

Case report

Histopathological and immunohistochemical studies of a spontaneous case of pulmonary adenomatosis in an adult ram: a case report

ABSTRACT

Aim: The aim of present study was 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 the history of respiratory distress. Detailed necropsy was performed. Tracheal mucosa showed mild congestion. 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 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. 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 the 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 which is caused by Jaagsiekte sheep retrovirus (JSRV) i.e., a beta-retrovirus which is responsible for causing the neoplastic transformation of type II pneumocytes and Clara cells. These cells typically secrete fluid lining the alveolar epithelium of lung. Excessive fluid secreted by the neoplastic cells can accumulate in 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 but they are not sensitive enough for diagnosis in the field. 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 present study is aimed to 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. Systemic necropsy was performed and tissue samples from trachea, nasal turbinate and lungs were collected in 10% neutral buffered formalin for histopathology. After fixation, routine paraffin embedding technique was used for the tissue processing [6]. Tissue sections of 4 μ m thickness were cut using rotary microtome (Yorco YSI 060 semi-automatic rotary microtome). The sections were stained with haematoxylin and eosin (H&E) [6]. 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 dehydrated 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 primary antibody at dilution of 1:400 and incubated overnight at 4 °C. 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 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 as positive.

Grossly, tracheal mucosa revealed mild congestion. Lungs were pale, firm in consistency and showed focal area of congestion and necrosis. On histopathological examination, lungs revealed the 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. (Fig. 1). Areas of necrosis, congestion and haemorrhages along with infiltration of mononuclear cells (mainly lymphocytes and few macrophages) were also observed (Fig. 2). 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. 3).

3. DISCUSSION

Ovine pulmonary adenocarcinoma is a transmissible contagious disease of adult sheep and goat which characterized by neoplastic transformation of type-II alveolar epithelial cells and Clara cells caused by retrovirus [7, 8]. 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 [9]. In the present study, pulmonary adenomatosis was observed in an adult ram. Microscopically, it revealed the presence of acinar or papillary-like structures in parenchyma and surrounded by the normal alveoli. Similar findings have been reported in sheep and goat by earlier workers [1, 5, 10]. Positive immunoreactivity for PCNA in neoplastic cells in the present study were similar to that documented in the earlier study [1] suggesting the active proliferation of neoplastic cells.

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.

REFERENCES

1. Singh R, Singh S, Singh R, Varshney R, Dhama K, Kumari S et al. Patho-Epidemiological study of jaagsiekte sheep retrovirus infection in the sheep and goats population, India. *Biol Rhythm Res.* 2018;51(8):1182-96.
2. Ali K, Farghali, HAMA, Shamaa AAE. Adenocarcinoma among sheep and goats in Kuwait. *Int J Natl Soc Sci* 2019;6(4):46-52.
3. WOAHA (World Organisation for Animal Health). *Ovine Pulmonary Adenocarcinoma (Adenomatosis)* In: *Terrestrial Manual*, 2021.
4. Cousens C, Gibson L, Finlayson J, Pritchard I, Dagleish MP. Prevalence of ovine pulmonary adenocarcinoma (Jaagsiekte) in a UK slaughterhouse sheep study. *Vet Rec.* 2015;176(16):413.
5. Abdullah MA. Ovine and Caprine Pulmonary Adenomatosis, At Duhok Abattoir, Iraq, First Prevalence and Pathological Study. *Egypt J Vet Sci.* 2023;54(1):117-24.
6. Luna LG, editor. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.* 3rd ed. McGraw Hill Book Co. New York; 1968.
7. Lopez A, Martinson SA. Respiratory system, mediastinum, and pleurae. In: Zachary JF, editor. *Pathologic basis of veterinary disease.* 6th ed. St. Louis, Missouri: Elsevier;2017.
8. Mansour KA, Al-Husseiny SH, Kshash QH, Jassim A. Clinical-histopathological and molecular study of ovine pulmonary adenocarcinoma in Awassi sheep in Al-Qadisiyah Province, Iraq. *Vet. World.* 2019;12(3):454-58.
9. Mohammed ZMA, Ibrahim WM. A Morphopathological study on ovine pulmonary adenocarcinoma in Libya. *Microbiol Res J Int.* 2022;32(2):23-32.
10. El-Mashad, Ismail AB, Moustafa, Ahmad S, Amin, Aziza et al. Pathological Studies on lung affections in sheep and goat at Qalyuobia Governorate. *Benha Vet Med J.* 2020;38(1):17-23.

