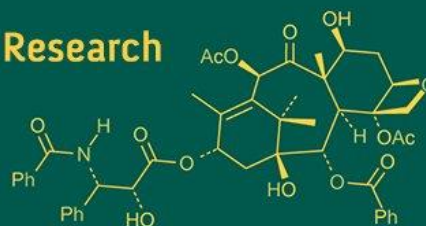
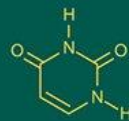
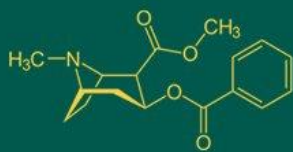


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## Prevalence and Pathology of suspected cases of SRLV infections in Farmed goats

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### Abstract

The present study examined pathoepidemiological aspect of Small Ruminant Lentivirus (SRLV) infection in 3,500 goats across farms near Mumbai from July to December 2024. Out of 3500 cases, 110 cases were suspected and clinical SRLV symptoms were observed in 3.14%, mainly as chronic respiratory distress (2.45%). Other conditions included combinations of mastitis and arthritis. Overall mortality rate was 1.17%, with lung lesions representing the most frequent finding (0.88%). Female goats had a higher prevalence (3.23%) than males (2.70%). Histopathological examination revealed various types of pneumonia, including fibrinous (26.82%) and interstitial (14.63%), along with other lesions such as congestion and oedema. Although PCR testing was negative for small ruminant lentivirus (SRLV), *Mycoplasma* spp. and ovine pulmonary adenocarcinoma (OPA) were detected in 9.92% and 1.41% of cases, respectively, suggesting that other infections can mimic SRLV-associated pathology.

**Keywords:** Small ruminant lentivirus, goats, prevalence, histopathology, PCR

### 1. Introduction

Lentiviruses, belonging to the Retroviridae family, are enveloped, single-stranded RNA viruses with a global distribution (Peterhans *et al.*, 2004) [20]. They infect a variety of hosts, including goats, horses, sheep, apes, and cats (Gufler *et al.*, 2007) [9]. Among them, Small Ruminant Lentiviruses (SRLVs) comprise a group of retroviruses that cause chronic, multisystemic, and progressively debilitating lifelong infections in sheep and goats. The major diseases associated with SRLVs include Caprine Arthritis Encephalitis Virus (CAEV), Maedi-Visna Virus (MVV), and Ovine Progressive Pneumonia Virus (OPPV). Clinically, Maedi is characterized by progressive interstitial pneumonia, while Visna presents as meningoencephalitis. CAE primarily causes polysynovitis/arthritis in adult goats and progressive paresis or leucoencephalomyelitis in kids.

Pathological and epidemiological studies can be conducted to establish correlations with lentiviral infections in goats. These infections are known to cause immunodeficiency and chronic inflammatory changes, and are unique in their ability to infect both dividing and non-dividing cells (Blacklaws, 2012; Zinc *et al.*, 1987) [1, 29]. SRLVs infect circulating monocytes and replicate extensively in tissue macrophages, leading to chronic inflammation in organs such as the lungs, mammary glands, central nervous system, and joints. Consequently, histopathological evaluation plays a critical role in the diagnosis of SRLV-associated diseases.

In India, the overall seropositivity of SRLVs ranges from 9.53% to 19.58% (Dar, 2017; Mishra, 2018; Kumar, 2019) [3, 16, 11], while PCR positivity is reported between 6.84% and 10.41% (Dar, 2017; Mishra, 2018; Kumar, 2019) [3, 17, 11]. Also, seropositivity toward CAEV found with prevalence rates of 4.5% (Didugu *et al.*, 2016) [4], 3.33% (Waseem *et al.*, 2015) [28], and 6.96% (Singh *et al.*, 2018) [24]. Currently, there are no known treatments or commercially available vaccines for CAEV. Therefore, controlling the spread and reducing the prevalence of SRLVs remains one of the most effective strategies for disease management. PCR is highly specific for the detection of CAEV proviral DNA in infected animals, with no cross-reactivity observed with other ruminant retroviruses (Clavijo and Thorsen, 1996) [2]. Many underreporting cases due to their subclinical nature calls for the

pathoepidemiological study of SRLV in goats. Thus, present study focuses on the determination of SRLV proviral DNA using PCR technique.

