2004).

Contagious caprine pleuropneumonia (CCPP) is one of the contagious respiratory diseases of high economic importance in goats in resource limited households of developing countries like India. It is caused by *Mycoplasma*

Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu, India.

**Corresponding Author:** P. Srinivasan, Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu, India. Email: srinipat2004@yahoo.com

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*capricolum* subsp. *capripneumoniae* (*M. capripneumoniae*), a member of the *Mycoplasma mycoides* "cluster". Economic losses associated with the disease include morbidity and mortality rates which might reach 100% in some flocks, decline or loss of production performance and amount incurred towards the prevention, control and treatment

(Parray *et al.*, 2019). A primary PPR viral infection causes remarkable immune-suppression that leads to complication by the secondary or opportunist organisms with high morbidity and mortality. In recent years, disease syndromes of multi factorial aetiology have been increasingly recognized with similar clinical signs and postmortem lesions. Hence identification of clinical and pathological changes in concurrent infection of PPR and CCPP will be helpful in diagnosis and adoption of appropriate therapeutic interventions and control strategies. The present study describes the clinicopathological findings, histopathology and detection of the genome of causative agent in tissues by polymerase chain reaction in goats affected with PPR and CCPP during natural outbreaks.

## MATERIALS AND METHODS

### History of disease outbreak

An outbreak of respiratory disease in a goat flock consisting of 45 Tellicherry cross bred animals in varying age groups was investigated during the month of February 2019 in Namakkal district of Tamil Nadu. The flock was maintained in an intensive system of management. The problem was noticed after introducing recently purchased ten kids of four months old. The outbreak was unresponsive to usual antibiotic therapy and was fast spreading within the flock. The clinical signs were pyrexia (Temperature 105°F), bilateral serous to mucopurulent oculo nasal discharge and diarrhoea. Six animals were died prior to the investigation and four animals showed the clinical signs. Necropsy on two goats were carried out during the investigation.

### Samples collection

Nasal (n=4), ocular (n=4) and rectal (n=4) swabs were collected from clinical cases using sterile swabs moisten in 500 µl of phosphate buffered saline (PBS) (0.1 M, pH 7.4). During necropsy, tissue samples from lung (n=2), spleen (n=2) and mesenteric lymph nodes (n=2) were collected in phosphate buffered saline (PBS) for the detection of PPRV and MCCP antigen. Samples were transported on ice and stored at -20°C until use. Heart blood and lung swab were collected and streaked on brain and heart infusion agar (Himedia Laboratories, Mumbai) for bacterial isolation. Tissues from heart, lung, liver, kidney, intestine, spleen and lymph node were collected in 10% neutral buffered formalin for histopathological examination.

### Polymerase chain reaction assay for pprv

RNA was extracted from 10 percent tissue suspensions using TRIZOL (Invitrogen, UK) and cDNA was synthesized using first strand cDNA synthesis kit (Invitrogen, UK) as per manufacturer's instructions. The primers specific for PPRV-M gene (Forward primer - 5' CTTGATACTCCCCAGAGATTTC 3' and reverse primer - 5' TTCTCCCATGAGCCGACTATGT 3') were designed based on the published sequence (Balamurugan *et al.*, 2006) which amplified 191 bp. PCR reaction was carried out with a reaction mixture (20 µl) containing 10 µl of amplicon master mix, 1 µl (20pmol/ µl)

each of forward and reverse primers, 2 µl of cDNA and 6µl of nuclease free water. Thermal cycling was performed in Gradient Thermocycler with the following cycling parameter *i.e.* initial denaturation at 94°C for five minutes, denaturation at 94°C for one minute, annealing at 55°C for one minute, extension at 72°C for one minute for 30 cycles and final extension at 70°C for five minutes.