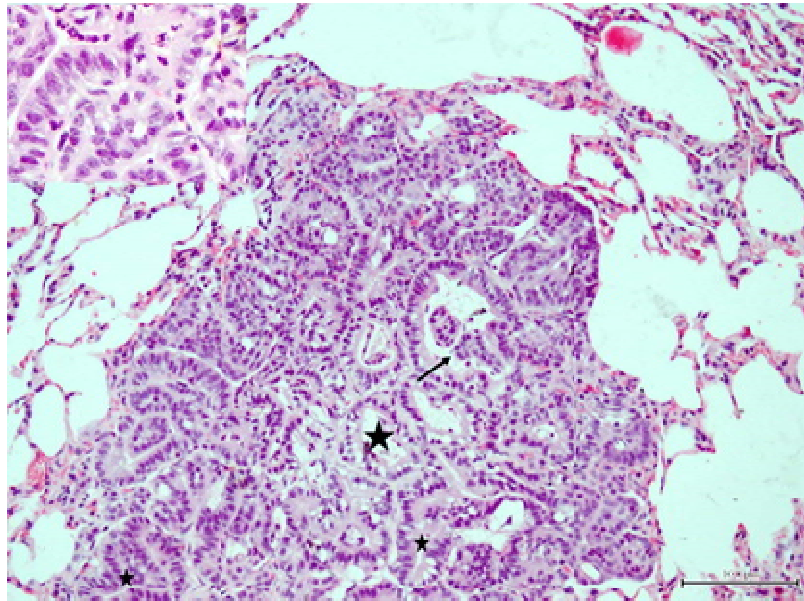


Fig. 1. Pulmonary adenomatosis: Lung section showing pulmonary adenocarcinoma characterized by the presence of neoplastic cuboidal to low columnar epithelial cells forming acinar or papillary-like structures in lung parenchyma, surrounded by normal alveoli (Inset: Higher magnification). H&E×200

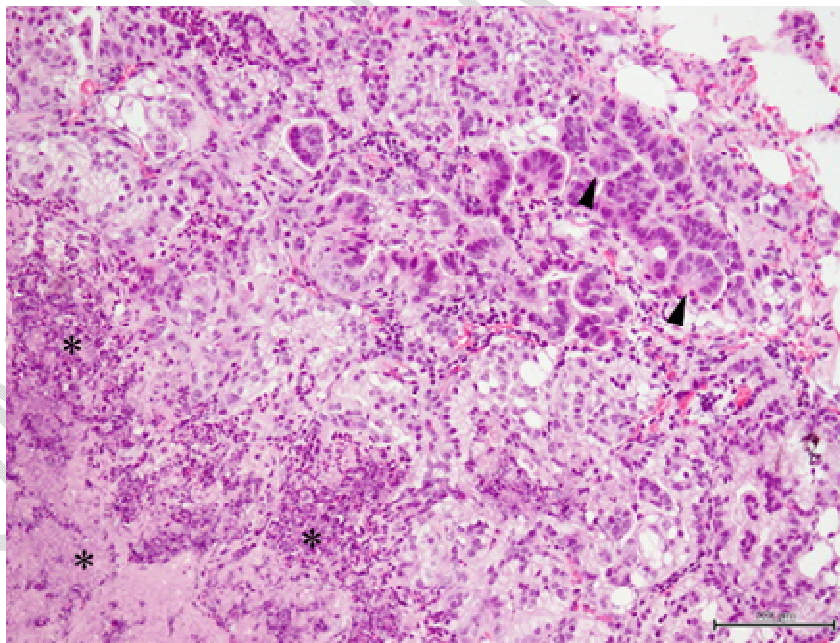


Fig. 2. Pulmonary adenomatosis: Lung section showing alveoli transforming into glandular structure (arrow heads) and large necrotic areas with mixed type of inflammatory cells (asterisks). H&E×200

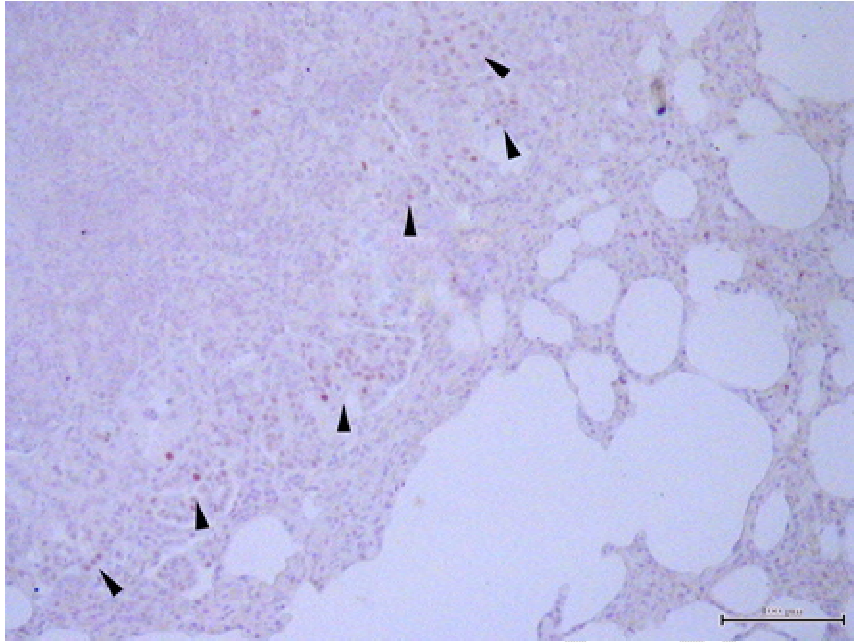


Fig. 3. Pulmonary adenomatosis: Neoplastic cells showing mild to moderate nuclear brown to brick red coloured immunostaining for proliferating cell nuclear antigen. IHC×200