## 2. Materials and Methods

The study was conducted at the Department of Veterinary Pathology, Mumbai Veterinary College, from July 2024 to February 2025. Prevalence (Sex-wise and month-wise) and clinical surveillance for Small Ruminant Lentivirus (SRLV) infection was carried out across 20 goat farms in and around Mumbai, where 3,500 goats were screened. From these, 110 goats over six months old showing suspected SRLV symptoms were selected. 110 Blood samples (6 ml each) were collected in both plain and EDTA vials for hematological and biochemical analysis. Hematological parameters were assessed using a Nihon Kohden Celtac analyzer, while serum biochemistry (total protein, albumin, and alkaline phosphatase) was analyzed using an Erba Chem-5X analyzer.

Additionally, 41 tissue samples (lung, joint, and udder) from diseased goats were preserved in 10% neutral buffered formalin and processed for histopathology using Haematoxylin and Eosin (H&E) staining. Synovial fluid from 11 arthritic goats was aspirated and evaluated for volume, color, and turbidity. Smears were stained with Leishman and Giemsa stains for cytological analysis. For molecular diagnosis, 141 samples (110 blood and 31 lung tissues) were screened for CAEV, MVV, JSRV, and Mycoplasma using nested PCR with specific primers. Details of these primers used in the study are mentioned in Table 1. DNA was extracted using the QIAGEN kit, and PCR products were visualized via 2% agarose gel electrophoresis under UV illumination. Cytological analysis of synovial fluid was performed for differential cell counts.

## 3. Results and Discussion

### 3.1 Prevalence of SRLV infection in goats Overall Prevalence

The overall prevalence of suspected Small Ruminant Lentivirus (SRLV) infection in goats across Mumbai was 3.14% (110/3500). Among these, chronic respiratory distress was the most commonly observed clinical sign, present in 78.18% (86/110) of affected goats, followed by mastitis (4.55%; 5/110), arthritis (2.73%; 3/110), and mixed-type infections (14.55%; 16/110) involving more than one clinical manifestation. The respiratory signs were further categorized into chronic cough, progressive weight loss, nasal discharge, and dyspnoea. Details of clinical signs showed by goats suspected of SRLV infection are summarized in Table 2.

During the study period, 41 out of 3500 goats (1.17%) died. Postmortem examination revealed lung lesions as the most frequent pathological finding, observed in 31 goats (0.88%). Combined lesions involving the lung and udder were found in 3 goats (0.08%), while 2 goats (0.05%) showed concurrent lung, joint, and udder lesions. Additionally, 1 goat (0.02%) exhibited joint lesions alone, and 4 goats (0.11%) had udder lesions without other organ involvement.

### 3.2 Hematobiochemical Examination

Hematobiochemical findings revealed no alterations in the blood samples of suspected cases when compared with normal healthy goats. Mild rise in neutrophils and low lymphocyte count was observed in certain cases. The

variation in WBC pattern suggested changes in immunity due to infections. Difference in leukocytes average and patterns, indicates growth-related physiological changes, and may also reflect some changes in immunity as a consequence of viral infection (Lipecka *et al.*, 2010)<sup>[14]</sup>. In the present study, results of biochemical examination are aligned with the study conducted by Garba *et al.*, (2019)<sup>[7]</sup>, who observed a significant increase in the levels of ALT, total protein, and globulin among the mastitis-positive goats.