### Polymerase chain reaction assay for mccc

Samples for polymerase chain reaction (PCR) were prepared as described by Woubit *et al.* (2004). About one gram samples from each lung tissue was taken and chopped with scissors and then grinded by mortar and pestle; mixed with 9 mL phosphate buffer solutions (PBS) and transferred to test tubes and subjected for DNA extraction. Mccc-specific primers Mccc-spe-F (5' - ATCAT TTTTAATCCCTTCAAG-3') and Mccc-spe-R (5' - TACT ATGAGTAATTATAATATATGCAA-3') were employed (Woubit *et al.*, 2004). PCR reaction was carried out with a reaction mixture (20 µl) containing 10 µl of amplicon master mix, 1 µl (20 pmol/ µl) each of forward and reverse primers, 2 µl of DNA and 6µl of nuclease free water. The Mccc-specific PCR was performed as follows: an initial denaturation step at 94°C for 2 min; amplification with 35 cycles of denaturation at 94°C for 30 s, annealing at 47°C for 15 s and extension at 72°C for 15 s and a final extension step at 72°C for 5 min. The amplified products were detected by staining with ethidium bromide (0.5 µg/ml) after electrophoresis at 80 volts for 2 h in 1.5% agarose gel. PCR products with a molecular size of 316 bp were considered indicative of Mccc.

### Histopathology

During histopathological processing, thin pieces of formalin fixed tissue samples were trimmed to a thickness of about 3 mm and kept in running tap water for overnight washing, then dehydration was done by subjecting the tissues through ascending grades of alcohol followed by clearing with acetone and then embedding in paraffin wax blocks and the tissues were then cut into 4-5 µm thick paraffin sections by microtome, stained with routine haematoxylin and eosin technique and finally mounted with DPX for histopathological examinations. (Suvama *et al.*, 2019).

## RESULTS AND DISCUSSION

In the recent past, outbreaks of respiratory disease with high mortality were noticed among goats under field condition and they are diagnosed as either PPR or Pasteurellosis. However, respiratory disease in goats are may also be caused by parainfluenza, Ovine progressive pneumonia, contagious caprine pleuropneumonia (CCPP), goat pox, contagious ecthyma, verminous pneumonia and many others (Emikpe *et al.*, 2010; Kul *et al.*, 2015). Diagnosis of such diseases based on clinical manifestations alone is very difficult as their signs are very similar at certain stage. Thus, laboratory confirmation is highly in dispensable for appropriate interventions to be made for the different diseases of goats especially PPR.

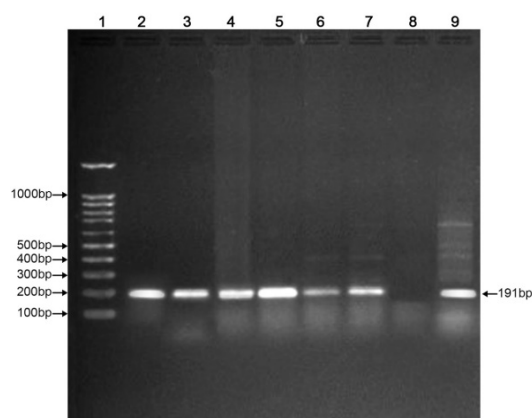
Out of 45 goats at risk, 23 showed clinical signs and 17 died of diseases with morbidity, mortality and case fatality rate of 51.11%, 37.77% and 73.91% respectively. In the present outbreak, high mortality was noticed as compared to previous records of 17.4% (Manimaran *et al.*, 2017) and 13.4% (Kumar *et al.*, 2014) during a natural outbreak of PPR in goats. Higher morbidity and mortality in the present study might be due to concurrent infection of CCPP and intensive system of management which facilitated the close contact and rapid spread of infection between the animals. Inclement dry cold weather during February coupled with poor nutrition might have enhanced the severity of the disease (Balamurugan *et al.*, 2012). Case fatality rate was highest in goats of 4-6 months age group as compared to adults. Maternally derived immunity to PPR normally persists up to 3-4 months of age (Singh *et al.*, 2004). At the end due to disappearance of immunity young animals are more susceptible to infection, which explain the higher mortality in 4-6 months age group. In addition, kidding twice a year results in a highly susceptible population (4-6 months of

