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Case Report

Pathology of natural cases of ovine pulmonary adenocarcinoma (Jaagsiekte) in goats

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Abstract

The pathological findings and the immunohistochemical and molecular diagnosis of two natural cases of pulmonary adenocarcinoma in goats are described. Ovine pulmonary adenocarcinoma (OPA), a contagious lung tumor, caused by an exogenous Jaagsiekte Sheep Retrovirus (JSRV), is reported commonly in sheep and rarely in goats. The affected lungs had a focally extensive and firm nodule or multiple nodules on the cranioventral region. On the cut surface, the nodules were greyish, granular and moist, resembling classical form of OPA. Microscopically, the lung sections showed unencapsulated nodules of neoplastic epithelial cells, from alveoli and bronchioles, forming papillary projections or acini. On the immunohistochemical analysis, JSRV capsid protein was demonstrated in the neoplastic epithelial cells. Genomic DNA was extracted from the lung tumor tissues and was subjected to U3-hn-PCR that further confirmed the presence of JSRV. The pathological findings in goats were similar to that of OPA affecting lungs of sheep and, to the author's knowledge, this is the first report of the disease in goats with immunohistochemical and PCR confirmation of JSRV.

Key words: goat, JSRV, lung, OPA, pathology.

Introduction

Ovine pulmonary adenocarcinoma (OPA), a contagious lung tumor in sheep is caused by an exogenous Jaagsiekte Sheep Retrovirus (JSRV) and is rarely reported in goats. It was described in South Africa during the 19th century and was called Jaagsiekte or Sheep Pulmonary Adenomatosis (SPA). Later on, various pathological and molecular studies, including sequencing of JSRV genome, were carried out in sheep (3, 9, 14, 21). Even though OPA is very common in sheep, its occurrence in goats is rare and most of the available reports are from India (4, 16, 19) and a few from other countries (1, 17). Experimental transmission of OPA to goats was carried out previously (18, 20). Renal and cardiac metastases of Jaagsiekte-like tumor was noticed in a goat in Saudi Arabia (1) and a histopathological study of naturally occurring OPA in a native goat was reported in Iran (17). Recently host species barriers to JSRV transmission and replication were studied in goats (6). The present paper describes the pathological findings and immunohistochemical and PCR diagnosis of two natural cases of ovine pulmonary adenocarcinoma in goats.

Case report

Two goat lung samples were collected from a local abattoir during routine meat inspection. Paraffin embedded tissue sections were prepared from both the lung samples and stained by H&E stain. Further, the lung samples were subjected to immunohistochemical and PCR analysis for JSRV. Immunostaining for JSRV capsid protein (CA) was carried out on paraffin sections using specific primary antibody (courtesy: Prof. M. Palmarini)

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and super-sensitive polymer-HRP detection kit (Bio Genex, USA). Genomic DNA from lung samples was extracted using DNA extraction kit (Qiagen) as per manufacturer's protocol and U3-hn-PCR was carried out in two rounds using primers P-1 (TGGGAGCTCTTTGGCAAAAGCC), P-III (CACCGGATTTTTACACAATCACCGG) and P-VI (TGATATTTCTGTGAAGCAGTGCC) specific to U3 gene of JSRV (13). PCR was carried out as described previously (8) and the amplified products at each round of PCR were detected by electrophoresis and visualized by UV gel documentation system.

Grossly, the two goat lung samples revealed locally extensive and firm nodules in the cranioventral part along with smaller nodules of 0.5-1.0 cm in diameter in the surrounding area. On the cut surface, the nodules were greyish, granular and moist, with oozing of mucus fluid (Fig. 1 and 2). The mediastinal lymph nodes were slightly enlarged and edematous.

