UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

CARACTERIZAÇÃO PATOLÓGICA DE LESÕES DE BOVINOS EM FRIGORÍFICOS

Andréia Vielmo

Porto Alegre
2022

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

CARACTERIZAÇÃO PATOLÓGICA DE LESÕES DE BOVINOS EM FRIGORÍFICOS

Autor: Me. Andréia Vielmo

Tese apresentada como requisito parcial para obtenção do grau de Doutor em Ciências Veterinárias da Universidade Federal do Rio Grande do Sul na área de concentração em Medicina Veterinária Preventiva e Patologia: Patologia Animal e Patologia Clínica

Orientador: Prof. Dr. David Driemeier

Coorientador: Prof. Dr. Welden Panziera

Porto Alegre
2022

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001

CIP - Catalogação na Publicação

Vielmo, Andréia
Caracterização patológica de lesões de bovinos em frigoríficos / Andréia Vielmo. -- 2022.
69 f.
Orientador: David Driemeier.

Coorientador: Welden Panziera.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Porto Alegre, BR-RS, 2022.

1. doenças de bovinos. 2. neoplasia. 3. doenças infecciosas. 4. histoquímica. I. Driemeier, David, orient. II. Panziera, Welden, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

ANDRÉIA VIELMO

CARACTERIZAÇÃO PATOLÓGICA DE LESÕES DE BOVINOS EM FRIGORÍFICOS

Aprovada em 31 de março de 2022

APROVADO POR:

_____

Prof. Dr. David Driemeier

Orientador e Presidente da Comissão

_____

Prof. Dr. Claudio Severo Lombardo de Barros

Membro da Comissão

_____

Profª. Drª. Paula Roberta Giaretta

Membro da Comissão

_____

Prof. Dr. Saulo Petinatti Pavarini

Membro da Comissão

RESUMO

O abatedouro frigorífico constitui uma relevante fonte de material para o auxílio de diagnóstico de enfermidades, muitas delas de caráter zoonótico. O exame de inspeção *post mortem* é importante para a identificação de lesões nos órgãos e/ou carcaças dos animais abatidos, as quais podem condicionar ou impedir o aproveitamento do produto para o consumo humano. Em razão disso, a tese teve como objetivo descrever macroscopicamente e microscopicamente as lesões neoplásicas e infecciosas de bovinos encontradas no abatedouro frigorífico com o intuito de auxiliar trabalhadores do serviço de inspeção e patologistas veterinários a identificar as lesões encontradas nas linhas de abate. Este estudo resultou em três trabalhos científicos: (1) *Primary hepatic neoplasms in cattle*. Nesse trabalho os neoplasmas hepáticos primários foram caracterizados através da macroscopia, histologia e achados imuno-histoquímicos. Dezenove neoplasmas foram identificados, sendo o carcinoma hepatocelular o neoplasma mais diagnosticado seguido pelo colangiocarcinoma. Vacas foram as mais acometidas. Os carcinomas hepatocelulares apresentaram-se macroscopicamente como massas solitárias ou nódulos multifocais, com superfície de corte esverdeada. Histologicamente, os padrões sólido e trabecular foram os mais predominantes. Todos os carcinomas hepatocelulares foram positivos na imuno-histoquímica anti-Hep Par-1. Os colangiocarcinomas apresentaram-se como nódulos multifocais, ocasionalmente com aspecto umbilicado e o arranjo histológico sólido. Na imuno-histoquímica anti-CK7 apenas um caso de colangiocarcinoma foi positivo. A imuno-histoquímica foi uma ferramenta importante para melhorar a acurácia no diagnóstico das neoplasias hepáticas primárias. Hep Par-1 é um marcador imuno-histoquímico útil para carcinomas hepatocelulares. (2) *Penile tuberculosis in a bull*. Esse relato de caso teve o objetivo de descrever um caso de tuberculose peniana causada por *Mycobacterium tuberculosis* var. bovis em um touro. Essa é uma localização anatômica incomum e é relevante para o diagnóstico diferencial de doenças granulomatosas nessa região. (3) *Histological and immunohistochemical features of carcinomas with pulmonary involvement in cattle*. Esse estudo teve como objetivo caracterizar através da histologia e imuno-histoquímica as neoplasias epiteliais pulmonares primárias e secundárias e, por meio disso, fornecer suporte ao patologista veterinário na análise histológica das lesões pulmonares. Dezenove neoplasias epiteliais com acometimento pulmonar foram identificadas. Adenocarcinoma foi a neoplasia primária mais comumente identificada, e CCE e carcinomas uterinos foram as neoplasias metastáticas mais frequentes. Todas as neoplasias foram positivas na imuno-histoquímica anti-panCK confirmando sua origem epitelial. A maioria das neoplasias primárias pulmonares demonstraram imunomarcação positiva para TTF-1. Não existem estudos caracterizando essas neoplasias em bovinos. Esses três trabalhos estão anexados na íntegra a esta tese.

