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Histopathological and Immunohistochemical Studies of a Spontaneous Case of Pulmonary Adenomatosis in an Adult Ram: A Case Report

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Case Report

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ABSTRACT

Aim: The present study aimed to describe histopathological findings and immunohistochemical expression of proliferating cell nuclear antigen (PCNA) in a spontaneous case of pulmonary adenomatosis in an adult ram.
Case Presentation: A carcass of an adult ram was presented for necropsy in the Post-mortem Hall of the Department of Veterinary Pathology, College of Veterinary Sciences of the Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar with a history of respiratory distress. A detailed

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necropsy was performed. The tracheal mucosa showed mild congestion. The lungs were pale and firm in consistency. On histopathological examination, nasal turbinate and trachea both showed the vascular changes along with mild infiltration of mononuclear cells. The sections of lungs revealed pulmonary adenocarcinoma characterized by the presence of cuboidal to low columnar epithelial cells forming acinar or papillary-like structures in the parenchyma and surrounded by the normal alveoli. Areas of necrosis along with infiltration of mononuclear cells were also evident. An Immunohistochemical study of the lung sections revealed mild to moderate immunopositive reactivity for the PCNA in the nuclei of neoplastic cells of adenocarcinoma. It indicated the proliferative activity of neoplastic cells.

Conclusion: Based on histopathological examination, the present case was diagnosed as pulmonary adenomatosis. PCNA expression indicated the presence of actively proliferating neoplastic cells.

Keywords: Proliferating cell nuclear antigen; pulmonary adenomatosis; ram; spontaneous.

1. INTRODUCTION

Production losses in small ruminants are mainly influenced by genetic factors, infectious agents, and environmental conditions, with infectious causes being especially significant [1]. Respiratory diseases are the most serious issues affecting small ruminants globally, often leading to significant mortality and decreased productivity [2]. Ovine pulmonary adenomatosis (OPA), also known as ovine pulmonary adenocarcinoma, Jaagsiekte or ovine pulmonary carcinoma, is a contagious lung tumour primarily affecting the sheep and, less frequently, goats [3]. It is an infectious form of lung tumour that is caused by Jaagsiekte sheep retrovirus (JSRV) i.e., a beta-retrovirus that is responsible for causing the neoplastic transformation of type II pneumocytes and Clara cells. These cells typically secrete fluid lining the alveolar epithelium of the lung. Excessive fluid secreted by the neoplastic cells can accumulate in the lungs and be discharged through the nose and mouth, potentially spreading the virus to other animals [4,5]. OPA diagnosis is challenging because of the lack of cellular or humoral immune response to viral proteins. Serological tests for JSRV antibodies are also unreliable [4], so at present there are no such screening test for diagnosis at the farm level. Although PCR-based techniques are useful in research, they are not sensitive enough for diagnosis in the field [6-9]. As a result, diagnosis of OPA primarily relies on postmortem examination of lungs which involves histopathology and occasionally immunohistochemistry (IHC) [1,4]. Few reports of OPA in sheep are documented in India; however, cases were documented from southern states of India based on pathomorphological and PCR results [1]. The aim of the present study was to describe histopathological findings and immunohistochemical expression of proliferating cell nuclear antigen (PCNA) in a spontaneous case of pulmonary adenomatosis in a ram.

2. CASE PRESENTATION

A carcass of an adult ram was presented to the Post-mortem Hall, Department of Veterinary Pathology, College of Veterinary Sciences of the Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. A systemic necropsy was performed by following the appropriate procedure described earlier [10]. The lungs were palpated and consistency was noted. The cut surfaces were thoroughly examined. The tissue samples from the trachea, nasal turbinate and lungs were collected in 10% neutral buffered formalin for histopathology. After fixation, a routine paraffin embedding technique was used for the tissue processing [11]. First of all, formalin fixed tissues were washed under tap water for overnight to remove the formalin. Then, tissues were dehydrated in ascending grades of ethanol (70, 80, 90 and 100%) for one hour each with three changes in 100% ethanol. Then cleared in benzene (two changes of 30 minutes each), followed by immersion into the liquid paraffin (3 changes of one hour each) for impregnation and infiltration in tissues. Then, the tissues were embedded in paraffin. Tissue sections of 4 µm thickness were cut using a rotary microtome (Yorco YSI 060 semi-automatic rotary microtome). The sections were stained with haematoxylin and eosin (H&E) [11]. After analyzing slides using light microscope, histopathological interpretation was done. For immunohistochemistry (IHC), paraffin embedded lung tissue sections were used for the expression of PCNA. Sections were taken on glass slides coated with 2% 3-aminopropyl-triethoxysilane in acetone. Sections were deparaffinized using

