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Case Report

Non jaagsiekte retrovirus associated adenosquamous lung carcinoma in a goat

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Abstract

An 11-year-old pet goat presented a clinical history of acute respiratory distress with ultrasound diagnosis of wide lung injury. The animal was euthanized due to welfare reasons. At necropsy, it was found pleural effusion and adhesion on the right cranioventral thoracic region. The right cranial and middle lung lobes were firm and light gray with a neocavity containing purulent exudate. From the middle lobe, there was a nodular proliferation occupying alveolar spaces, densely cellular and composed by cuboid-columnar epithelial cells arranged in papillae and acini (60%), as well as polygonal cells arranged in nests with squamous differentiation (40%). Marked pleomorphism, anisocytosis and anisocariosis were also noted. A total of 39 mitosis figures for ten fields at 400x magnification were counted. Lung samples were negative for jaagsiekte retrovirus (JSRV) by PCR. Immunostaining for TTF1 and P53 occurred in zones of adenomatous and squamous differentiation, respectively. In MIB-1, 14% (82/594) of immunolabeled cells were observed in the squamous component. In conclusion, the histopathological and immunohistochemical characteristics confirmed the diagnosis of a pulmonary adenosquamous carcinoma, without JSRV involvement, in goat species.

Key words: retrovirus; adenomatous; squamous; lung neoplasm; small ruminant

Introduction

In humans, lung cancer is the most diagnosed neoplasm in the world, with carcinomas being the most prevalent (2). Adenosquamous carcinoma is a less common and aggressive form of non-small cell lung carcinoma (13).

In veterinary medicine, and especially in small ruminants, reports on primary lung cancers are scarce. In these species, most cases are associated with the jaagsiekte retrovirus (JSRV) that causes ovine lung adenocarcinoma (OPA). The OPA is endemic in several countries in Europe, Africa, Asia, and the Americas (14). Therefore, the investigation of JSRV in cases of lung cancer in such species is important to differentiate individual cases from those with infectious potential. In this sense, it is describe a rare case of adenosquamous lung carcinoma in goats.

Case report

An 11-year-old pet goat showed a clinical history of respiratory distress, excessive salivation and inappetence. Clinical examination revealed: tachycardia, tachypnea, ruminal atony (only borborygmus), moderate dehydration (10%), drooling and pale conjunctival mucosa. Respiratory examination revealed: dilated nostrils, restrictive mixed dyspnea with increased abdominal component, auscultation

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of fluid in the trachea and fine crackles in the mid-ventral fields of the lung, increase in the lung auscultation field up to the 11th intercostal space (ICS), productive and strong cough (when stimulated). Ultrasound scan showed: 1) an intense amount of anechoic fluid compatible with bilateral pleural effusion (12 cm deep) (Fig. 1A); 2) segments of consolidated lung in the right 6th ICS (Fig. 1B); 3) pyothorax and pleural abscess in the right 5th ICS (Fig. 1C & 1D); 4) heart unchanged, but displaced upwards on the left side due to great amount of fluid; 5) increased lung field up to the 11th ICS; 6) mid-dorsal lung field unchanged. The animal underwent to ultrasound-guided puncture in the ventral right 6th ICS using a needle (18G) for relief of clinical signs. A total of 1.2 liters of translucent, straw-colored fluid that clotted in the serum tube were aspirated and the animal showed an immediate improvement in the breathing pattern. A blood sample was also collected for whole blood count. The animal was treated with intravenous fluid therapy, non-steroidal anti-inflammatory drugs (flunixin meglumine; 2.2 mg/kg) and antibiotics (30.000 IU/kg of benzathine penicillin; 2.5 mg/kg of enrofloxacin). The whole blood count showed platelet aggregates, leukocytosis with discrete regenerative left shift in neutrophils (21.287 cells/µL), absolute neutrophilia (20.010 cells/µL), lymphopenia (426 cells/µL), monocytosis (639 cells/µL) and eosinopenia (0 cells/µL). After 48 hours, the animal returned to show a severe respiratory distress and due to the wide lung injury seen by ultrasound and advanced age, it was indicated for euthanasia.