### 3.3 Gross Examination

On gross examination, 35 (85.36%) out of 41 lung samples were found abnormal based upon visual examination, palpation, size, location, texture, consistency, and colour. Whereas, 7/41 (17.07%) dead goats showed mastitis. Lung lesions were further classified as bronchopneumonia, interstitial pneumonia, cyst, nodules, consolidation, OPA and miscellaneous (emphysema, atelectasis, congestion, haemorrhages) lesions. Affected joints appeared swollen due to an increased amount of synovial fluid or pus accumulation due to bacterial infection (septic arthritis). Highest prevalence of lung lesions was observed in miscellaneous lesions (17.07%) while least prevalent lesion was cyst, noticed only in two lungs (4.8% of the affected cases). Similar kind of classification and study observed by Lee *et al.*, (2017)<sup>[12]</sup>, as miscellaneous lesions showed the highest frequency while cyst lesions had the lowest prevalence.

Gross examination of lung tissues revealed abnormalities in 35 out of 41 samples (85.36%), based on visual inspection, palpation, and assessment of size, location, texture, consistency, and color. Lung lesions were categorized into bronchopneumonia, interstitial pneumonia, cysts, nodules, consolidation, ovine pulmonary adenocarcinoma (OPA), and miscellaneous lesions (including emphysema, atelectasis, congestion, and haemorrhages). Among the pulmonary lesions, miscellaneous lesions were the most prevalent, observed in 17.07% of cases, whereas cystic lesions were the least common, identified in only two lungs (4.8%). These findings are consistent with observations made by Lee *et al.*, (2017)<sup>[12]</sup>, who reported a similar classification pattern, with miscellaneous lesions being the most frequent and cysts the least prevalent.

Affected joints were found to be swollen due to excessive synovial fluid or purulent material, indicative of septic arthritis likely caused by bacterial infection. Additionally, 7 of the 41 diseased goats (17.07%) exhibited signs of mastitis.

### 3.4 Histopathological Evaluation

Histopathological evaluation of 35 abnormal lung tissues revealed bronchopneumonia as the most common lesion (17/35 cases), followed by miscellaneous lesions (10/35), interstitial pneumonia (6/35), and ovine pulmonary adenocarcinoma (OPA; 2/35). These findings are consistent with Javed (2017) and Singh *et al.*, (2017), who also recorded bronchopneumonia as the predominant pathology. Histopathological lesions in lung tissues of goats suspected for SRLV infection are detailed in Table 3.

Fibrinous bronchopneumonia was characterized by inflammatory exudates, mainly neutrophils and macrophages within bronchioles and adjacent alveolar spaces. Suppurative bronchopneumonia showed similar exudation along with pulmonary oedema, indicative of acute

inflammation. These features align with reports by Singh *et al.*, (2017) <sup>[23]</sup>, Mahile *et al.*, (2022) <sup>[15]</sup>, and Kokate (2022) <sup>[10]</sup>.

Interstitial pneumonia exhibited thickened inter-alveolar and interlobular septa (Fig.2), congestion, oedema, neutrophilic infiltration, and capillary engorgement which are consistent with the findings of El-Mashad *et al.*, (2020) <sup>[5]</sup> and Valecha (2020). <sup>[27]</sup> Additional findings included mononuclear cell infiltration (Fig.1), fibroblast proliferation with collagen deposition, hyperplasia of type II pneumocytes, bronchus-associated lymphoid tissue (BALT), and smooth muscle proliferation. Similar observations were reported by Georgsson *et al.*, (1971), Dousti *et al.*, (2020), Mahile *et al.*, (2022) <sup>[15]</sup>, and Kokate (2022) <sup>[10]</sup>. In contrast, Madhav *et al.*, (2015) noted lymphoid nodules and multinucleated giant cells.

Other lung lesions included congestion (7.31%), congestion with oedema (4.8%), congestion with haemorrhage (2.43%), pulmonary oedema (7.31%), and emphysema with atelectasis (2.43%), aligning with Valecha (2022) <sup>[27]</sup>.