age) during winter and monsoon seasons. Moreover, the Tellicherry cross breed animals are highly susceptible to PPR and CCPP than other local breeds (Sounderarajan *et al.*, 2006). Interestingly, the outbreak was noticed after the introducing the newly purchased animals from a local sandy into the existing flock. PPRV is transmitted through direct contact between infected and susceptible animals. Hence purchase of potentially infected animals and their subsequent introduction into naïve flocks might transmit the virus to susceptible goats (Singh *et al.*, 2004).

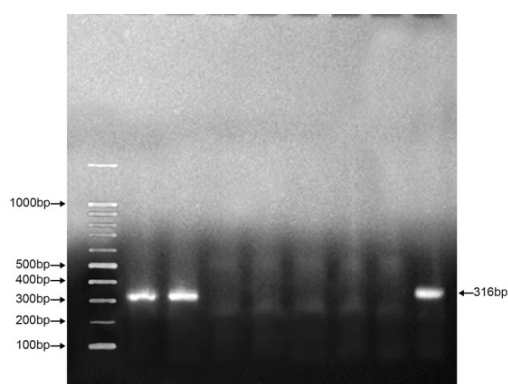
Lungs and liver impression smears did not show any pathogenic bacteria. Cultural examination of heart blood and lungs did not yield any bacterial growth on aerobic incubation ruling out the possibility of other bacterial infections. Pooled nasal, ocular and rectal swab as well as the tissue samples of lung and spleen were positive for PPRV by RT-PCR with M gene specific primers. Amplification of 191 bp (Fig 1) product of M gene confirmed the identity of PPRV. A distinct single band was obtained by specific amplification of 316 bp amplicon characteristic of 16S rRNA gene fragment of *Mycoplasma capricolum* sub sp. *capripneumoniae* (Fig 2) in lung tissue whereas nasal swabs collected from live animals were negative. This is in agreement with the findings of Bolske *et al.* (1996) who also reported failure to detect MCCP in dried nasal swabs from a herd with CCPP due to low prevalence of the organism in the nasal cavity particularly during later stages of the disease and presence of PCR inhibitory substances. The prevalence of mycoplasma infection in goats in various states of India has been acknowledged earlier (Ingle *et al.*, 2008; Shaheen *et al.*, 2001; Abraham *et al.*, 2015). The presence of mycoplasmosis in goats of Tamil Nadu has also been reported earlier (Manimaran *et al.*, 2020), but confirmed reports on concurrent occurrence of CCPP and PPR could not be obtained on perusal of available literature.

Clinical signs observed in the affected goats were anorexia, depression, high fever (105°F), congested mucus membrane, mucoid nasal and ocular discharge (Fig 3), difficulty in breathing, violent coughing, reluctance to move, soiling of perianal region with faeces due to diarrhoea and emaciation. In addition, mild erosion on oral mucosa (Fig 4) as well as on the lips and dental pad were also observed. The clinical signs observed in the present outbreak were suggestive of PPR (Ahmed *et al.*, 2005; Kwiatek *et al.*, 2007). However similar clinical signs are also exhibited by other respiratory diseases of goats (Kgotlele *et al.*, 2019). Under field conditions, difficulty in differentiating clinical signs manifested by different respiratory diseases in goats has been mentioned as a major limiting factor in diagnosis especially for PPR and CCPP (Mbyuzi *et al.* 2015).