xylene and rehydrated with descending grades of alcohol. Thereafter, heat-induced epitope retrieval was carried out by immersing tissue sections in 0.01M citrate buffer (pH-6) to microwave irradiation (15 cycles of 2 minutes each). For blocking of endogenous peroxidase activity, tissue sections were immersed in 3% hydrogen peroxide for 45 minutes. Blocking of non-specific sites was carried out by incubating tissue sections with 5% normal goat serum (Sigma) in 1% bovine serum albumin (Himedia) prepared in phosphate buffer saline (PBS). Mouse monoclonal anti-PCNA antibody (Sigma Aldrich) was used as the primary antibody at a dilution of 1:400 and incubated overnight at 4 ºC. Duplicate section, incubated with 1% bovine serum albumin without primary antibody served as negative control. Then, sections were incubated with anti-mouse secondary antibody followed by Extravidin peroxidase (1:20 dilution; Sigma Aldrich) for 45 mins each. Then, 3-Amino-9-ethylcarbazole (AEC; Sigma Chemicals, USA) staining substrate was applied to the moist tissue sections for colour development. The sections were washed three times with PBS for 5 minutes each following each step from antigen retrieval to colour development. Gill's Haematoxylin was used as a counterstain (Sigma). Then, slides were rinsed with distilled water and mounted with aqueous CC mount (Sigma Aldrich). Nuclear staining of brick red to brown coloured in neoplastic cells was considered positive. For, determination of PCNA index, hot spot area of slide (area with neoplastic cells) was examined at high power field (40× objective) of light microscope was examined as per the method described earlier. A total of 1000 neoplastic cells were examined and the neoplastic cells which showed brick red to brown stained nuclei were counted irrespective of staining intensity. The immunopositive cells for PCNA were expressed as percentage. The intensity of immunopositive cells was recorded.

Grossly, the tracheal mucosa revealed mild congestion. Lungs were pale, firm in consistency and showed focal area of congestion and necrosis (Fig. 1). The cut surfaces did not reveal oozing out of any discharge from it. On histopathological examination, the lungs revealed pulmonary adenocarcinoma characterized by the presence of cuboidal to low columnar epithelial cells forming acinar or papillary-like structures in the parenchyma, surrounded by the normal alveoli. (Figs. 2 & 3). Areas of necrosis, congestion and haemorrhages along with infiltration of mononuclear cells (mainly lymphocytes and a few macrophages) were also observed (Fig. 4). Mild congestion and infiltration of mononuclear cells was evident in the trachea and nasal turbinate. IHC revealed mild to moderate immunopositive reaction for PCNA in the nuclei of the neoplastic cells and it appeared brown to brick red coloured (Fig. 5). The PCNA immunoreactivity was 37.8%.

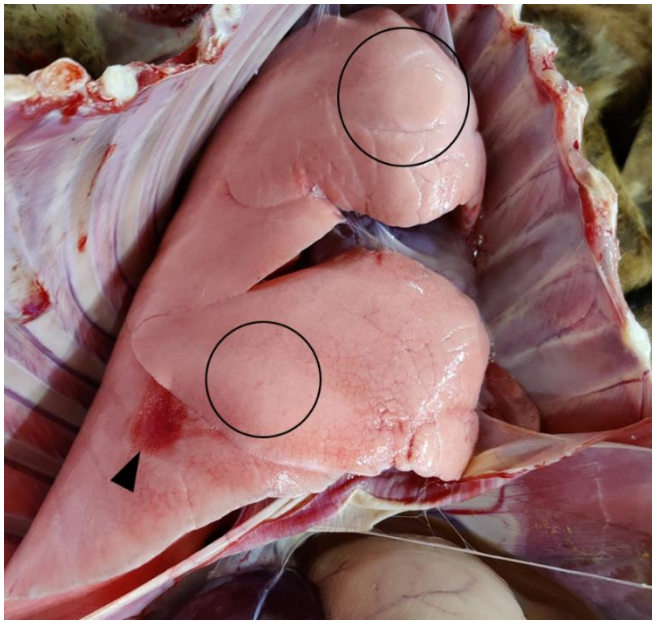

Fig. 1. Photograph of lungs showing focal area of congestion (arrow head) and multiple pale areas (encircled)

