

Primary Pulmonary Adenocarcinoma in a Cow

Adony Querubino de Andrade Neto¹, Rodolfo José Cavalcanti Souto², Jobson Filipe de Paula Cajueiro²,
Carla Lopes de Mendonça², David Driemeier³, José Claudio de Almeida Souza⁴,
Fábio de Souza Mendonça⁵ & José Augusto Bastos Afonso²

ABSTRACT

Background: Primary neoplasms of the respiratory tract are rare in cattle, and they present with nonspecific clinical signs and are usually found at post-mortem. Pulmonary adenocarcinoma of the acinar type is uncommon, and information about this neoplasm in cattle is scarce. This paper aims to describe the clinical, laboratory, and pathological findings in a cow with this neoplasm.

Case: A 10-year-old, adult, mixed-breed Holstein cow weighing 300 kg was referred to the Garanhuns Cattle Clinic of the Campus of Federal Rural University of Pernambuco, Brazil, with a history of decreased appetite, tiredness, weight loss, and difficulty in breathing for two months. The animal had been treated at the farm of origin with enrofloxacin, florfenicol, and flunixin meglumine. The animal's appetite improved, but no improvement in the respiratory symptoms was observed. On examination at our center, the cow was in an orthopedic position, with neck extension and elbow abduction; and it remained in a recumbent position. The cow had neutrophilia, normochromic normocytic anemia, and hyperfibrinogemia. The body condition score (BCS) was 1 (BCS ranges from 1 to 5), and the cow had moderate enophthalmia, serous secretions in nostrils, tachycardia, and tachypnea. It also had increased breathing intensity; increased breath sounds in the cranial regions of both lungs; areas of reduced breath sounds in the medial portions of the lungs; intermittent wheezing in the cranial region of the left lung medially and in the cranial region of the right lung medially; intermittent crepitations in the cranial region of the right lung medially; reduced thoracic expansion; and expiratory dyspnea. Pulmonary ultrasonography revealed hyperechogenic multifocal structures in both lungs. In view of the severe clinical condition and unfavorable prognosis, the owner opted for euthanasia. Necropsy revealed that there was a significant amount of yellow fluid in the thoracic cavity, and the cut surfaces of the lungs were covered with fibrin and pleural adhesions. The lungs had irregular surfaces with multifocal nodules of various sizes and firm consistency. The texture of the cut nodules was solid, and the nodules had a yellowish color. Histologically, the pulmonary nodules were composed of neoplastic cells. They were pleomorphic, infiltrative epithelial cells with little cytoplasm; large and spherical nuclei with abundant euchromatin; occasional anisokaryosis; visible nucleolus; and numerous mitotic figures. Results of anti-cytokeratin immunohistochemistry were strongly positive for cytokeratin in the lungs and lymph nodes.

Discussion: The diagnosis of acinar pulmonary adenocarcinoma was based on clinical, laboratory, histopathological, and immunohistochemical findings. Clinical signs and lung ultrasound suggested a severe respiratory disease. Pulmonary tumors are usually solid, and they show areas of central necrosis that resemble granulomas. However, the histopathological findings in the current case were compatible with a primary pulmonary neoplasm. Primary pulmonary neoplasms, although rare, should be considered in the differential diagnosis of weight loss, cachexia, and respiratory symptoms in adult cattle. The morphological pattern and the strong immunoreactivity on immunohistochemical examination were conclusive for a diagnosis of a primary pulmonary adenocarcinoma of the acinar type because these tumors specifically contain cytokeratin.

Keywords: respiratory disease, histopathology, cytokeratin, neoplasia, lung.

Descritores: doença respiratória, histopatologia, citoqueratina, neoplasia, pulmão.

DOI: 10.22456/1679-9216.90277

Received: 4 November 2018

Accepted: 20 February 2019

Published: 14 March 2019

¹Programa de Pós-graduação em Ciência Veterinária, ²Clínica de Bovinos de Garanhuns, ⁴Campus Unidade Acadêmica de Garanhuns & ⁵Laboratório de Diagnóstico Animal, Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco (UFRPE), Recife, PE, Brazil. ³Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária (UFRGS), Porto Alegre, RS, Brazil. CORRESPONDENCE: A.Q. Andrade Neto [adony_neto@hotmail.com - Tel.: +55 (73) 9.9158.8662]. Clínica de Bovinos de Garanhuns, Campus Unidade Acadêmica de Garanhuns. Av. Bom Pastor, s/n, Caixa Postal 152. Bairro Boa Vista. CEP 55292-272 Garanhuns, PE, Brazil.