Palavras-chave: doenças de bovinos, neoplasia, doenças infecciosas, imuno-histoquímica.

ABSTRACT

The slaughterhouse is a relevant source of samples to improve surveillance of diseases, especially those of zoonotic nature. The post-mortem organs and/or carcasses inspection is decisive to recognize gross lesions with public health impact and avoid human consumption. Therefore, the thesis aimed to describe gross and microscopic aspects of neoplastic and infectious lesions of cattle found at the slaughterhouse concerning to help inspection service workers and veterinary pathologists to recognize those lesions observed in slaughter lines. This study developed three scientific manuscripts: (1) Primary hepatic neoplasms in cattle. This work characterized primary hepatic neoplasms through gross, histology, and immunohistochemical findings. Nineteen neoplasms were identified, with hepatocellular carcinoma being the most diagnosed neoplasm, followed by cholangiocarcinoma. Cows were the most affected. Hepatocellular carcinomas presented grossly as solitary masses or multifocal nodules, and greenish on the cut surface. Histologically, solid and trabecular patterns were the most predominant arrangement. All hepatocellular carcinomas were positively immunolabelled by anti-Hep Par-1. Cholangiocarcinomas occurred as multifocal nodules, sometimes with an umbilicated gross appearance and a solid histological arrangement. In the anti-CK7 immunohistochemistry, only one case of cholangiocarcinoma was positively immunolabelled. Immunohistochemistry was an important tool to improve accuracy in primary liver neoplasms diagnostic. Hep Par-1 is a useful immunohistochemical marker for hepatocellular carcinomas. (2) Penile tuberculosis in a bull. This case report aimed to describe a case of penile tuberculosis caused by Mycobacterium tuberculosis var. bovis *in a bull. This is an uncommon anatomical location and is relevant for the differential diagnosis of granulomatous diseases in this region. (3) Histological and immunohistochemical features of carcinomas with pulmonary involvement in cattle. This study aimed to characterize the primary and secondary lung epithelial neoplasms through histology and immunohistochemistry and, thereby, provide support for veterinary pathologists in lung lesions histological analysis. Nineteen epithelial neoplasms with lung involvement were identified. Adenocarcinoma was the most common primary neoplasm, and SCC and uterine carcinomas were the most frequent metastatic neoplasms. All neoplasms were positive on anti-panCK immunohistochemistry, confirming their epithelial origin. Most primary lung neoplasms demonstrated positive immunolabeling for TTF-1. To the best of the author's knowledge, there were no studies characterizing these neoplasms in cattle. The full papers are attached to this thesis.*

Keywords*: diseases of cattle, neoplasia, infectious diseases, immunohistochemistry.*

SUMÁRIO

1. INTRODUÇÃO

O Brasil possui destaque mundial na produção e exportação da carne bovina. Com um rebanho de aproximadamente 187,55 milhões de bovinos a pecuária brasileira registrou em 2020 abate de aproximadamente 41,5 milhões de bovinos. Nesse mesmo ano, o Brasil teve aumento de 8% nas exportações de carne bovina em relação a 2019. Do total de carne produzida, 73,93% tiveram como destino o mercado interno, enquanto 26,07% foram destinadas às exportações, com aumento de 9,8% no volume de carne *in natura.* Dentre os estados brasileiros produtores, o Rio Grande do Sul possui importante participação nesse cenário. O rebanho bovino gaúcho é estimado em 12.433.747 bovinos, o que representa 6,63% do rebanho total brasileiro. No ano de 2020 o Produto Interno Bruto (PIB) da pecuária de corte brasileira cresceu 20,8% em relação ao ano anterior e totalizou 10% do PIB total (ABIEC, 2021).