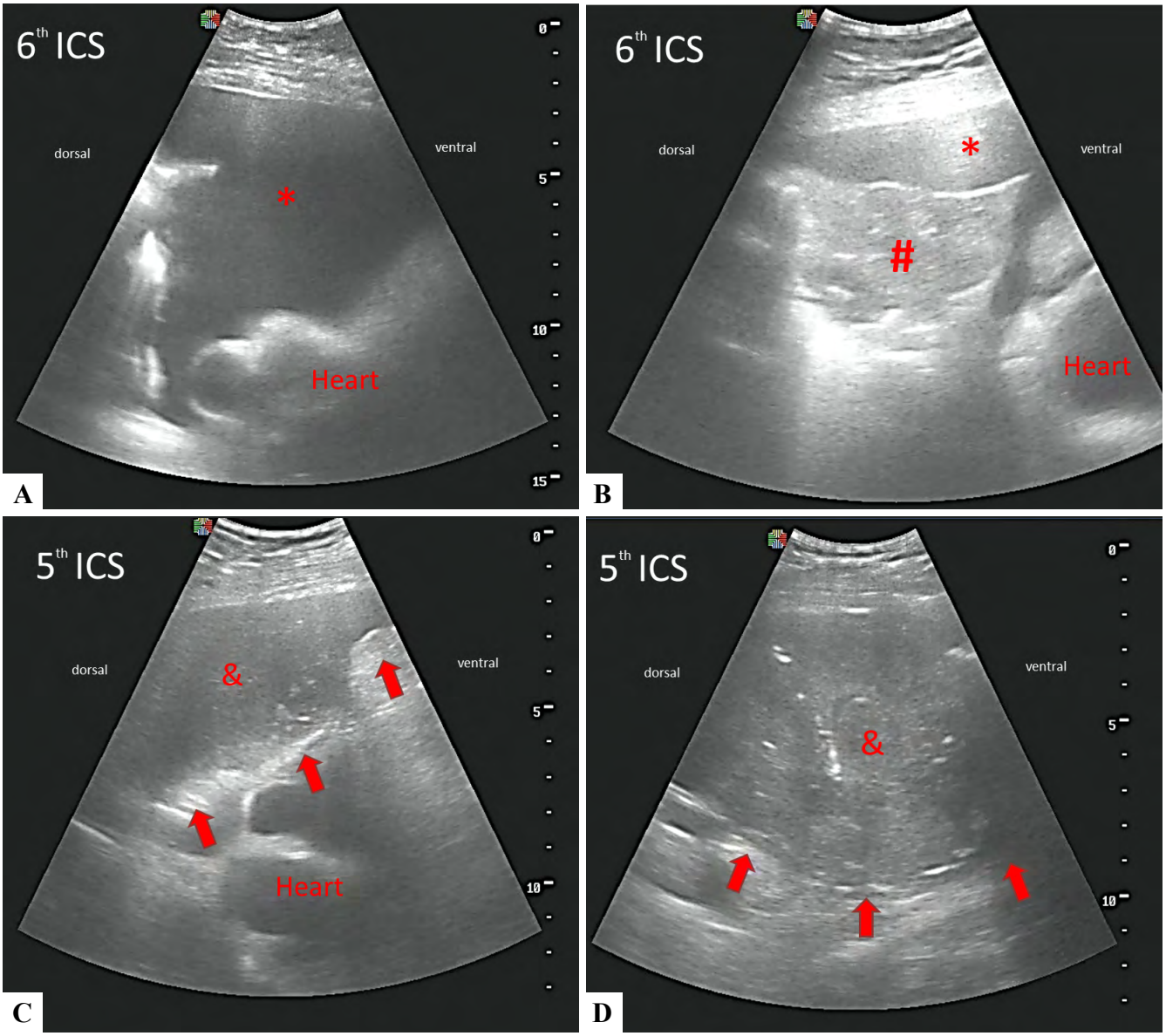

Figure 1. Mode-B ultrasound image of caprine lung between 5-6 th ICS. **A.** Intense amount of anechoic fluid compatible with pleural effusion (*). **B.** Pulmonary consolidation segment (#). **C.** Suggestive image of an encapsulated abscess (&). **D.** Suggestive image of pyothorax. 7.5 MhZ transducer and 10-15cm depth

