



Detection of Ovine Pulmonary Adenocarcinoma (OPA) in sheep from district Budgam, Jammu and Kashmir, India

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ABSTRACT

An outbreak of Ovine Pulmonary Adenocarcinoma (OPA) ensued in a flock of Merino sheep in Temperate western sub zone of lesser Himalayas in Pir Panjal region of Jammu and Kashmir, India. The affected animals exhibited symptoms of labored breathing, copious bronchial discharge on elevating hindquarter and moist lung sounds. Despite treatment, all affected animals succumbed, indicating a 100% case fatality rate with higher prevalence in male (12/16) than female (4/16) and most of the deaths in spring and summer at Highland pasture (13/16) than in winter (3/16), indicating exercise intolerance. A comprehensive diagnostic approach was employed incorporating pathological, histopathological and hemi-nested PCR technique, to confirm the presence of Jaagsiekte sheep retrovirus (JSRV), the etiological agent responsible for Ovine Pulmonary Adenocarcinoma (OPA). The study reports the first confirmed natural case of OPA in Jammu and Kashmir, India, with a prevalence of 1.67% in the affected flock. The findings suggest that young sheep can be infected through colostrum and milk from carrier dam. The JSRV was confirmed by PCR techniques with Post-mortem examination revealed typical gross lesions and histopathological findings, including papillomatous ingrowths and hyperplasia of bronchial epithelium and type 2 pneumocyte proliferation.

Keywords: Jaagsiekte, Jammu and Kashmir, Mortality, Retrovirus, Sheep

Ovine Pulmonary Adenocarcinoma (OPA) is a highly contagious lung infection caused by Jaagsiekte Sheep Retrovirus (JSRV), leading to neoplastic transformation of alveolar and bronchiolar secretory epithelial cells. The condition was initially known as Jaagsketi in South Africa during 1800s from the word Afrikaans for “Chasing sickness” (Shivasharanappa *et al.* 2023). JSRV is a single-stranded, positive-sense RNA virus belonging to the genus Beta retrovirus, family *Retroviridae* (Gray *et al.* 2019). Ovine Pulmonary Adenocarcinoma (OPA) primarily affects sheep, goats, and mouflons, but not other livestock species. Typically, natural cases occur in adult sheep between 1-4 years old (Quintas *et al.* 2021), although tumors have been reported in sheep as young as 2 months and as old as 11 years (Salvatori *et al.* 2004, Rai 2000). JSRV replicates in tumor cells, releasing the virus into the airways, where it is expelled through respiratory secretions. The transmission occurs through close contact like housing and manger feeding that allows inhalation of infected aerosols and ingestion of colostrum and milk. Despite its significance, OPA is not a notifiable disease, resulting in

limited epidemiological investigations (Shivasharanappa *et al.* 2023). OPA is one among the five ice berg diseases along with Johnes Disease, Maedi Visna (MV), Border Disease and Caseous Lymphadenitis (Henderson 2022). OPA is not present in Australia and New Zealand and has been eradicated from Iceland (WOAH Terrestrial Manual 2021). Dolly, the first genetically modified livestock was also affected by this virus resulting in lung tumour and was finally euthanized at the Roslin Institute on Feb 14, 2003 at the age of 6 and a half years (Shivasharanappa *et al.* 2023). Youssef *et al.*, 2015 and Gray *et al.* 2019 report that this disease has similar histomorphological features and tumour inducing pathway as lung carcinoma in humans. Thus, making this disease in sheep as a good animal model for an experimental study and understanding retrovirus lung carcinoma in humans (Shivasharanappa *et al.*, 2023). This study is first of its kind in Jammu and Kashmir that briefly discuss the OPA, its epidemiology, pathogenesis and diagnosis

MATERIAL AND METHODS

Time period and study area: The present study was conducted from April 2023 to January 2024, in temperate zone at Government Sheep Breeding Farm Kralpathri located at 33.9016°N latitude and 74.6124°E longitude at an altitude of 2210 meters (7250 feet) in the hills of