Para garantir a qualidade da carne brasileira e seus subprodutos na mesa dos consumidores, à nível de mercado interno e externo, é de extrema importância um sistema de inspeção sanitário qualificado para garantir a segurança sanitária (DUPUY *et al*., 2014; VIDAL *et al*., 2015). A inspeção nos frigoríficos consiste em observar e examinar a carcaça e os órgãos, em busca de condições anormais que limitem ou impeçam o aproveitamento do produto ou matéria-prima para o consumo humano. A realização do serviço de inspeção sanitária é de responsabilidade do serviço veterinário oficial, representado por médicos veterinários e auxiliares de inspeção nas linhas de abate. O uso não regulamentado e inadequado de produtos de origem animal, assim como o desperdício de alimentos, pode ter forte impacto econômico para indústria e produtores, além de comprometer a segurança alimentar (VIAL *et al*., 2015; VIDAL *et al*., 2015; JEDREJEK *et al*., 2016).

O abatedouro frigorífico constitui uma relevante fonte de material para o auxílio de diagnóstico de enfermidades, muitas delas de caráter zoonótico, (UNGAR *et al*., 1990). Além disso, essas doenças acarretam prejuízos econômicos e têm importância em saúde pública (SCHENK; SCHENK, 1982; BIDONE *et al*., 2021). Em bovinos, as principais condenações descritas na literatura brasileira ocorrem devido a doenças parasitárias (cisticercose [*Cysticercus bovis*], hidatidose e fasciolose) (MARQUES *et al*., 2008; DUTRA *et al*., 2010; SANTOS *et al*., 2010; FRUET *et al*., 2013; TESSELE et al., 2013a; PANZIERA *et al*., 2017; BIDONE *et al*., 2021), doenças bacterianas, principalmente,

tuberculose (SANTOS *et al*., 2010; FURLANETTO *et al*., 2012) e neoplásicas (TESSELE; BARROS, 2016).

Dentre as doenças bacterianas, a tuberculose é uma doença zoonótica, frequentemente diagnosticada na rotina dos frigoríficos e possui distribuição mundial. A enfermidade é causada principalmente por *Mycobacterium tuberculosis* var. bovis e apresenta alta prevalência nos países em desenvolvimento (CARVALHO *et al*., 2016). Entretanto, no Brasil, os dados epidemiológicos da condição são escassos e não descrevem apropriadamente o *status* no território (QUEIROZ *et al*., 2016). A tuberculose tem impactos socioeconômicos importantes, reduzindo a produtividade da pecuária, devido ao descarte precoce de animais de alto valor zootécnico, redução no ganho de peso dos animais afetados e perda na exportação de produtos de origem animal na indústria, principalmente, a carne (CARVALHO *et al*., 2016). Além das doenças infecciosas, neoplasias também podem ser detectadas no exame *post mortem* de animais abatidos. Os tumores mais frequentemente encontrados na espécie bovina são o linfoma associado ao vírus da leucose enzoótica bovina, carcinoma de células escamosas e o feocromocitoma (TESSELE; BARROS, 2016). No entanto, trabalhos sobre a frequência, diagnóstico e caracterização de neoplasias nessa espécie são escassos. Segundo recomendações do Regulamento Industrial de Inspeção dos Produtos de Origem Animal (Ministério da Agricultura, 2017), as carcaças, partes delas, ou órgãos afetados por tumores malignos devem ser condenadas, independente da ocorrência de metástases. A falta de segurança e experiência técnica em diagnosticar e diferenciar diversas neoplasias de forma que o destino mais adequado das carcaças e vísceras seja selecionado, pode gerar perdas econômicas pela condenação de órgãos e carcaças que não precisariam ser totalmente condenados. Além disso, algumas neoplasias identificadas nas linhas de abate, são consideradas diagnósticos diferenciais de doenças infecciosas (CULLEN; STALKER, 2016; KAMELLI *et al*., 2016; BOWN *et al*., 2017; FACCIN *et al*., 2018).