Histopathological examination of joints showed oedematous and congested synovial membranes, hypertrophied or necrotic villi, and mild inflammatory infiltration, indicative of synovitis. Among seven mammary gland samples examined, four showed atrophy and fibrosis, two had mastitis (one with neutrophilic and one with lymphocytic infiltration), and one appeared normal.

### 3.5 Cytological Examination

Cytological analysis was performed on synovial fluid from 11 goats with arthritis outlined in Table 4. In 4 cases, the fluid appeared clear and colourless with minimal neutrophil presence, suggestive of myositis or periarticular trauma. In 3 goats, the synovial fluid was pale yellow and watery, a feature consistent with chronic hemorrhage and bilirubin pigment formation, as reported by Waseem *et al.*, (2015) <sup>[28]</sup> and Moraes *et al.*, (2010) <sup>[18]</sup>. The largest volume (6 mL) was collected from a hock joint and was serosanguineous

with low viscosity, aligning with findings from Singh *et al.*, (1997) <sup>[22]</sup> and Tyagi and Singh (1993) <sup>[26]</sup>, who noted increased fluid volume in infectious arthritis. Microscopic examination revealed a predominance of neutrophils (91%) and mononuclear cells (9%). Differential cell counts showed that 7 out of 11 samples had elevated neutrophil levels, while the remaining exhibited mononuclear cell predominance.

### 3.6 Molecular detection by PCR

Molecular detection using PCR revealed that none of the samples tested positive for Maedi- Visna virus (MVV) or Caprine Arthritis Encephalitis Virus (CAEV). This negative result is most likely due to a low concentration of viral genetic material, which may have limited the PCR's ability to detect CAEV. As noted by Ramirez *et al.*, (2009), the sensitivity of the primers and the selection of the target region within the viral genome can significantly influence detection outcomes. The high genetic variability of small ruminant lentiviruses (SRLVs) further complicates PCR-based diagnosis, reducing the assay's effectiveness, as highlighted by Peterhans *et al.*, (2004) <sup>[20]</sup>.

In the present study, Ovine Pulmonary Adenocarcinoma (OPA) showed a prevalence rate of 1.41%, which aligns with findings reported by Sonawane *et al.*, (2016) <sup>[25]</sup>. PCR analysis of blood and tissue samples revealed no positive results in blood; however, out of 31 lung tissue samples, 2 tested positive for the U3 LTR gene (Fig.3), indicating a lung tissue prevalence of 6.45%. These findings confirm the presence of Jaagsiekte sheep retrovirus (JSRV) in goat populations in and around the Mumbai region. Furthermore, Mycoplasma was detected with an overall prevalence of 9.92% through PCR in the same geographic area. Results of PCR amplified products of Mycoplasma are featured in Fig.4. These results are consistent with the findings of Lin *et al.*, (2022) <sup>[13]</sup>, who reported a Mycoplasma prevalence ranging from 8.20% to 24.59% in dairy goats between 2017 and 2020.

**Table 1:** Details of PCR Primers of Retroviruses used in the study

| Primer name                  | Sequence (5'- 3')                                    | Gene | Product size (bp) | References                                    |
|------------------------------|------------------------------------------------------|------|-------------------|-----------------------------------------------|
| CAEV-F CAEV-R                | ATGCAGCATGGCCTCGTGTCT<br>CTTAATGCATTATTACCCATTGCA    | Gag  | 308               | Leroux <i>et al.</i> , 1997                   |
| MVV-Fex MVV-Rex              | TGACACAGCAAATGTAACCGCAAG<br>CCACGTTGGGCGCCAGCTGCGAGA | LTR  | 276               | Preziuso <i>et al.</i> , 2013                 |
| MVV-Fin MVV-Rin              | AAGTCATGTA(G/T)CAGCTGATGCTT<br>TTGCACGGAATTAGTAACG   | LTR  | 199               | Preziuso <i>et al.</i> , 2013                 |
| JSRV-3d-F JSRV-4d-R          | TGGGAGCTCTTTGGCAAAAGCC<br>CACCGGATTTTTACACAATCACCGG  | U3   | 176               | Palmarini <i>et al.</i> , 1996                |
| 309-MYC- U-F<br>309-MYC- U-R | GGCGAATGGGTGAGTAACACG<br>CGGATAACGCTTGCGACCTATG      | Uvf  | 450               | Wong-Lee <i>et al.</i> , 1993 <sup>[12]</sup> |