Externally, perianal region was smeared with fecal materials. Necropsy of two goats revealed mild erosion of oral mucosa, hard palate and tongue (Fig 4). Trachea contained profuse frothy exudate and the mucosa congested. Extensive pleuritis was observed with accumulation of clear straw coloured fluid with fibrin



**Fig 1:** PPR: Amplification of 'M' gene (191 bp) of PPRV. Lane: 1: DNA ladder (100 bp), 2 and 3: Positive sample of dead animal, 4 to 7: Positive samples of live animals 8: Negative control, 9: Positive Control



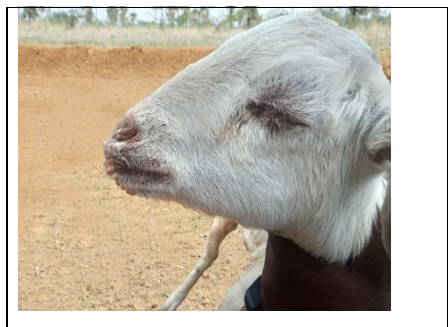
**Fig 2:** CCPP: PCR amplification of 16S rRNA gene (316 bp) of MCCP. Lane: 1: DNA ladder (100 bp), 2 and 3: Positive sample of dead animal, 4 to 7: Samples of live animals 8: Negative control 9: Positive Control.



flocculations in the thoracic cavity. Large fibrin clots were found adhering to the lungs. Lungs showed greyish pink consolidation of cranial lobes and anterior parts of diaphragmatic lobes with presence of frothy exudates and severe adhesions to thoracic wall (Fig 5). On cut section consolidated areas were granular in appearance (Fig 6). Bronchial and mediastinal lymph nodes are oedematous and congested. Intestinal mucosa are severely congested and there was no worms. Mesenteric lymph nodes are oedematous. Liver showed moderate enlargement with bile stasis. Kidney was moderately congested. These findings

are similar to those reported in previous study of PPR with concurrent infections of CCPP (Abraham *et al.*, 2015, Kgotlele *et al.*, 2019, Teshome *et al.*, 2019).

Histopathological examination of lungs of PPRV and CCPP positive animals showed congestion of pulmonary capillaries. Alveolar lumen contained serofibrinous exudation, infiltration of neutrophils, mononuclear and alveolar syncytial cells (Fig 7). Intracytoplasmic and intranuclear inclusion bodies were noticed in the syncytia (Fig 8). Alveolar walls and interlobular septa were thickened by the presence of edema and proliferation of type II



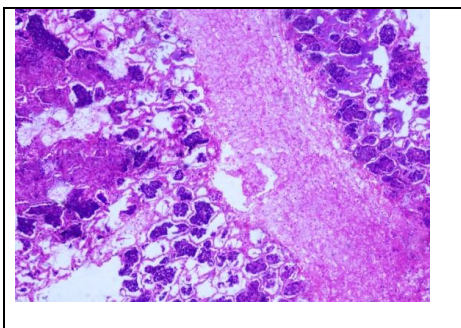
**Fig 3:** PPR + CCPP: Mucoïd nasal and ocular discharge.



**Fig 6:** PPR + CCPP: Lungs: Cut section of the consolidated area showing granular appearance.



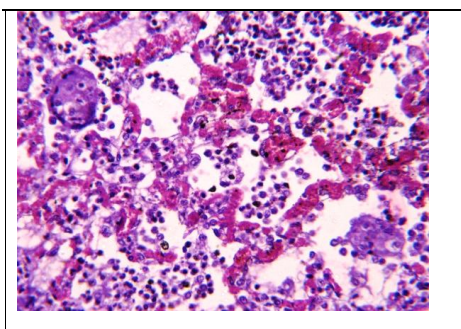
**Fig 4:** PPR + CCPP: Mild erosion of oral mucosa.



**Fig 7:** PPR + CCPP: Lung: Alveolar lumen contained serofibrinous exudation, infiltration of neutrophils and mononuclear cell and thickening of interlobular septa. H & E x 400.



**Fig 5:** PPR + CCPP: Lungs: Serofibrinous pleuritis, diffuse consolidation of cranial lobes of right lung with fibrin clots adherent to the rib cages.