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Doodhpathri. The topography of the farm is undulating hills covered by forest land spread over a vast area (Bashir *et al.* 2020b). The farm maintains Kashmir Merino and Australian Merino Sheep. The animals were dewormed regularly and dipped twice a year after shearing. The flocks were vaccinated against enterotoxaemia, foot and mouth disease, sheep pox and PPR as per recommended schedule. Sheep are intensively stall fed from 15th Nov to 15th April. During spring (16th April to 15th June) and Autumn (16th Sep to 14th Nov) sheep are allowed to graze for min 8 hours on farm land, community grazing land and nearby forest land. During summer (16th June to 15th Sep) sheep are migrate for 8-10 hours on foot to Highland Pasture Ashtar located at 34.2199°N and 74.7736°E at an altitude of 3450 meters (11,300 feet) in the western zone of lesser Himalayas, in search of highly nutritious pasture grass. During migration, sheep are exposed to varying climatic conditions wherein ambient temperature fluctuates from sub-zero during the night to 21-32°C during the day. During nights sheep are kept in temporary paddocks and let out for grazing during day for about 10 to 12 h (Bashir *et al.* 2020a).

Total 16 naturally infected sheep displayed varying symptoms of progressive anorexia, open-mouth breathing, moist respiratory sounds (rales and wheezes), dyspnoea, and copious amounts of bronchial fluids, which progressed to laboured abdominal breathing and frothy exudate secretion upon lifting the hind limbs (Wheelbarrow test) (Fig 1) and generalized weakness. Rectal temperature was within normal range but heart rate was elevated. These symptoms are consistent with previous reports (Shivasharanappa *et al.* 2023). Coughing and inappetence are not common (WOAH Terrestrial Manual 2021). The treatment regimen included symptomatic management with antibiotics, analgesics, antihistamines, bronchodilators, and tonics. Despite treatment, the affected animals succumbed to the disease. Post-mortem (PM) examination of these animals was conducted and morbid samples like lung fluid and affected lung tissue, kidney and heart samples were

collected aseptically in sterile containers. The samples for histopathology were fixed in 10% formalin and samples for RNA extraction were transported in ice to the Disease Investigation Laboratory (DIL) Nowshera, Srinagar, for complete investigation including molecular detection of the agent involved. For histopathology, lung, kidney and heart samples were processed by paraffin embedding and the sections were stained by H&E dye.

The genomic RNA was isolated from the bronchial fluids using commercial RNA extraction kit (RNA sure) as per the method described by the manufacturer. cDNA was synthesised using cDNA synthesis kit (Thermoscientific) following manufacturer's instructions. This was followed by heminested PCR consisting of two rounds using primers P-I (TGGGAGCTCTTTGGCAAAAGCC), P-III (CACCGGATTTTACACAATCACCGG) and P-VI (TGATATTTCTGTGAAGCAGTGCC) specific to U3 gene of JSRV. PCR was carried out and the amplified products at each round of PCR were detected by electrophoresis and visualized by UV gel documentation system (Devi *et al.* 2014).

RESULTS AND DISCUSSION

This study reports the first confirmed natural case of Ovine Pulmonary Adenocarcinoma (OPA) in Jammu and Kashmir, India. Damodaran (1960) reported first confirmed case of OPA in India. Kumar *et al.* 2014a reported this disease in Pondicherry and Chennai on the samples collected from the slaughter houses. 16 out of 954 sheep were diagnosed with Ovine Pulmonary Adenocarcinoma (OPA), having prevalence of 1.67% with higher prevalence in male than female (Table 1). The flock prevalence in this study was comparable to the 2.46% as reported by Devi *et al.* (2014) in Indian sheep. However, it was lower than the prevalence rates reported by Kumar *et al.* (2014b) and Sonawane *et al.* (2016), which were 4.87% and 8%, respectively.