Alguns estudos relacionados com a caracterização de lesões de bovinos encontradas em frigoríficos demonstram a grande variabilidade de apresentações morfológicas das condições abordadas (TESSELE *et al*., 2013a; TESSELE *et al*., 2013b; TESSELE *et al*., 2014; TESSELE; BARROS, 2016; PANZIERA *et al*., 2017; PANZIERA *et al*., 2020), e existe ainda, a necessidade de mais estudos de caracterização com diferentes enfoques. Em muitos casos, para a diferenciação entre as lesões, as avaliações histológica, histoquímica, imuno-histoquímica, microbiológica ou molecular, devem ser utilizadas

(PANZIERA *et al*., 2017; BIANCHI *et al*., 2019). O reconhecimento de doenças granulomatosas durante a inspeção, como a tuberculose, é altamente relevante para a vigilância e o controle da infecção em animais e rebanhos (DOMINGO *et al*., 2014).

Entende-se que um estudo detalhado sobre as características macroscópicas, histológicas, histoquímicas, imuno-histoquímicas e moleculares de lesões infecciosas e neoplásicas observadas em bovinos abatidos em frigoríficos auxiliaria os médicos veterinários responsáveis pelos serviços de inspeção veterinário oficial a reconhecê-las e, possivelmente, diferenciá-las. Isso permitiria o destino apropriado para as carcaças e vísceras dos animais, evitando assim, problemas relacionados à saúde pública e maiores perdas econômicas para a indústria e produtores. Dessa forma, o objetivo dessa tese foi descrever lesões neoplásicas hepáticas e pulmonares em bovinos, e relatar um caso de tuberculose no trato reprodutor de um touro. Desse estudo resultaram três trabalhos científicos. A introdução, material e métodos, resultados e discussão obtidos em cada um desses trabalhos está descrita a seguir.

2. ARTIGO 1

Nesse item é apresentado o artigo intitulado:

Primary hepatic neoplasms in cattle

Andréia Vielmo, Welden Panziera, Matheus V. Bianchi, Fernando F. Argenta, Cíntia De Lorenzo, Luís A. Vielmo, Saulo P. Pavarini and David Driemeier

(Artigo publicado na revista *Pesquisa Veterinária Brasileira 40(6):409-416, 2020*)

Pesq. Vet. Bras. 40(6):409-416,2020

Primary hepatic neoplasms in cattle[1]

Andréia Vielmo[2*], Welden Panziera[2], Matheus V. Bianchi[2], Fernando F. Argenta[2], Cintia De Lorenzo[2], Saulo P. Pavarini[2] e David Driemeier[2]

ABSTRACT. - Vielmo A., Panziera W., Bianchi, M.V., Argenta F.F., Lorenzo C., Pavarini S.P. & Driemeier D. 2020. **Primary hepatic neoplasms in cattle.** *Pesquisa Veterinária Brasileira 40(6)409-416*. Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9090, Prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: andreiavielmo@yahoo.com.br.