INTRODUCTION

Primary neoplasms of the respiratory tract are rare in cattle, and they present with nonspecific clinical signs and are usually found a post-mortem [6,12]. Although the lungs are a frequent site of metastasis, primary pulmonary neoplasms have a low incidence when compared to primary neoplasms of other organs [2].

In cattle, pulmonary tumors of epithelial origin can originate from bronchial epithelial cells, goblet cells, or alveolar-lining cells. These neoplasms can be histologically classified into four types: papillary, acinar, solid, and mixed. The papillary and the acinar type are more frequent in animals [3]. An adenocarcinoma is a malignant tumor originating from pulmonary epithelial cells [4]; it is the most frequently reported type of the pulmonary neoplasms in cattle [8].

The first detailed report of a pulmonary adenocarcinoma in a cow in Brazil was by Viott *et al.* [16], and it was subsequently followed by reports by Sousa *et al.* [12] and Santarosa *et al.* [10]. Other cases have been reported in retrospective studies of neoplasms diagnosed during necropsies performed in veterinary hospitals [6,9].

However, due to the rare occurrence of pulmonary adenocarcinomas in this species, little is known about the clinical, laboratory, and pathological aspects of this disease. Therefore, the aim of this study was to report the occurrence of a primary pulmonary adenocarcinoma and to describe the clinical, laboratory, and pathological findings in a cow with this neoplasm.

CASE

A 10-year-old, adult, mixed-breed Holstein cow weighing 300 kg was referred to the Garanhuns Cattle Clinic of the Campus of Federal Rural University of Pernambuco with a 2-month history of decreased appetite, tiredness, weight loss, and difficulty in breathing. At the farm of origin, the animal had been treated with enrofloxacin [Enrofloxacin¹ 10%, 5 mg kg⁻¹, subcutaneously, for 10 days]; florfenicol [Nuflor², 20 mg kg⁻¹, intramuscular, 3 times every 48 h]; and flunixin meglumine [Desflan³, 2.2 mg kg⁻¹, intramuscular, for 5 days]. The animal's appetite improved, but no improvement in the respiratory symptoms was observed. On examination, the cow was apathetic, and it remained in the sternal decubitus position, rising only with assistance. When standing, the animal assumed an orthopedic position, with neck extension and elbow abduction. The body condition score (BCS) was 1 [BCS ranges from 1 to 5]

(Figure 1A). The cow had moderate enophthalmia, serous secretion in both nostrils, tachycardia (heart rate, 88 beats per minute), tachypnea (respiratory rate, 48 breaths per minute). It also had increased breathing intensity; increased breath sounds in the cranial regions of both lungs; areas of reduced breath sounds in the medial portions of the lungs; intermittent wheezing in the cranial region of the left lung medially and in the cranial region of the right lung medially; intermittent crepitations in the cranial region of the right lung medially; reduced thoracic expansion; and expiratory dyspnea.

Blood samples were collected by jugular venipuncture in Vacutainer siliconized tubes containing 10% EDTA (Vacuette[®])⁴ for measurements of blood count, total plasma protein (TPP), and plasma fibrinogen [5]. The complete blood count (CBC) revealed leukocytes were within the reference range; however, there was neutrophilia and normocytic normochromic anemia. The cow also had hyperfibrinogenemia.

Lung ultrasound [14] revealed multifocal hyperechogenic structures suggestive of a granulomatous inflammatory process in both lungs. Other alterations, such as dilatation of the caudal vena cava and a hydrothorax of moderate echogenicity, were observed (Figure 1B).

In view of the severe clinical condition and unfavorable prognosis, the owner opted for euthanasia [7]. Necropsy revealed there was a significant amount of yellow fluid in the thoracic cavity, and the cut surfaces of the lungs were covered with fibrin and pleural adhesions. The lungs had irregular surfaces with multifocal nodular formations of various sizes and firm consistency (Figure 1C). The texture of the cut nodules was solid, and the nodules had a yellowish color (Figure 1D). Several thrombi were observed in the pulmonary veins, and alveolar and interstitial emphysema were also observed. In the omentum, multifocal nodular structures smaller than 0.5 cm, similar to those observed in the lungs, were also noted. The capsular surface of the liver had areas of scarring fibrous retraction and adherence to the diaphragm. The hepatic portal vein was dilated and hardened due to thrombosis. Fragments of the pulmonary nodules and regional lymph nodes were collected, fixed in 10% formaldehyde, and routinely processed and stained with hematoxylin and eosin (HE) for histopathological evaluation.