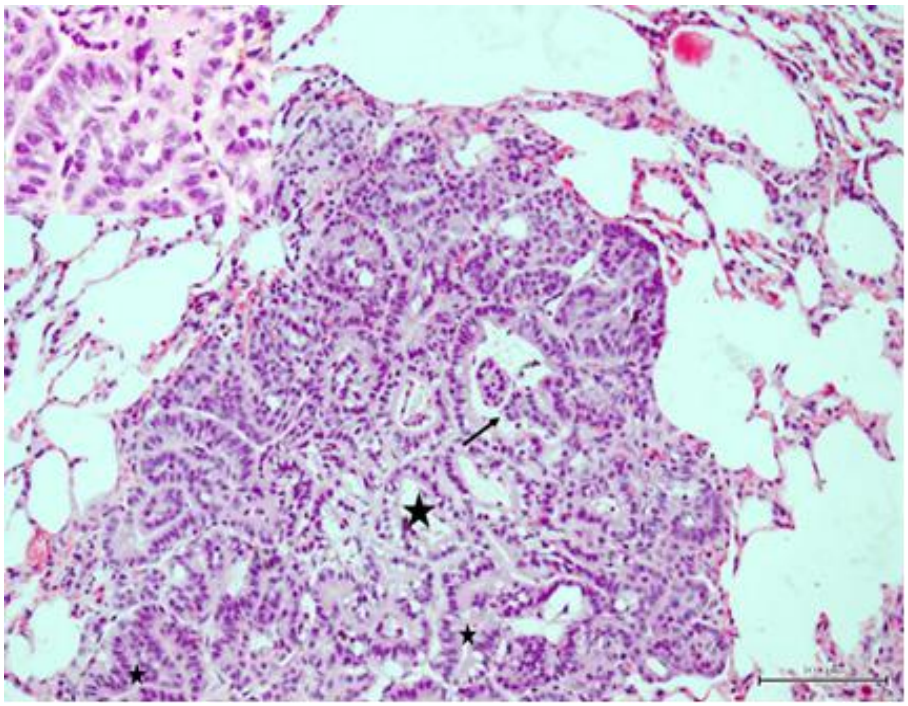

Fig. 2. Pulmonary adenomatosis: Lung section showing pulmonary adenocarcinoma characterized by the presence of neoplastic cuboidal to low columnar epithelial cells forming acinar (stars) or papillary-like structures (arrow) in lung parenchyma, surrounded by normal alveoli (Inset: Higher magnification). H&E×200