Primary hepatic neoplasms are mostly detected in cattle as incidental findings in slaughterhouses or diagnosed at the necropsy, wherein it may be related to the cause of death. A proper characterization of primary hepatic neoplasms is essential to an accurate diagnosis, especially at the slaughter lines, in order to reduce erroneous condemnations. This work aimed to characterize the gross, histological, and immunohistochemical features of primary liver neoplasms detected in slaughtered cattle in Southern Brazil. Nineteen primary hepatic neoplasms were identified. Grossly, these lesions were classified according to their distribution as focal, multifocal, or diffuse. Histologically, the shape and arrangement of the cells, as well as possible malignant features were evaluated. Immunohistochemistry (IHC) was also performed for biliary epithelium (CK7) and hepatocytes (Hep Par-1) markers. Hepatocellular carcinoma (84.2%) was the most frequently detected hepatic neoplasm, followed by cholangiocarcinoma (15.8%), and these were only identified in adult cows. Hepatocellular carcinomas occurred as solitary masses or multifocal nodules, which on the cut surface were often green. Cholangiocarcinomas occurred as multifocal nodules, occasionally showing an umbilicated appearance. Histologically, hepatocellular carcinomas had mostly trabecular and solid patterns, while cholangiocarcinomas presented mostly a solid arrangement. Upon IHC, all hepatocellular carcinomas were immunolabeled for anti-HeP Par-1, ranging from mild (25%), moderate (31.2%), to marked (43.7%), while immunolabeling for anti-CK7 was detected only in one case of cholangiocarcinoma.

INDEX TERMS: Neoplasms; cattle diseases; abattoir study; hepatic tumors; hepatocellular carcinoma; cholangiocarcinoma; hepatic markers.

RESUMO.- [**Neoplasmas hepáticos primários de bovinos.**] Os neoplasmas hepáticos primários são detectados em bovinos principalmente como achados incidentais em matadouros ou diagnosticados na necropsia, quando podem estar relacionados à causa da morte. A caracterização adequada dos tumores hepáticos primários é essencial para obter diagnósticos precisos, especialmente nas linhas de abate, com o propósito de reduzir condenações errôneas. Este trabalho teve o objetivo de determinar as características macroscópicas, histológicas e imuno-histoquímicas dos neoplasmas primários do fígado de

[1] Received on Mach 10, 2020
Accepted for publication on March 24, 2020
[2] Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brasil. *Autor para correspondência: andreiavielmo@yahoo.com.br.

bovinos abatidos em um matadouro-frigorífico no Sul Brasil. Dezenove neoplasias hepáticas primárias foram identificadas. Macroscopicamente, os tumores hepáticos foram classificados de acordo com sua distribuição como, focais, multifocais ou difusos. Histologicamente, a forma e o arranjo das células e possíveis características malignas foram avaliados. Também foi realizada imuno-histoquímica (IHQ) para marcadores de epitélio biliar (anti-CK7) e hepatócitos (anti-Hep Par-1). O carcinoma hepatocelular (84,2%) foi o neoplasma hepático mais frequentemente detectado, seguido pelo colangiocarcinoma (15,8%). Esses tumoes foram identificados apenas em vacas adultas. Os carcinomas hepatocelulares eram vistos como massas solitárias ou nódulos multifocais que na superfície de corte geralmente eram esverdeados. Os colangiocarcinomas foram observados como nódulos multifocais, ocasionalmente com aspecto umbilicado. Histologicamente, os padrões mais observados nos carcinomas hepatocelulares foram trabeculares e sólidos, enquanto nos colangiocarcinomas o arranjo sólido foi o mais frequente. Na IHQ todos os carcinomas hepatocelulares foram marcados por anti-HeP Par-1, com marcação que variou de leve (25%), moderada (31,2%) a acentuada (43,7%); imunomarcação para anti-CK7 foi detectada em apenas um caso de colangiocarcinoma.

TERMOS DE INDEXAÇÃO: Neoplasmas, bovinos, doenças de bovinos, estudo em abatedouro, tumores de fígado, carcinoma hepatocelular, colangiocarcinoma, marcadores hepáticos.