Microscopic examination showed nodular lung lesions that consisted of pleomorphic infiltrative epithelial cells with little cytoplasm; large and spherical nuclei

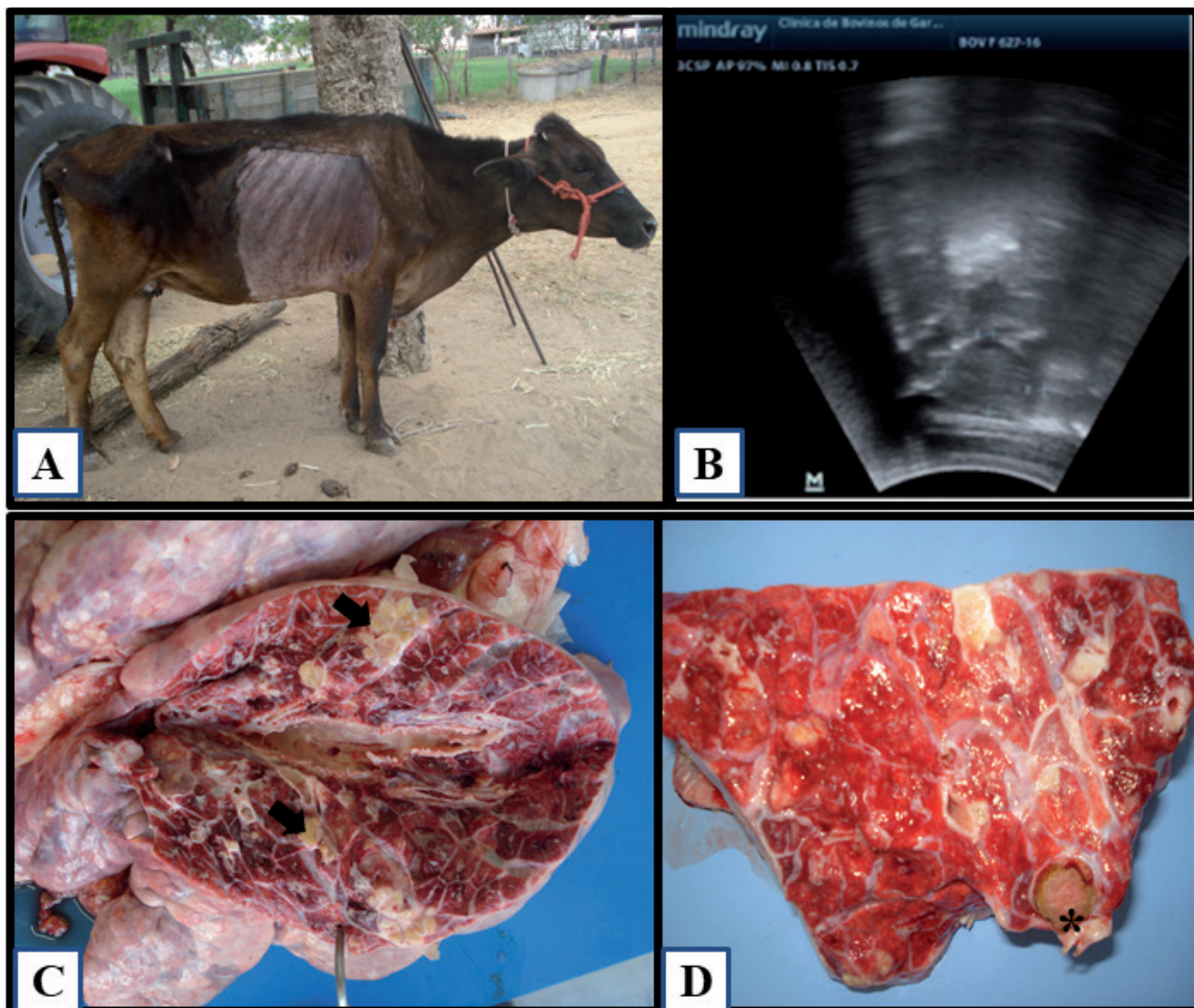


Figure 1. A- Cow was apathetic and cachectic, and it was in an orthopedic position, with neck extension and elbow abduction. B- Ultrasound image of the lung obtained at the 9th right intercostal space: the lung showed heterogeneous echogenicity in the parenchyma and hypoechoic areas of varying sizes; there were also circular, homogenous, non-capsulated hypoechoic structures suggestive of nodules; and there were no reverberation artifacts. C- Multifocal nodules in the lungs (arrows). D- A section of the lung with nodules that were solid and yellowish*.