**Table 2:** Details of clinical signs showed by goats suspected of SRLV infection

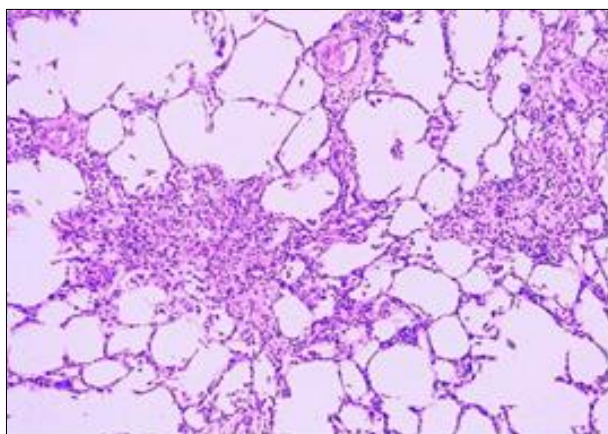
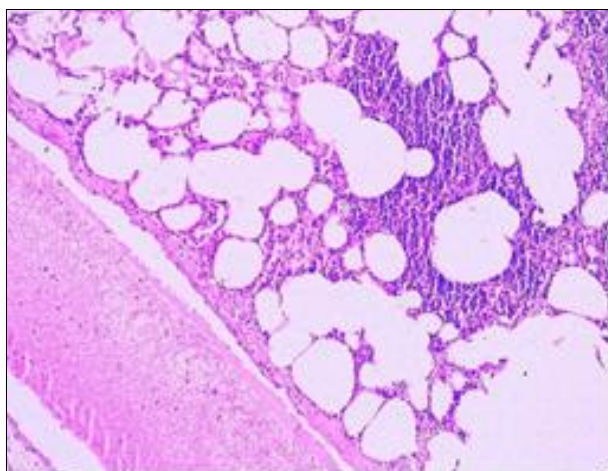
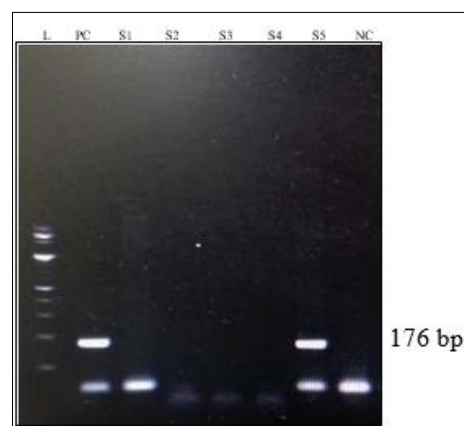
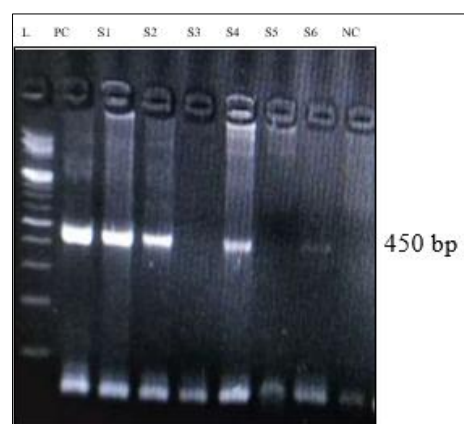
| Clinical Signs                                                                 | Number of goats | Prevalence |
|--------------------------------------------------------------------------------|-----------------|------------|
| Total number of suspected cases for SRLV infection                             | 110             | 03.14%     |
| Total number of cases showing chronic respiratory distress only                | 86              | 02.45%     |
| Total number of cases showing chronic respiratory distress + Clinical Mastitis | 11              | 00.31%     |
| Total number of cases showing chronic respiratory distress + Arthritis         | 4               | 00.11%     |
| Total number of cases showing clinical signs of Arthritis only                 | 3               | 00.08%     |
| Total number of cases showing clinical signs of Mastitis only                  | 5               | 00.14%     |
| Total number of cases showing clinical sign of mastitis + Arthritis            | 1               | 00.02%     |