INTRODUCTION

Primary hepatic neoplasms (PHN) are uncommon in domestic animals, except dogs, and are generally hepatocellular or cholangiocellular in origin (Cullen & Stalker 2016). In cattle, PHN frequency varies geographically, with incidences ranging from 3.1 and 10% in South Africa and in the United Kingdom (UK), respectively (Anderson & Sandinson 1968; Bastianello 1982). Recent data from Brazilian studies (Lucena et al. 2011, Tessele & Barros 2014, Reis et al. 2017) indicate lower frequencies of 1.88%-4.6% of liver tumors when compared to other studies (Anderson & Sandinson 1968). Overall, lymphoma (enzootic bovine leucosis) and squamous cell carcinoma are the most common detected bovine neoplasms. The latter may occur in the upper alimentary tract, the eyes and adnexa, and in vulva (Lucena et al. 2011, Rosa et al. 2012, Carvalho et al. 2014, Reis et al. 2017).

Primary tumors that originate from hepatocytes may include adenomas, hepatocellular carcinomas, and hepatoblastomas, while those originated from the biliary epithelium are classified as adenomas, biliary cystadenomas, cholangiocarcinomas, or biliary cystadenocarcinomas (Head et al. 2003, Cullen 2017). Mixed hepatocellular and cholangiocellular carcinomas are rarely observed and have histological characteristics of both cell origins: hepatocytes and biliary epithelium (Cullen 2017).

The diagnosis of PHN is not always obtained based solely on histological characteristics; on these instances, the immunohistochemical exams are essential to establish a conclusive diagnosis. Several immunohistochemical markers may assist in the differential diagnosis of liver tumors (Chan & Yeh 2010), such as hepatocyte paraffin 1 (Hep Par-1), which is used to label neoplastic hepatocytes and, thus identify hepatocellular carcinomas (Chu et al. 2002), and cytokeratin 7 (CK7), which labels bile duct epithelial cells (Yabuchita et al. 2001), allowing a proper recognition of cholangiocarcinomas.

The characterization of the PHN is important to improve the accuracy of identification in the meat inspection lines. According to the Industrial Regulation of Animal Producer Inspection (Ministério da Agricultura 2017), carcasses, part of them, or organs affected by malignant tumors must be condemned, regardless of the occurrence of metastases. Thus, the difficulty in the diagnosis may lead to an incorrect destination of the carcass (Freitas 1999). In order to assist veterinary pathologists and meat inspectors, as well to improve diagnostic accuracy, this study aimed to characterize the gross, microscopic, and immunohistochemical aspects of PHN detected in slaughtered cattle in Southern Brazil.

MATERIAL AND METHODS

From 2015 to 2016, liver samples of cattle slaughtered in an abattoir of the municipality of Santa Maria, Rio Grande do Sul state (29°46'54.2"S; 53°46'38.8"W) were collected. These samples, previously fixed in 10% formalin, were sent for gross and histological evaluation after condemnation at the meat inspection line. Metastases were also sent for analysis.

The slaughtered cattle were from different regions of the state. For this study, only those livers with PHN were selected. The samples were photographed, trimmed, routinely processed for histology, and stained by hematoxylin and eosin (HE). Data regarding sex and age (estimated by dentition) (Food Safety Inspection Service 2013) of the cattle involved were obtained from the slaughterhouse files.

Grossly, PHN were classified according to their distribution into focal, multifocal, or diffuse. Focal tumors appeared as a single nodule or mass in the liver. Tumors distributed as multiple nodules or masses with a random distribution in the liver parenchyma were included in the multifocal category. Diffuse neoplasms affected all or most parts of the liver. In this latter pattern, there was an increase in the whole volume of the organ, accompanied by changes in color and consistency of the affected part.

Histological evaluation was performed according to previously established criteria (Cullen 2017). The following features were considered: (1) cellular arrangement; (2) stromal pattern; (3) cell pleomorphism; (4) vascular or lymphatic invasion; (5) necrosis, hemorrhage, and intratumoral inflammatory infiltrate; (6) intratumoral vascular spaces; and (7) mitotic index. The degree of mitoses was evaluated by three independent veterinary pathologists and considered as mild (less than or equal to one mitosis per high power field - 400x), moderate (2-4 mitosis per high power field), and marked (greater than five mitoses per high power field).