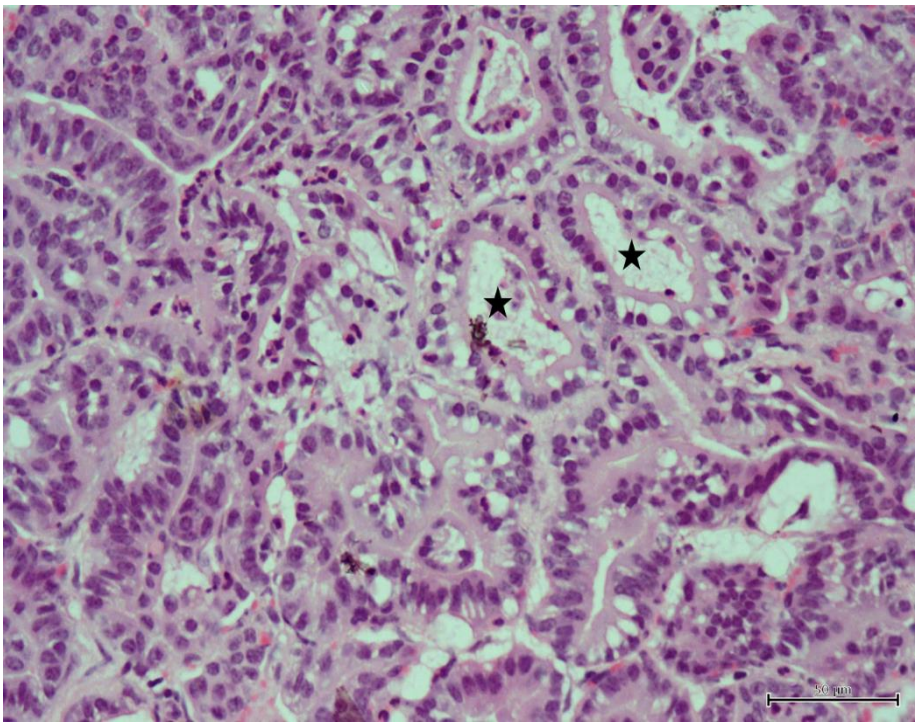

Fig. 3. Pulmonary adenomatosis: Lung section showing pulmonary adenocarcinoma characterized by the presence of neoplastic cuboidal to low columnar epithelial cells forming acinar structures (stars) in lung parenchyma. H&E×400

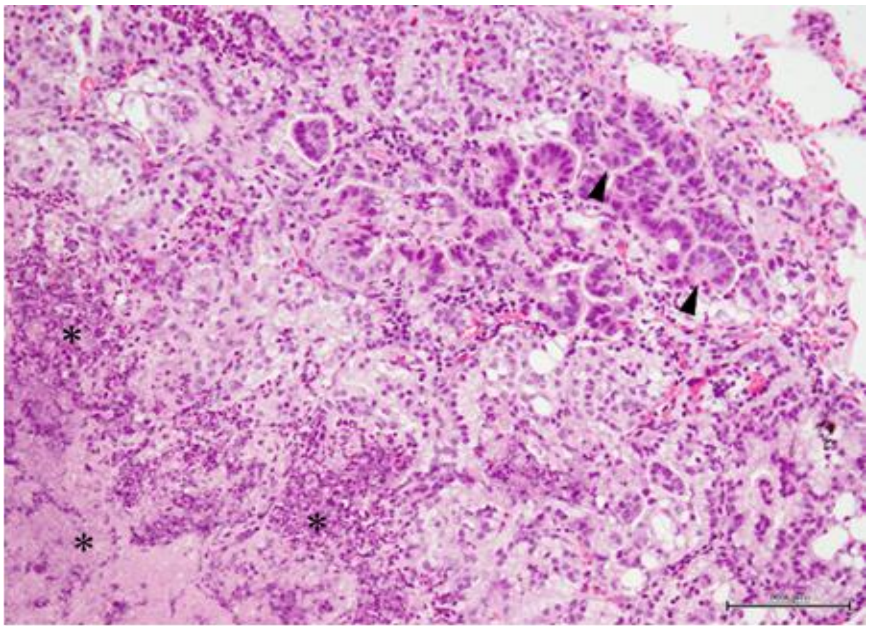

Fig. 4. Pulmonary adenomatosis: Lung section showing alveoli transforming into glandular structure (arrow heads) and large necrotic areas with mixed type of inflammatory cells (asterisks). H&E×200