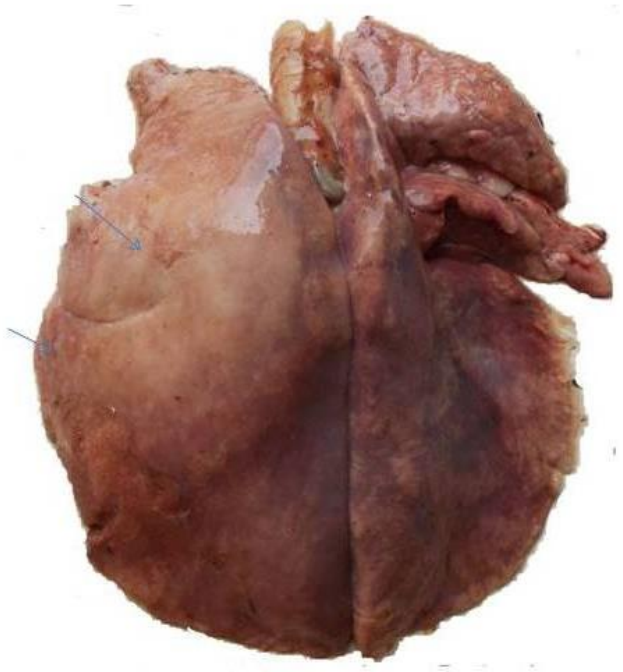


Figure 1. Goat - ovine pulmonary adenocarcinoma - Left lung (arrows) showing diffuse firm nodular consolidation in cranioventral part along with small tumor nodules in the surrounding area.

Histologically, the lung sections revealed variably sized, unencapsulated, nodules of proliferation of neoplastic alveolar and bronchiolar epithelial cells forming papillary projections into the lumen (Fig. 3). The projections caused alveolar dilatation and were supported by thin connective tissue stalk. Additionally, neoplastic epithelial cells were also arranged in acinar pattern and were lined by either single or multiple layers of epithelial cells. In scattered areas, the bronchiolar papillary projections filled the lumen and were continuous with the terminal bronchiolar epithelium. The neoplastic cells were well differentiated, cuboidal or columnar with round to oval nuclei and occasionally vacuolated cytoplasm. There was increased mitotic activity. A few neutrophils and macrophages infiltrated the surrounding alveoli along with mild infiltration of mononuclear cells and fibroblasts in the stroma. No metastasis was evident in the mediastinal lymph nodes.



Figure 2. Goat - ovine pulmonary adenocarcinoma - Cut section of lung with greyish, granular and moist surface.

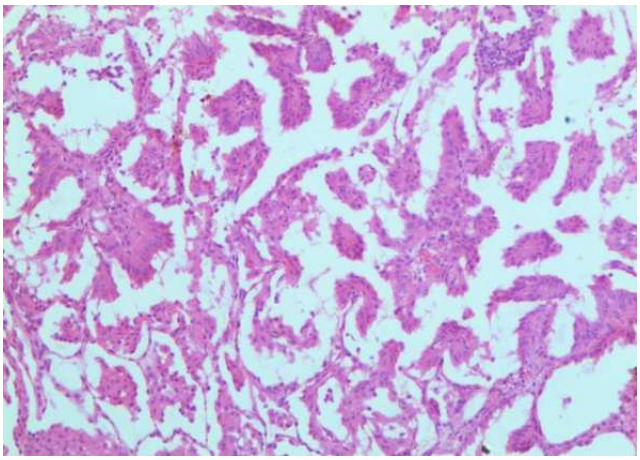


Figure 3. Goat - ovine pulmonary adenocarcinoma - Lung: Note proliferation of alveolar epithelium with papillary projections. HE, obj. 10x.

On immunostaining, JSRV capsid protein positive immunoreaction was indicated by the presence of intracytoplasmic fine brown granular staining in the proliferated alveolar or bronchiolar epithelial cells (Fig. 4). Positive staining was also noticed in a few alveolar macrophages and sloughed epithelial cells.

On U3-hn-PCR, an expected size of amplified product of 176 bp was found in first round and 133 bp in the second round. There was no amplification in the controls.

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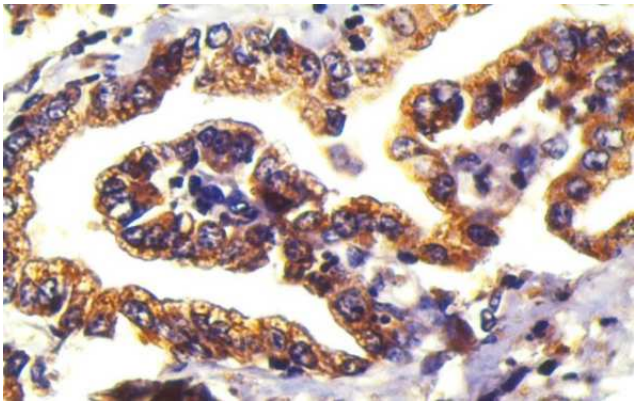


Figure 4. Goat - ovine pulmonary adenocarcinoma - Lung: IHC- JSRV- capsid protein: Note intracytoplasmic brown granular staining in the neoplastic alveolar epithelial cells, obj.40x.