with abundant euchromatin; occasional anisokaryosis; visible nucleoli; and several mitotic figures. The mitotic index was high, with approximately 3-4 mitotic cells per field of view [40x magnification] (Figure 2A). The neoplastic cells replacing the alveolar tissue showed an acinar arrangement with a maximum of two layers of cells. Necrotic cells and a mononuclear inflammatory infiltrate, consisting mainly of lymphocytes and macrophages, were frequently observed in these acini. The acini were surrounded by a fibrovascular tissue, which was composed of well-differentiated fibroblasts and delicate collagen fibers organized at the periphery; the acini occasionally contained interspersed neoplastic epithelial cells, some of which had binucleations.

In addition, edema, congestion, and diffuse areas of polymorphonuclear and mononuclear inflammatory infiltration were noted in the parenchyma (Figure 2B).

Results of immunohistochemical examination using anti-cytokeratin antibodies (Anti-Cytokeratin antibody [34BE12], ab191208®)⁵ were strongly positive for cytokeratin in the lungs (Figure 2C) and lymph nodes (Figure 2D); the staining was moderate in the cytoplasm, and it was diffuse in the neoplastic cells. Based on the clinical, anatomopathological, histopathological, and immunohistochemical findings, the tumor was diagnosed to be a pulmonary adenocarcinoma with acinar pattern presenting with metastases to the regional lymph nodes and greater omentum.