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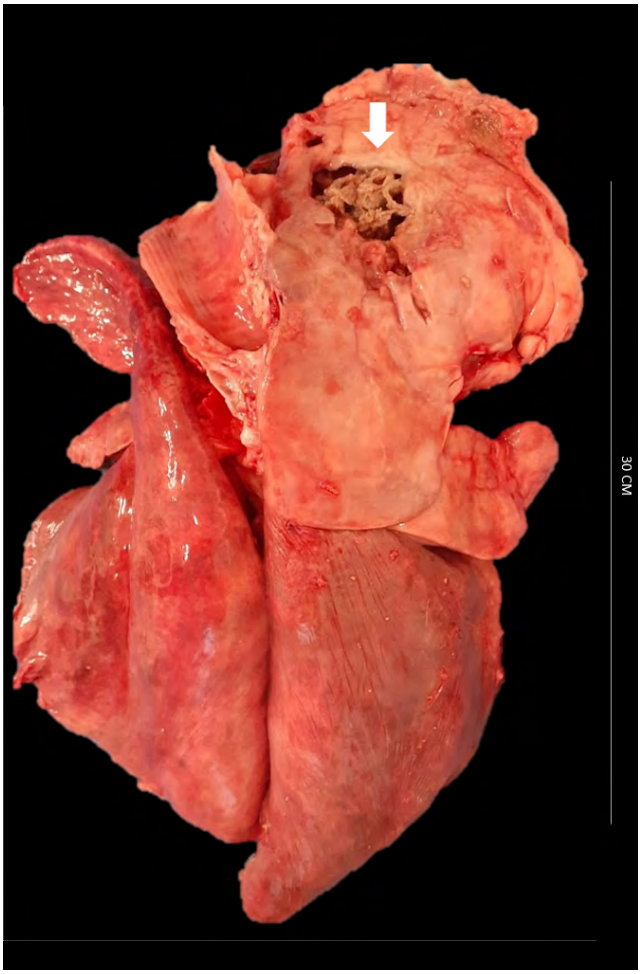

Figure 2. Goat, mixed breed, pulmonary adenosquamous carcinoma. Pulmonary lobes, cranial and middle, right firm, and light gray. Neocavity (11x11x8 cm) in the right cranial lobe with purulent exudate (white arrow) suggestive of abscess and chronic suppurative bronchopneumonia. Pulmonary adenosquamous carcinoma in the right middle lobe. Bar: 30cm.

At necropsy, pleural effusion and adhesion on the right cranioventral thoracic region were observed. The right cranial and middle lung lobes were firm and light gray. Also, the right cranial lobe had a neocavity formation (11x11x8 cm) with purulent exudate suggestive of abscess and chronic suppurative bronchopneumonia (Fig. 2). Tissues fragments were collected and fixed in 10% formalin during 48 hours. Tissue samples were processed and stained with HE. Samples of the purulent content were collected for bacterial culture and antibiogram being detected *Escherichia coli*, *Staphylococcus pseudintermedius* e *Klebsiella* sp.

In the histological sections from the middle lung lobe were observed nodular proliferation, densely cellular, well demarcated, surrounded by fibrous connective tissue, infiltrative, of epithelial cells, cuboid-columnar and polygonal cells, arranged in papillae and acini (60%) (Fig. 3) and others arranged in nests with differentiation into squamous cells (40%) (Fig. 4), occupying alveolar spaces, supported by preexisting stroma. The cells show marked pleomorphism, anisocytosis (large and eosinophilic cytoplasm, occasionally with a ciliated apical border) and anisokaryosis (oval nuclei, in different locations - central, paracentral, and peripheral, karyomegaly and binucleations), as well as a coarse chromatin, every so often evident nucleolus. A total of 39 typical and atypical mitosis in ten fields, at 400x magnification, were counted. There was also an accentuated mixed inflammatory infiltrate, with a necrotic center, degenerated neutrophils, surrounded by macrophages, multinucleated giant cells, lymphocytes, and plasma cells, and surrounded by a fibrous capsule in focal areas. In the right cranial lobe, there was moderate mixed intra-alveolar and multifocal peribronchial inflammatory infiltrate, marked focal fibrosis, moderate edema, and multifocal emphysema.