In order to assess desmoplasia levels and mucin content, histological sections of the neoplasms were stained by Masson's trichrome (MT) and Periodic Acid Schiff (PAS), respectively. Selected sections of the neoplasms were submitted to immunohistochemistry (IHC) using the universal polymer method marked with peroxidase (MACH 4 Universal HRP-Polymer - Biocare Medical) for the biliary epithelium [monoclonal antibody anti-cytokeratin (anti-CK7); clone/brand:M7018/Dako; dilution 1:40; antigenic retrieval proteinase K] and hepatocytes [monoclonal antibody anti-hepatocyte paraffin 1(anti-Hep Par-1); clone/brand: M7018/Dako; dilution 1:40; antigenic retrieval citrate buffer pH6.0 for 40min at 96°C]. For both protocols, revelation was obtained by using the chromogen 3-amino-9ethylcarbazole (AEC) and counterstain with Mayer´s hematoxylin. As a positive control, a section of the liver previously tested was used, and the same material was used as a negative control, by replacing the primary antibody with phosphate-buffered saline. The immunolabeling intensity was classified as absent, mild, moderate, or marked.

RESULTS

Nineteen primary hepatic neoplasms were identified during the period studied, of which 16 (84.2%) were hepatocellular carcinomas and three (15.8%) cholangiocarcinomas. All affected cattle were adults above 3-year-old. Female cattle were mostly affected, both in cases of hepatocellular carcinomas (93.75%, 15/16), and in cases of cholangiocarcinomas (66.7%, 2/3). Grossly, hepatocellular carcinomas were focal in 43.7% (Fig. 1A) of the cases, multifocal in 43.7% (Fig. 1B), and diffuse in 12.5% (Fig. 1C). The focal pattern occurred as nodules measuring 4-17 cm in diameter, which occupied and partially effaced the affected hepatic lobe. The multifocal pattern occurred as random nodular areas, ranging from 1-20 cm in diameter. In the diffuse pattern, the entire organ was affected. The neoplastic masses were white, yellow or red, and soft or firm. The cut surface showed white, yellow, dark green (Fig. 1D) and red areas. Occasionally there were extensive friable areas (necrosis) intermixed with the tumor mass.

Histologically, hepatocellular carcinomas had a solid pattern in half of the cases (Fig. 2A) and trabecular pattern in the other half (Fig. 2B). In the solid pattern, the hepatic

architecture was disorganized by the proliferation of dense mantles of neoplastic hepatocytes. Trabeculae of varying thickness formed by neoplastic cells characterized the trabecular pattern. Neoplastic cells were polygonal, with distinct cytoplasmic boundaries, abundant and faint eosinophilic, sometimes vacuolated, cytoplasm. The nucleus was rounded, with finely stippled chromatin, containing one to five evident nucleoli. Binucleated or multinucleated neoplastic hepatocytes were observed in 56.2% of the tumors (Fig. 2C). Inflammatory infiltrate of lymphocytes, plasma cells, neutrophils, and macrophages occurred in 87.5% of hepatocellular carcinomas; while in 25% of the cases, there were intratumoral vascular spaces, and in 6.25% there was discrete multifocal mineralization. Vascular invasion (blood or lymphatic) occurred in 37.5% of the cases (Fig. 2D). Among the tumors with vascular dissemination, 25% had a solid pattern and 12.5% the trabecular pattern. Extrahepatic metastasis (to mediastinal lymph node) of a solid pattern hepatocellular carcinoma occurred in one case (Fig. 2E). Additional histological characteristics of hepatocellular carcinomas are in Table 1.

IHC for anti-Hep Par-1 revealed an intracytoplasmic granular immunolabeling ranging from mild (25% of cases), moderate (31.25% of cases), moderate (31.2% of cases) and marked (43.7%) (Fig. 2F). IHC anti-CK7 did not reveal any labeling for hepatocellular carcinomas.

Grossly, all three cholangiocarcinomas had a multifocal pattern of liver involvement, which was characterized by slightly irregular, occasionally umbilicated, and firm nodules of 1-6 cm in diameter, randomly distributed in the liver parenchyma. On the cut surface, these nodules were white to yellow.