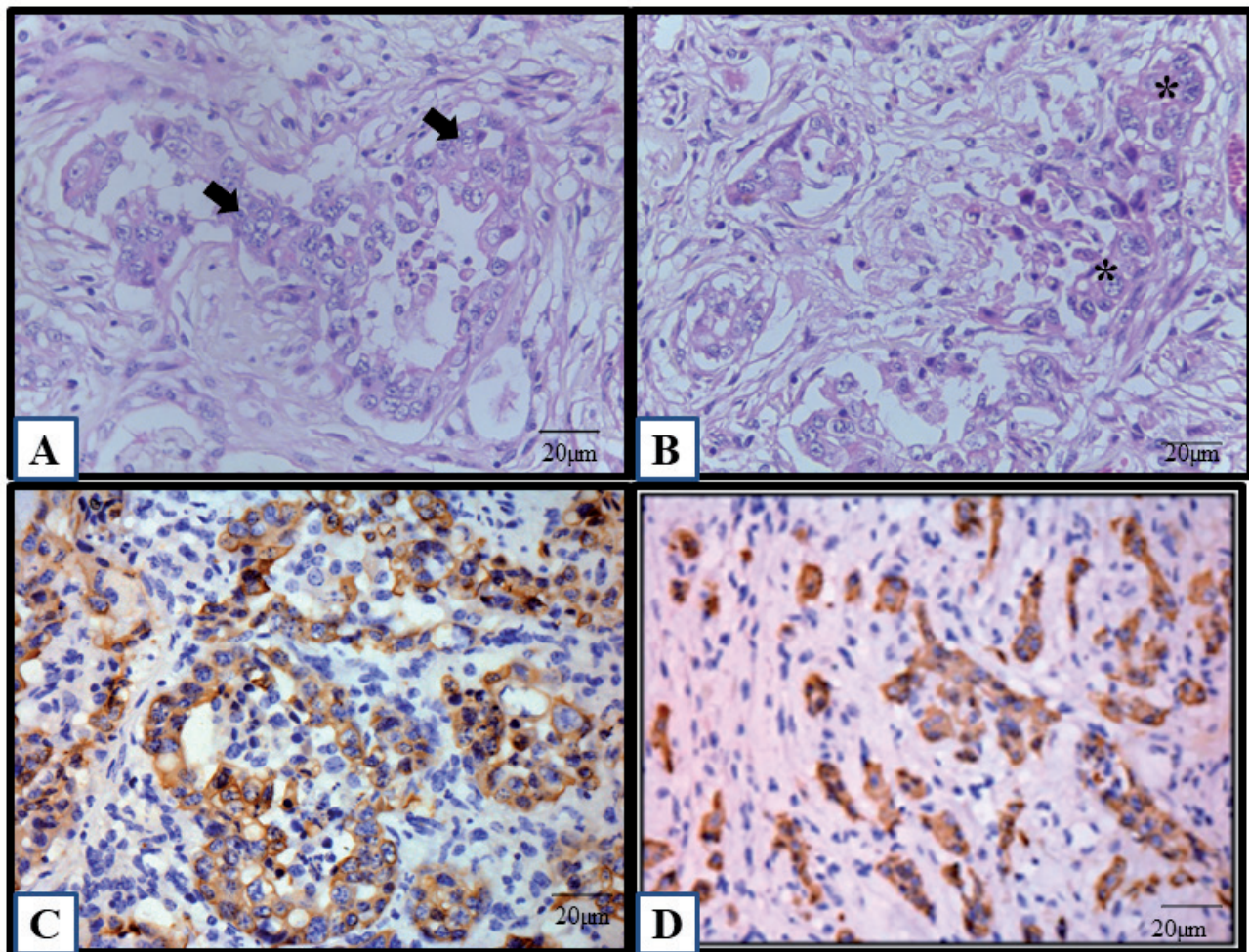


Figure 2. A- Section of the lung with neoplastic epithelial cells that were pleomorphic and infiltrative (arrow) [HE; magnification, 20x]. B- Neoplastic alveolar cells arranged in acini, necrotic areas, and a mononuclear inflammatory infiltrate that was mainly consisting of lymphocytes and macrophages* [HE; magnification, 20x]. C- Immunohistochemical examination of the lung was strongly positive for cytokeratin. [CK AE1/AE3; magnification, 20x]. D- Lymph node immunohistochemical examination was strongly positive for cytokeratin [CK, AE1/AE3; magnification, 20x].

DISCUSSION

In cattle, pulmonary adenocarcinoma must be differentiated from other neoplasms, especially mesotheliomas and primary neoplasms of the uterus and pancreas [6,13]; from lymphomas associated with infection with bovine retrovirus (enzootic bovine leukemia virus [BLV] infection); and from bovine tuberculosis associated with *Mycobacterium bovis* [3].

Adenocarcinomas have uniform basal nuclei that form acini, a characteristic that is absent in mesotheliomas, which have granular cells with irregular nuclei and primitive acini [12]. Pulmonary metastases of endometrial adenocarcinomas consist of large amounts of fibrous tissue, and they also contain highly pleomorphic glandular epithelial cells. Furthermore, macroscopic examination revealed nodules in the uterine tissue and the abdominal cavity [13]. Pancreatic

adenocarcinomas exhibit distinct cellular patterns, with cells with visible nuclei; the cells are generally uniform, are oval, and have dispersed chromatin. The cells can form poorly differentiated acini or tubules. In pancreatic adenocarcinomas, areas of hemorrhage, mineralization, or necrosis are also frequently present [8]. In the present study, metastases were found in the lymph nodes and greater omentum, and similar findings have been reported by Viott *et al.* [16] and Santarosa *et al.* [10].

Bovine leukosis is easily identified by histopathologic examination because it has a characteristic distribution of clusters of neoplastic lymphocytes in different organs, both lymphoid and non-lymphoid [11]. In bovine tuberculosis, the histological findings consist of multifocal lesions of caseous necrosis. The lesions contain homogeneous eosinophilic material, nuclei, and scarce nuclear remains, with varying degrees of multifo-

cal mineralization. Adjacent to necrotic area, there is a granulomatous inflammation with abundant epithelioid macrophages, multinucleated Langhans-like giant cells, and scarce-to-abundant lymphocytes [1].

Clinical signs and lung ultrasound suggested a severe respiratory disease. The necropsy findings of multifocal nodules with a solid texture and a yellowish color in the lungs suggested tuberculosis. Pulmonary tumors are usually solid, and they show areas of central necrosis that resemble granulomas [4]. However, the histopathological findings in the current case were compatible with a primary pulmonary neoplasm.

In humans, pulmonary adenocarcinoma has been associated with *Mycobacterium avium* complex infection [15]. However, there is no report showing this association in cattle; histological sections of animals with pulmonary adenocarcinoma that were stained with Ziehl-Neelsen were negative [3,10,12].

Primary pulmonary neoplasms, although rare, should be considered in the differential diagnosis of

weight loss, cachexia, and respiratory symptoms in adult cattle. The morphological pattern and the strong immunoreactivity on immunohistochemical examination were conclusive for a diagnosis of a primary pulmonary adenocarcinoma of the acinar type because these tumors specifically contain cytokeratin [4]. Tumors of this type are frequently associated with metastases, making the prognosis unfavorable. Therefore, they often lead to animals' deaths [8].

MANUFACTURERS

¹Tortuga Saúde Animal. São Paulo, SP, Brazil.