**Table 3:** Details of histopathological lesions in lung tissues of goats suspected for SRLV infection

| Histopathological lesions    | No. of lungs affected | Frequency of occurrence (%) |
|------------------------------|-----------------------|-----------------------------|
| Fibrinous Bronchopneumonia   | 11                    | 26.82%                      |
| Suppurative Bronchopneumonia | 5                     | 12.19%                      |
| Interstitial pneumonia       | 6                     | 14.63%                      |
| Pulmonary adenomatosis       | 2                     | 4.87%                       |
| Congestion                   | 3                     | 7.31%                       |
| Congestion and Oedema        | 2                     | 4.87%                       |
| Congestion and Haemorrhages  | 1                     | 2.43%                       |
| Pulmonary oedema             | 3                     | 7.31%                       |
| Emphysema and Atelectasis    | 2                     | 4.87%                       |

**Table 4:** Details of synovial fluid analysis from dead goats suspected of SRLV infection

| Case no.    | Volume (ml) | Colour       | Viscosity | Neutrophil | Mononuclear cells |
|-------------|-------------|--------------|-----------|------------|-------------------|
| Case No. 1  | 4           | Yellow       | Watery    | 87         | 13                |
| Case No. 2  | N           | Colourless   | Clear     | Absent     | 84                |
| Case No. 3  | 3.5         | Reddish      | Watery    | 83         | 17                |
| Case No. 4  | N           | Colourless   | Clear     | 2          | 98                |
| Case No. 5  | 6           | Reddish      | Watery    | 91         | 9                 |
| Case No. 6  | 4.2         | Straw colour | Watery    | 93         | 7                 |
| Case No. 7  | 3.6         | Reddish      | Watery    | 79         | 21                |
| Case No. 8  | N           | colourless   | Clear     | Absent     | 93                |
| Case No. 9  | 5           | Yellow       | Watery    | 94         | 6                 |
| Case No. 10 | 4.7         | Yellow       | Watery    | 89         | 11                |
| Case No. 11 | N           | Colourless   | Clear     | 1          | 92                |

**Fig 1:** Interstitial pneumonia: Section of lung showing infiltration of lymphomononuclear cells in the interstitium. H&E X100**Fig 2:** Interstitial pneumonia: Section lung with interstitial cell infiltration, thickened interlobular septa. Note the absence of any exudate or cellular infiltration in the alveolar space. H&E X100.**Fig 3:** Agarose gel electrophoresis (2%) showing specific PCR amplified product of OPA with U3- LTR gene primers as 176bp, positive & negative control and 100bp ladder. Sample no.5 positive for OPA**Fig 4:** Agarose gel electrophoresis 2% showing specific PCR amplified products of Mycoplasma genus with primers as 450bp, positive and negative control and 100 bp ladder

#### 4. Conclusion

Clinical signs indicative of small ruminant lentivirus (SRLV) infection was detected in 3.14% of goats, accompanied by a mortality rate of 1.17%. Hematobiochemical evaluations of suspected cases were inconclusive. In contrast, gross and histopathological assessments of lung tissues revealed a 9.75% prevalence of maedi-like interstitial pneumonia. Molecular analysis of SRLV-negative samples detected Jaagsiekte sheep retrovirus (JSRV) in 1.41% and *Mycoplasma* species in 9.92% of cases, underscoring the necessity for differential diagnosis. These results emphasize the critical role of combining histopathology with molecular techniques for precise identification of respiratory diseases in goats.

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