Immunohistochemistry was used to confirm the cellular origin of the neoplasm using antibodies TTF1, P53

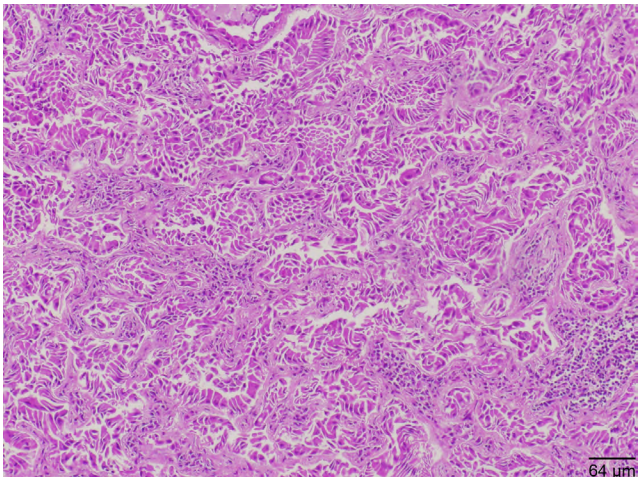

Figure 3. Goat, mixed breed, pulmonary adenosquamous carcinoma. Cuboid-columnar epithelial cells arranged in papillae and acini comprising 60% of the neoplasm. HE. Bar, 64 µm.

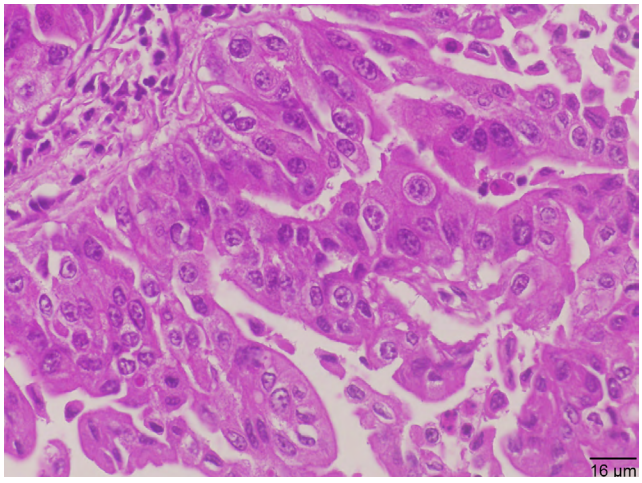

Figure 4. Goat, mixed breed, pulmonary adenosquamous carcinoma. Polygonal epithelial cells arranged in nests in squamous cells differentiation comprising 40% of the neoplasm. HE. Bar, 64 µm.

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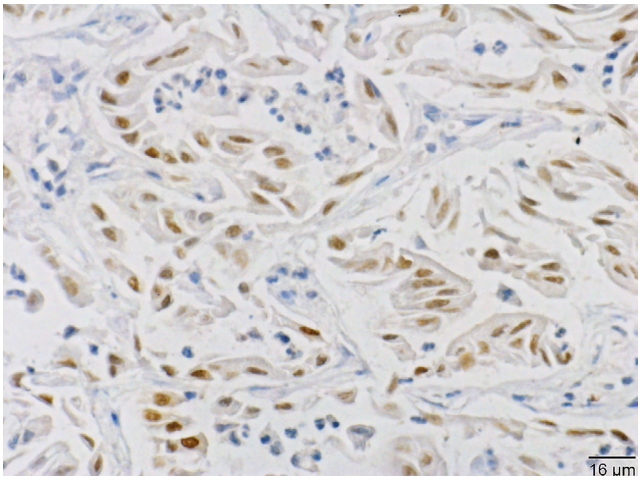

Figure 5. Goat, mixed breed, pulmonary adenosquamous carcinoma. Positive immunostaining for thyroid transcription factor-1 (TTF1) in areas of adenomatous differentiation. IHC. Bar, 16 µm.