²Intervet Schering-Plough MSD Saúde Animal. São Paulo, SP, Brazil.

³Ourofino Saúde Animal. Cravinhos, SP, Brazil.

⁴Greiner Bio-One Produtos Médicos Hospitalares Ltda. Americana, SP, Brazil.

⁵Abcam - Immunohistochemistry (IHC) kits and reagents. Cambridge, UK.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

- 1 **Andreazza D., Boos G.S., Boabaid F.M., Wouters A.T.B., Wouters F., Souza S.O., Menegat M.B. & Driemeier D. 2015.** Caracterização histológica e imuno-histoquímica das lesões de tuberculose em bovinos e de linfadenite granulomatosa em suínos. *Pesquisa Veterinária Brasileira*. 35(2): 129-136.
- 2 **Baba A.I. & Câtoi C. 2007.** Tumors of the respiratory system. In: *Comparative Oncology*. Bucharest: The Publishing house of the Romanian Academy, pp.01-99.
- 3 **Barley J.P. 2011.** Pulmonary papillary adenocarcinoma in an adult cow. *Veterinary Ireland*. 1(12): 674-675.
- 4 **Caswell J.L. & Williams K.J. 2016.** Respiratory tract. In: Maxie M.G. (Ed). *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th edn. St. Louis: Elsevier, pp.495-500.
- 5 **Jain N.C. 1993.** *Essentials of Veterinary Hematology*. Philadelphia: Lea & Febiger, 417p.
- 6 **Lucena R.B., Rissi D.R., Kommers G.D., Pierezan F., Oliveira Filho J.C., Macêdo J.T.S.A., Flores M.M. & Barros C.S.L. 2011.** A retrospective study of 586 tumors in Brazilian cattle. *Journal of Comparative Pathology*. 145(1): 20-24.
- 7 **Luna S.P.L. & Teixeira M.W. 2007.** Eutanásia: considerações éticas e indicações técnicas. *Revista CFMV*. 13(41): 60-69.
- 8 **Meuten D.J. 2002.** *Tumors in Domestic Animals*. 4th edn. Ames: Iowa State Press, 788p.
- 9 **Reis M.O., Slaviero M., Lorenzetti M.P., Cruz R.A.S., Guimarães L.L.B., Pavarini S.P., Driemeier D. & Sonne L. 2017.** Neoplasmas bovinos diagnosticados no Setor de Patologia Veterinária da UFRGS, Porto Alegre (2005-2014). *Pesquisa Veterinária Brasileira*. 37(2): 105-109.
- 10 **Santarosa B.P., Dantas G.N., Rocha N.S., Rodrigues C.A., Chiacchio S.B., Oliveira-Filho J. P., Amorim R.M. & Gonçalves R.C. 2015.** Adenocarcinoma pulmonar em vaca nelore: Relato de caso. *Veterinária e Zootecnia*. 22(4): 569-574.
- 11 **Silva Filho A.P., Afonso J.A.B., Souza J.C.A., Riet Correa F., Dantas A.F., Dantas A.C., Costa N.A. & Mendonça C.L. 2011.** Linfossarcoma em bovinos no Agreste Meridional de Pernambuco. *Pesquisa Veterinária Brasileira*. 31(7): 591-597.

- 12 Sousa D.Z., Rivera L.C., Quevedo D.C., Gorino A.C., Biagio S.C. & Laufer R.A. 2014.** Pulmonary adenocarcinoma in cattle. *Revista MVZ Córdoba*. 19(3): 4358-4363.
- 13 Stilwell G. & Peleteiro M.C. 2010.** Uterine adenocarcinoma with pulmonary, liver and mesentery metastasis in a Holstein cow. *Veterinary Medicine International*. 2010: Article ID 540830, 7 pages.
- 14 Streeter R.N. & Step D.L. 2007.** Diagnostic ultrasonography in ruminants. *Veterinary Clinics of North America: Food Animal Practice*. 23(3): 541-574.
- 15 Tamura A., Hebisawa A., Kusaka K., Hirose T., Suzuki J., Yamane A., Nagai H., Fukami T., Ohta K. & Takahashi F. 2016.** Relationship between lung cancer and Mycobacterium avium complex isolated using bronchoscopy. *The Open Respiratory Medicine Journal*. 10(1): 20-28.
- 16 Viott A.M., Langohr I.M., Vannucci F.A., Almeida A.P., Leite R.C. & Ecco R. 2010.** Adenocarcinoma pulmonar em um bovino. *Ciência Rural*. 40(2): 454-457.