**Fig 8:** PPR + CCPP: Lung: Alveolar wall was thickened and lumen contained syncytial cells with eosinophilic inclusions. H & E x 400.

pneumocytes. Massive infiltrations of lymphocyte and macrophages were noticed around in bronchi, bronchioles and blood vessels. Spleen and lymph node showed variable degrees of lymphoid cell depletion. Degenerative and necrotic changes were noticed in liver and kidney (Abraham *et al.*, 2015; Manimaran *et al.*, 2017).

## CONCLUSION

The present study indicates outbreaks with respiratory signs under field condition in goats often occur as complicated one. This results in difficulties in diagnosis, persistency of the outbreaks due to misdiagnosis, partial diagnosis and improper control strategies. Adopting suitable molecular diagnostic procedure like PCR based detection technique in conjunction with pathology for confirmation of etiological agents in complicated cases could play a better role in the epidemiological investigation and devising control strategies including developing of simple multiple disease diagnostic kits and use of multivalent vaccines.

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## REFERENCES

- Abraham, S.S., Asha, T.T., Julie, B., Prathiush, P.R., Nandakumar, S. and Prasad, P.M. (2015). Pathological and molecular characterization of contagious caprine pleuropneumonia (CCPP) outbreak in Kerala. *Indian Journal of Veterinary Pathology*. 39: 121-124.
- Ahmed, K., Jamal, S.M., Ali, Q. and Hussain M. (2005). An outbreak of peste des petits ruminants virus (PPRV) in goat flock in Okara, Pakistan. *Pakistan Veterinary Journal*. 25: 146-148.
- Balamurugan, V., Saravanan, P., Sen, A., Rajak, K.K., Venkatesan, G., Krishnamoorthy, P., Bhanuprakash, V. and Singh, R.K. (2012). Prevalence of Peste des Petits ruminants among sheep and goats in India. *Journal of Veterinary Science*. 13: 279-285.
- Balamurugan, V., Sen, A., Saravanan, P., Singh, R.P., Singh, R.K., Rasool, T.J. and Bandyopadhyay, S. K. (2006). One-step multiplex RT-PCR assay for the detection of *Peste des petits ruminants* virus in clinical samples. *Veterinary Research Communication*. 30: 655-666.
- Bolske, G.B., Mattsson, J.G., Bascunana, C.R., Bergstrom, K., Wesonga, H. and Johansson, K.E. (1996). Diagnosis of contagious caprine pleuropneumonia by detection and identification of *Mycoplasma capricolum subsp. capripneumoniae* by PCR and restriction enzyme analysis. *Journal of Clinical Microbiology* 34: 785-791.
- Emikpe, B.O., Sabri, M.Y., Akpayie, S.O. and Zamri-Saad, M. (2010). Experimental infection of peste des Petits Ruminant virus and Mannheimia haemolytica A2 in goats: immunolocalisation of Mannheimia haemolytica antigens. *Veterinary Research Communication*. 34: 569-578
- Ingle, V.C., Sivakumar, P., Kalorey, D.R., Pote, D.E., Dhamanna, P.P.S., Chavhan, S.K., Nagdive, A.A. and Hatkar, D.N. (2008). Seroprevalence of contagious caprine pleuropneumonia in goats in Nagpur district of Vidarbha region. *Veterinary World*. 1: 270-271.
- Kgottele, T., Chota, A.C., Chubwa, C.C., Nyasebwa, O., Lyimo, B., Torsson E, Karimuribo, E., Kasanga C. J., Wensman J. J., Misinzo, G., Shirima, G. and Kusiluka, L. (2019). Detection of Peste des petits ruminants and concurrent secondary diseases in sheep and goats in Ngorongoro district, Tanzania. *Comparative Clinical Pathology*. 28: 755-759.