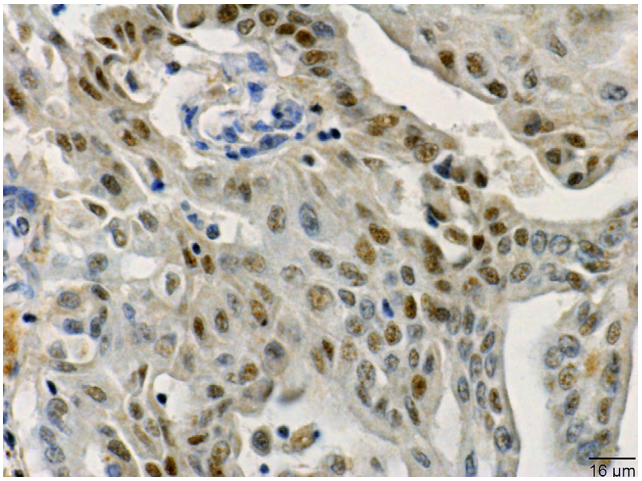

Figure 6. Goat, mixed breed, pulmonary adenosquamous carcinoma. P53 immunoreactivity in areas of squamous differentiation. IHC. Bar, 16 µm.

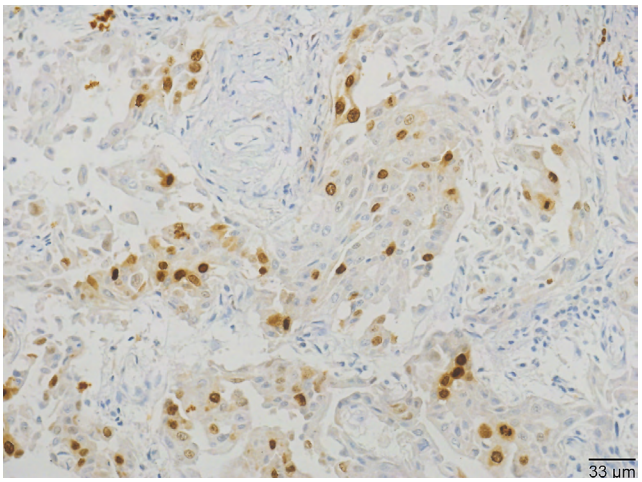

Figure 7. Goat, adenosquamous lung carcinoma. MIB-1 revealed focal areas of immunomarked cells in the squamous component.IHC. Bar, 33 µm.

and MIB-1. For immunohistochemistry, deparaffinized 3µm sections of formalin-fixed paraffin-embedded (FFPE) tissues silanized slides were submitted to antigen retrieval (citric acid solution 10mM pH 6.0 in a pressure cooker, for 3 min, at 120º C). Endogenous peroxidase was blocked with 6% hydrogen peroxide for 30 min followed by overnight incubation with monoclonal mouse anti-p53 at 1:100 (clone DO7, Dako Carpinteria, CA, USA), monoclonal mouse anti-Ki-67 at 1:200 (MIB-1, Dako Carpinteria, CA, USA) and monoclonal mouse Anti- thyroid transcription factor at 1:100 (TTF-1, clone SPT24, Novocastra-Leica, Nussloch, Germany). Signal was amplified by Novolink polymer detection system (Leica Biosystems, Newcastle, UK) for 60 min, and visualization was achieved by diaminobenzidine (Sigma D5637, MO, USA) chromogen for 3 min. The samples were counterstained with Harris Hematoxylin for 20 seconds followed by dehydration and slide preparation with synthetic resin. Tissue sections in which the primary antibodies were replaced by non-immune serum of those species where antibodies were raised served as negative controls. All antibodies used for the immunohistochemistry study were designed for humans' proteins, but the cross-reactivity in goats was confirmed through positive internal controls.

Nuclear immunostaining for TTF1 occurred in areas of adenomatous differentiation (Fig. 5), while P53 was positive in areas of squamous differentiation (Fig. 6). MIB-1 revealed focal areas, 14% (82/594) of immunostained cells in the squamous component (Fig. 7).