Although OPA is rare in sheep under 7-9 months, most clinical cases occur in animals over two years old (Table 1) that can be attributed to its long incubation period (WOAH Terrestrial Manual 2021) of 6 months to 4 years (Shivasharanappa *et al.* 2023). However, Sonawane *et al.*

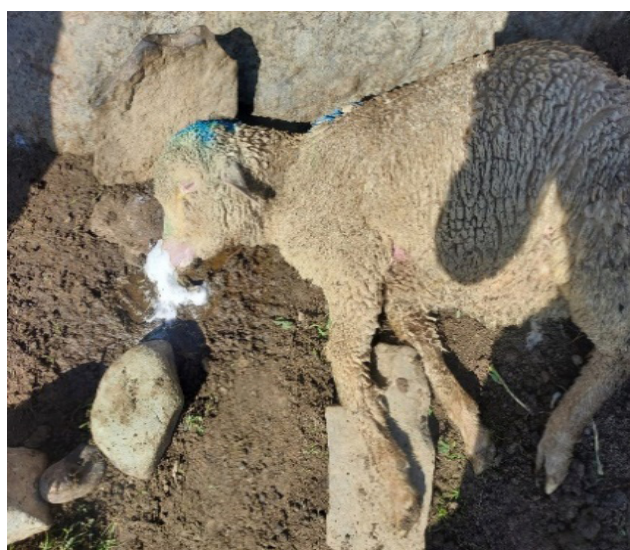


Fig. 1. Frothy Lung fluid discharge in OPA affected dead sheep

Table 1. Distribution of animals affected with Ovine Pulmonary Adenocarcinoma

Sex	N	%
Male	12/16	75.00
Female	4/16	25.00
Age	N	%
6-12 months	3/16	18.75%
13-18months	5/16	31.25%
19-24 months	4/16	25.00%
25 and above	4/16	25.00%
Season	N	%
Winter	3/16	18.75%
Spring	2/16	12.50%
Summer at HLP	11/16	68.75%

(2016) reported OPA in sheep as young as 3-6 months without clinical signs or gross lesions. Notably, young sheep between 6 months and 3 years of age were affected in this study also, suggesting potential infection through colostrum and milk from carrier Ewe to new born lambs by crossing gut epithelial lining and infecting somatic cells and macrophages (Shivasharanappa *et al.* 2023). Despite treatment, all the 16 animals succumbed to the disease within one to two months, showing 100% case fatality rate. The disease's non-responsiveness to antibiotics and symptomatic treatment aligns with previous findings (Griffiths *et al.* 2010).

Detecting JSRV in its preclinical stage is challenging due to the lack of diagnostic tests, likely attributed to the absence of T-cell responses and circulating JSRV-specific antibodies in naturally infected or experimentally infected sheep. However, the World Organisation for Animal Health (OIE) recommends using PCR and RT-PCR methods targeting the LTR region of JSRV for epidemiological studies and diagnosing OPA, particularly in samples from lungs, mediastinal lymph nodes, bronchoalveolar lavage fluid, and peripheral blood mononuclear cells (Shivasharanappa *et al.* 2023) and also imaging techniques like transthoracic ultrasound (Cousens & Scott, 2015), computed tomography, X-rays and colour CT Scan (Quintas *et al.* 2021) can also help in detecting subclinical cases. In this study, most cases of OPA occur after sheep migrate to highland pasture, suggesting exercise intolerance may play a role. For this reason, disease is also known as "Driving sickness" (Henderson 2022). Most deaths were observed in spring and summer at highland pasture with temperatures below 10°C, suggesting environmental and climate factors contributing to OPA's clinical manifestation. These findings were in consonance with Griffiths *et al.* (2010) who linked high mortality rates to adverse climate and nutritional restrictions.

Post-mortem examination revealed frothy nasal discharge, consolidated lungs, haemorrhagic trachea, pus in lungs which may be due to secondary infection, enlarged heavy lungs with firm nodules mainly on craino ventral