Discussion

There are a few reports of OPA in goats, though its incidence is low and ranges from 0.72 to 4.4% (4, 19). The earlier reports are based on the findings of gross and histopathological lesions in abattoir or *postmortem* lung samples. In Saudi Arabia, renal and cardiac metastases of Jaagsiekte like tumor (1) and coexistence of nasal and pulmonary retrovirus induced adenocarcinomas in goats (2) were recorded.

The gross lesions of lungs are similar to classical form of OPA in sheep with moist and greyish surfaces (7, 9). The terms 'classical' and 'atypical' SPA were used to describe the lesions of lungs in SPA (7, 9). Grossly, the classical form is characterized by firm, greyish to purple neoplastic nodules that, invariably, are moist and contain large amounts of fluid filling the air ways in the cranioventral portion of the lungs. The atypical form is characterized by solitary nodules in the diaphragmatic lobe and several smaller nodules in the other lobes. In naturally occurring OPA of goats in Iran, the lungs were heavy and moist and the affected areas were solid and light grey. On the cut surface, numerous small, slightly elevated, white to grey nodules were described (17). In another case of pulmonary retrovirus induced adenocarcinomas in a goat, multiple purplish, circular or irregular, tumor nodules of different sizes were present in the lungs, with metastases to the liver and left kidney (2).

The histological findings are consistent with classical form of OPA in sheep (5, 7, 9, 11) and goats (17). The histopathological findings in both forms of OPA in sheep are almost similar, but there is increased infiltration of mononuclear cells and connective tissue in the tumor stroma in the atypical form (7, 9).

In the present study, immunostaining was performed for JSRV capsid protein to determine the presence of viral protein in the proliferated epithelial cells of lung. Positive immunoreaction was indicated by the presence of intracytoplasmic fine brown granular material in the proliferated alveolar or bronchiolar epithelial cells. These findings were similar with those observed in OPA lung sections of sheep in immunohistochemical studies for JSRV capsid protein (5, 9, 12, 15).

In the present study, U3-hn-PCR was conducted to confirm the presence of JSRV proviral DNA in the two goat lung samples and the PCR results were similar as observed in sheep OPA (9, 10, 13). Sheep and goats contain copies of endogenous retroviruses in their genome which closely resemble JSRV. There are sequence differences in U3 region of LTR of exogenous JSRV that can differentiate both exogenous and endogenous JSRV (3) and the primers designed from that area can be used for diagnostic confirmation of OPA.

Overall, the gross and histopathological lesions in the two natural cases of goats are similar to those of OPA affected lungs of sheep and goats. However, morphological differences were noticed between lesions in lambs and goat kids, on experimental inoculation of JSRV (6). In inoculated lambs, the tumors were diffusely infiltrative with marked consolidation in cranioventral lung lobes, while, in goat kids, multifocal well-circumscribed, pale and firm nodules were described. Histological examination of sections from infected goat kids showed large, well-circumscribed nodules of brochioloalveolar carcinoma, while, in infected lambs, the tumor nodules consisted of proliferated epithelial cells with acinar or papillary pattern with infiltration to the adjacent alveoli. Thus, in infected goats, JSRV induces neoplastic lesions that appear to be circumscribed compared to those observed in infected sheep. In addition, the results of this study suggested that goat lung alveolar proliferating cells, targeted for viral carcinogenesis, are not permissive to viral replication, but can be transformed by JSRV and the goat cells are somewhat restrictive for one or more steps of the JSRV replication cycle.

These two naturally infected goat lung samples revealed lesions similar to those of naturally OPA affected sheep. In sheep, experimentally induced OPA resulted in a large number of small widely disseminated tumor nodules. Large monocentric coalescing masses, involving one or more lung lobes were found in natural cases of OPA (15). The morphological differences in natural and experimental OPA cases in sheep and goats might be due to the differences in the nature of disease, dose of inoculation, course of the disease and age of the animals. However, further molecular studies are required to know the factors that are responsible for species differences in susceptibility and low incidence of OPA in goats when compared to sheep.

Conclusion

Two natural cases of OPA in goats were diagnosed based on gross and histopathology findings, immunohistochemistry and molecular detection of JSRV. To the author's knowledge this is the first report of natural

cases of OPA in goats with immunohistochemical and PCR confirmation of JSRV.

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