PCR was performed on lung samples from paraffin blocks for JSRV identification. Two 9-µm sections were conditioned in 1.5mL sterile microtubes. Dewaxing and lysis were conducted as described by (4) followed by RNA extraction using the Biogene DNA/RNA viral (Bioclin-Quibasa, Brazil) according to manufacturer's instructions. The hemi-nested PCR for JSRV, based on primers PI JSRV 5'-TGGGAGCTCTTTGGCAAAAGCC-3', PIII JSRV 5'-CACCGGATTTTTACACAATCACCGG-3' and PVI JSRV 5'-TGATATTTCTGTGAAGCAGTGCC-3' that amplified the U3 region of the virus and for the housekeeping gene 18S RNA wit primers 5'- CAGCCACCCGAGATTGAGCA -3 and 5'-TAGTAGCGACGGGCGGTGTG-3' in order to confirm the integrity of the DNA, were performed with GoTaq® Probe 1-Step RT-qPCR System (Promega, WI, USA). Thermal cycler conditions were 15 min at 45°C, 5 min at 95°C; 40 cycles of 95°C for 30 sec, 59°C (first round) and 57°C (second round) for 60 sec, and 72°C for 30 sec; and a final extension step at 72°C for 10 min. PCR products stained with GelRed (Biotium, CA, USA) were electrophoresed in 2% agarose gel and visualized under UV illumination (Uvitec, Cambridge, UK). PCR was negative for JSRV and detectable for endogenous control of the reaction, showing that the DNA was extracted properly.

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Discussion

To the authors' knowledge, this is a rare report of primary lung cancer not related to JSRV, in goat species. Other lung tumor of bronchioloalveolar origin was reported in a senile goat and confirmed by immunohistochemistry the alveolar cell origin type II by marking tumor cells for surfactant proteins C and B (12). In the present report, there was positivity for the TTF1 antibody, evidencing the adenomatous areas of the neoplasm. Also, a retrospective study on 102 tumors in 100 goats only pointed to the lung as a site of metastasis by other neoplasms (9).

The thoracic transcutaneous ultrasonography assisted in the diagnosis and extension of lung injury, in addition to determining the patient's poor prognosis. This imaging technique has also been investigated for the detection of in vivo lesions of OPA and has been proposed for its eradication in affected flocks (5). However, this methodology does not fully cover the lung field and does not guarantee the detection of tumors smaller than 2 cm. In the present report, the ultrasound image obtained was compatible with a suppurative pleuropneumonia, and it was not possible to verify the primary neoplasm as reported previously (5).

The OPA is the main lung tumor described and diagnosed in sheep, associated with JSRV, and which occurs less frequently in goats (11). Therefore, it is an important differential diagnosis in cases of lung cancer in small ruminants (12). In this study, lung samples were negative for JSRV. Although the PCR was performed in paraffin samples, which can reduce the sensitivity of the technique, the integrity of the genetic material in the tissues was confirmed by the amplification of the endogenous gene. There are few reports of the disease with confirmed viral involvement in Latin America (3,10). In Brazil, there are only two cases diagnosed in small ruminants with a histopathological description, but no evidence of viral involvement (1,6). Thus, continuous epidemiological surveillance programs are important to monitor the viral spreading and this infectious disease in the American continent.

In humans, adenosquamous lung tumors is a distinct category classified by WHO that shows squamous and glandular components with each comprising at least 10% of the tumor (15). Squamous differentiation into adenosquamous tumor consists of groups and nests of polygonal cells with eosinophilic cytoplasm and a squamous appearance (13). The neoplastic cells in the present study exhibited two patterns, cuboidal-columnar arranged in papillae and acini and polygonal arranged in nests with areas of central keratinization, which suggested adenosquamous carcinoma (ASC).

Immunostaining for TTF1 occurred in multifocal areas of adenomatous neoplastic proliferation. For the diagnosis of ASC, the co-expression of ADC (adenocarcinoma) and SCC (squamous cell carcinoma) markers in the same tumor cells is not necessary, as ASC consists of two different types of tumor, individual tumor cells do not show bidirectional differentiation (13). The expression of P53 was evidenced by IHC in multifocal areas of the neoplasm in the squamous component. Mutation in P53 leads to unregulated cell proliferation. In humans, this protein, when overexpressed, is a marker of more aggressive tumors and worse prognosis (4). In squamous cell carcinoma of the bovine horn, an association between mutation in P53 and extension of tumor malignancy was detected (8). This protein acts to suppress and inhibit tumor growth (4), being an important marker in neoplasms with squamous differentiation. MIB-1 is a nuclear protein expressed in all cell cycle phases (8). This marker showed areas of cell proliferation in the squamous component. In conclusion, the histopathological and immunohistochemical characteristics confirmed the diagnosis of pulmonary adenosquamous carcinoma cancer not related to JSRV in goat species.

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