surfaces (Fig 2), and frothy fluid in trachea and bronchi is the prominent finding, correlating the outward symptoms with lesions observed. These findings are in consonance with the necropsy findings by WOAHS Terrestrial Manual 2021. Enlarged mediastinal lymph nodes were observed, consistent with previous reports of enlargement and oedema in mediastinal lymph nodes (Shivasharanappa *et al.* 2023). Additionally, pulmonary oedema was noted, characterized by infiltration of lymphocytes, neutrophils, and macrophages in alveolar septa, along with mild congestion. The lungs also showed alveolar epithelial proliferation, lymphocytic infiltration, and obstruction of alveolar and bronchial lumens with papillomatous ingrowths, hyperplasia of bronchial epithelium, and proliferation of Type 2 pneumocytes. These findings align with previous studies (Palmarini and Fan 2001) that suggest this transmissible tumour originates from alveolar type 2 pneumocytes and Clara cells in the lungs. Furthermore, interstitial nephritis and severe intertubular haemorrhage were observed in the kidney medulla, and the heart showed shrunken myofibers and degeneration of myocardium with pyknotic nuclei with multifocal infiltration of polymorphonuclear and mononuclear cells. In this study, U3 hn PCR was conducted to confirm the presence of proviral JSRV DNA in the bronchial fluid samples. The desired product amplified was of 176 bp using above said primers on (Fig 3). There is sequence difference in U3 region of LTR with enJSRV 47bp longer than exJSRV that helps to differentiate between the enJSRV and exJSRV. Most of the enJSRV are defective and silent. Hence not able to cause tumour like conditions unlike exJSRV (Devi *et al.* 2014)

According to WOAHS Terrestrial Manual 2021 there is no known risk of human infection with JSRV and currently no vaccination is available for the control of disease. Some of the control measures can be by removing the clinically affected animals from the flock, reducing stocking density, providing more time outdoor than inside the shed and regular cleaning/disinfection of feeding mangers (Henderson 2022). Removing new born lambs from the

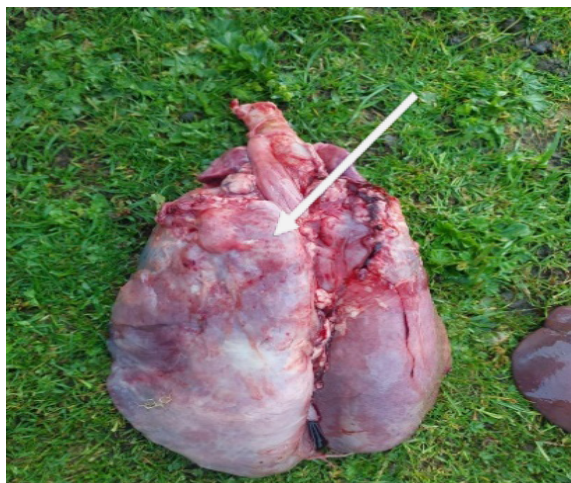


Fig. 2. Increase in lung size with typical nodular tumorous growth

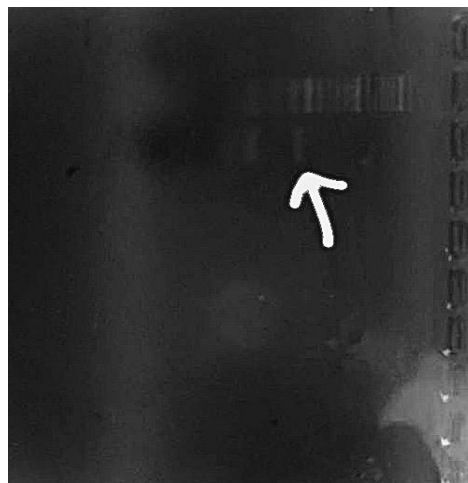


Fig. 3. U3 region of JSRV LTR

affected dams and feeding them preheated colostrum/milk can also be a preventive measure against OPA (Voigt *et al.* 2007).

CONCLUSION

This study reports the first confirmed natural cases of Ovine Pulmonary Adenocarcinoma (OPA) in Merino sheep from Jammu and Kashmir, based on clinical signs, pathological lesions and molecular detection of Jaagsiekte sheep retrovirus (JSRV) using hemi-nested PCR. The study highlights the importance of surveillance and early detection of OPA to prevent its spread and reduce economic losses to the sheep farmers. No serodiagnostic test is available for subclinical cases. Only method of prevention in current scenario is by detection of clinical cases and slaughtering them which is economically not viable in field conditions. Hence focus should be on developing molecular tests for early detection of cases. Further studies are needed to determine the prevalence and molecular epidemiology of JSRV in sheep populations of Jammu and Kashmir.

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