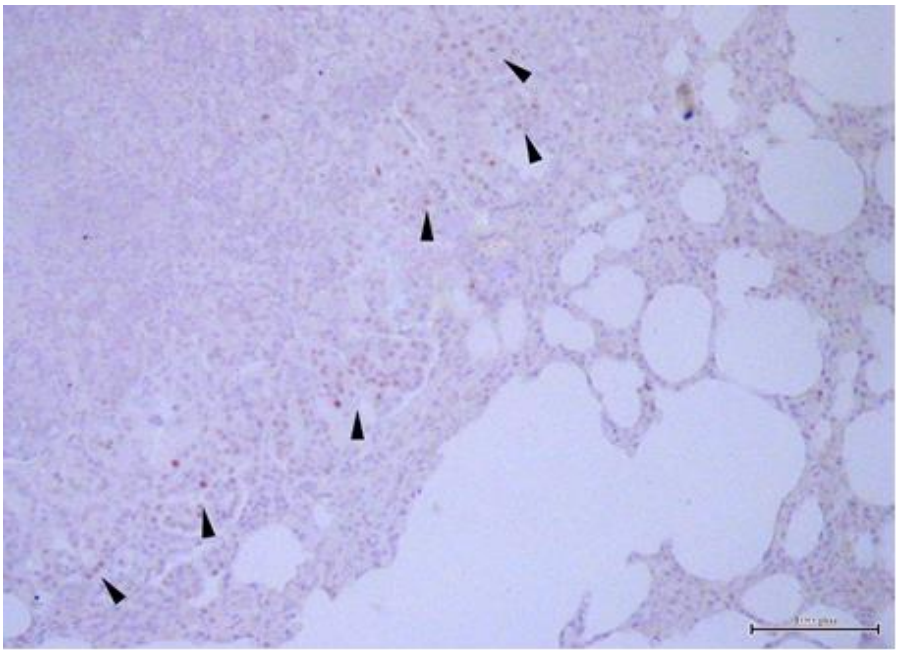

Fig. 5. Pulmonary adenomatosis: Neoplastic cells showing mild to moderate nuclear brown to brick red coloured immunostaining (arrow heads) for proliferating cell nuclear antigen. IHC×200

3. DISCUSSION

Ovine pulmonary adenocarcinoma is a transmissible contagious disease of adult sheep and goats characterized by neoplastic transformation of type-II alveolar epithelial cells and Clara cells caused by retrovirus [12,13]. Using modern techniques, many methods have been developed for the diagnosis of pulmonary adenomatosis but necropsy and histopathological examination will remain a gold standard for its diagnosis [14]. In the present study, pulmonary adenomatosis was observed in an adult ram. Grossly, lungs appeared pale, firm

and revealed focal area of congestion and necrosis. Besides, the earlier researchers also reported the presence of varying size of foci coalescing to form nodules in lungs. In the present case, histopathologically lungs revealed acinar or papillary-like structures in parenchyma and surrounded by the normal alveoli. Histopathological findings were in corroboration with earlier researchers [15]. Additionally, they also noticed the fibrous and thickened interstitial connective tissue [15]. Oda and Youssef [16] observed randomly distributed, well-differentiated epithelial cells arranged mostly in cystic or papillary acinar-like structures or rarely in solid aggregates.

Proliferating cell nuclear antigen (PCNA) is a widely used biomarker for assessing cellular proliferation. It is expressed in the nucleus and is involved in DNA synthesis and cell cycle regulation [17]. PCNA is maximally expressed during 'S' phase of cell-cycle and is rarely expressed in the stationary phase. However, PCNA can also be observed in other phases of the cell cycle and in non-dividing cells owing to its half-life of 8-20 hours [18]. In the present report, positive immunoreactivity for PCNA was observed in neoplastic cells, with a PCNA index of 37.8%, suggesting the active proliferation of neoplastic cells. However, no immunostaining was observed in the adjacent normal lung alveolar epithelium. Pawaiya and Kumar [15] in their study on nine cases of pulmonary adenocarcinoma reported similar histopathological findings and immunostaining for PCNA, but a PCNA index of 73.9% was observed. These differences in PCNA indices can be attributed to the varying chronicity of the cases and maturation stages of the neoplastic cells. By using imaging diagnostic techniques such as computed tomography, ultrasonography, and X-rays can be used for disease diagnosis in preclinical form. Polymerase chain reaction assay and immunohistochemistry can be utilized to detect and confirm the presence of JSRV [19]. Furthermore, different types of molecular markers can also be identified for understanding the pathogenesis of disease to elucidate the molecular mechanisms of transformation of normal lung parenchyma to tumorous cells and their proliferation. This understanding will be beneficial for aiding in disease diagnosis.

4. CONCLUSION

In conclusion, based on histopathological examination the present case was diagnosed as pulmonary adenomatosis. IHC indicated the presence of proliferating neoplastic cells within lung parenchyma. Nevertheless, the present report validates the existence of ovine pulmonary adenomatosis within the sheep flocks in the Haryana region. Furthermore, more targeted investigations on a larger scale are required to assess the prevalence of this contagious disease and its impact on economic losses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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