- Kul.O., Atmaca, H.T. and Munir, M. (2015). Pathology of *Peste des petits ruminants* virus infection in small ruminants and concurrent infections in Munir M(ed) *peste des petits ruminants* virus. Springer-Verlag, Berlin Heidelberg. pp119-131.
- Kumar, N., Maharchandani, S., Kashyap, S.K., Singh, S.V., Sharma, S., Chaubey, K.K. and Ly, H. (2014). *Peste des petits ruminants* virus infection of small ruminants: a comprehensive review. *Viruses*. 6: 2287-2327.
- Kwiatk, O., Minet, C., Grillet, C., Hurard, C., Carlsson, E., Karimov, B., Abbina, E., Diallo, A. and Libeau, G. (2007). *Peste des petits ruminants* (PPR) outbreaks in Tajikistan. *Journal of Comparative Pathology*. 13: 111-119.
- Manimaran, K., Balakrishnan, S., Sangeetha, A., Dhanalakshmi, M., Karthik, K., Selvaraj, J. and Sivakumar. T. (2020). Molecular diagnosis of caprine mycoplasmosis in Cauvery delta region of Tamil Nadu. *Journal of Entomology and Zoology Studies*. 8: 949-953
- Manimaran, K., Selvaraj, J., Jaisree, S., Aravindh Babu, R.P., Hemalatha, S., Raja A. and Roy, P. (2017). An outbreak of peste des petitis ruminants in sheep and goats at Salem district of Tamil Nadu, India. *Indian Journal of Animal Research*. 51: 332-335.
- Mbyuzi, A.O., Komba, E.V.J., Cordery-cotter, R., Magwishwa, H.B., Kimera, S.I. and Kamra, D.M. (2015). Descriptive survey of peste des petits ruminants and CCPP outbreaks in traditional goat flocks in Southern Tanzania: producers' concerns, knowledge and attitudes. *Livestock Research for Rural Development*. 27: 65-68.
- Parray, O.R., Yatoo, M.I., Muheet Bhat, R.A., Malik, H.U., Bashir, S.T. and Magray, S.N. (2019). Seroepidemiology and risk factor analysis of contagious caprine pleuropneumonia in Himalayan Pashmina Goats. *Small Ruminants Research*. 171: 23-36.
- Shaheen, M., Haque, S. and Thakur, D.K. (2001). Contagious caprine pleuropneumonia in kids in Ranchi. *Indian Journal of Animal Science*. 71: 136- 138.
- Singh, R.P. (2011). Control strategies for *Peste des petits ruminants* in smallruminants of India. *Revue Scientifique et Technique* (International Office of Epizootics). 30: 879-887.
- Singh, R.P., Sreenivasa, B.P., Dhar, P., Shah, L.C., and Bandyopadhyay, S.K. (2004). Development of a monoclonal antibody based competitive- ELISA for detection and titration of antibodies to *Peste des petits ruminants* (PPR) virus. *Veterinary Microbiology*. 98: 3-15.
- Singh, R.P., Saravanan, P., Sreenivasa, B.P., Singh, R.K. and Bandyopadhyay, S.K. (2004). Prevalence and distribution of *Peste des petits ruminants virus* infection in small ruminants in India. *Revue Scientifique et Technique*. 23: 807-819.

- Soundararajan, C., Sivakumar, T., Ramesh, S., Muthukrishnan, S. and Palanidurai, R. (2006). *Peste des petits ruminants* among sheep and goats in an organized farm in Tamil Nadu. *Indian Veterinary Journal*. 83: 1045-1047.
- Suvarna, S.K., Layton, C. and Bancroft, J.D. (2019). *Bancroft's Theory and Practice of Histological Techniques*. 8<sup>th</sup> Edn, Elsevier, United states.
- Teshome, D., Sori, T., Sacchini, F. and Wieland, B. (2019). Epidemiological investigations of contagious caprine pleuropneumonia in selected districts of Borana zone, Southern Oromia, Ethiopia. *Tropical Animal Health Production*. 51: 703-711.
- Woubit, A., Lorenzon, S., Petraud, A. and Silvan, L.M. (2004). A specific PCR for the identification of *Mycoplasma capripneumonia*, the causative agent of contagious caprine pleuropneumonia. *Veterinary Microbiology*. 104: 125-132.