Histologically, the solid pattern occurred in two out of three cases (Fig. 3A); in the remaining case, the neoplasm was arranged in acini or irregular ducts (Fig. 3B). In both histological patterns, neoplastic cells partially effaced the liver parenchyma. The cells varied from cuboidal to rounded, with indistinct cytoplasmic borders, moderate and faint eosinophilic cytoplasm. Nuclei were predominantly oval, with finely stippled chromatin and one to three evident nucleoli. Cellular pleomorphism was moderate in two cases and marked in the other, in which there was marked anisocytosis and anisokaryosis (Fig. 3C). The mitotic index was mild (1/3), moderate (1/3), or marked (1/3). Fibrous connective tissue proliferation was observed in HE stained sections (Fig. 3D) and evidenced by the MT technique (Fig. 3E), in which it was mild in one of the tumors and marked in the other two (scirrhous cholangiocarcinomas). Mild (two cases) and moderate (one case) areas of necrosis and hemorrhage were also observed within the neoplasm parenchyma. Vascular invasion was found in two cases, and in one of the tumors, there was pulmonary metastasis. In all three cases, there was mild intratumoral inflammatory infiltrate of lymphocytes, plasma cells, neutrophils, and macrophages. A mild amount of mucin was evidenced within the neoplastic ducts in two cases by the PAS technique. Upon IHC evaluation, only one cholangiocarcinoma had marked intracytoplasmic and membranous anti-CK7 immunolabeling (Fig. 3F). No case of cholangiocarcinoma was positive in the IHC for anti-Hep Par-1.

DISCUSSION

The diagnosis of all PHN cases in this study was obtained through the association of the gross, histopathological, histochemical, and immunohistochemical findings. Liver neoplasms are considered uncommon when compared to other tumors of cattle (Lucena et al. 2011, Tessele & Barros 2016, Reis et al. 2017). Usually, PHN in cattle are incidental findings of slaughterhouses (Tessele & Barros, 2016) or are diagnosed at the necropsy, wherein it may be related to the cause of death (Reis et al. 2017).

All cattle affected in this study were all adults, aged three years or older, similar to that reported by other authors (Anderson & Sandinson, 1968, Wettimuny 1969, Braun et al. 2005, Jeong et al. 2005). Females were more affected than males, and, although few studies assess a possible sex predilection for the development of liver tumors in cattle (Wettimuny

1969, Braun et al. 20050), in dogs and cats it is known that there is no such predisposition (Patnaik et al. 1981, Trigo et al. 1982, Lawrence et al. 1994, Liptak et al. 2004, Flores et al. 2013, van Sprundel et al. 2014). Probably females were overrepresented in this study since these are slaughtered later, and are thus, in the so-called "cancer age" as previously postulated (Reis et al. 2017).

In the present study, there was a predominance of hepatocellular carcinomas over cholangiocarcinomas. Most studies in cattle demonstrated similar ratios (Bastianello 1982, Bettini & Marcato 1992, Lucena et al. 2011), with occasional exceptions (Anderson & Sandinson 1968). PHN in this study had distinct gross morphological patterns. The vast majority of hepatocellular carcinomas were characterized by solitary masses or multifocal nodules, which are patterns commonly described in cattle (Anderson & Sandinson 1968, Wettimuny 1969). Observation of this pattern can assist in a presumptive diagnosis at the gross examination. The diffuse pattern was an unusual presentation for hepatocellular carcinomas and observed in only two cases, which suggests a prolonged clinical evolution, which allowed an almost complete replacement of the healthy parenchyma.

The parenchyma of hepatocellular carcinomas may present a wide range of colors caused by hemorrhages, areas of necrosis and cholestasis, including gray-white, red, brown, yellow and dark green (Wetimuny 1969, Betini & Marcato 1992). These variations occurred in the hepatocellular carcinomas of the current study. The green areas are a critical macroscopic characteristic and, combined with the pattern distribution of the neoplasm, provide valuable clues for a gross presumptive diagnosis. Metastases from hepatocellular carcinomas are uncommon (Bettini & Marcato 1992), but when they occur, the sites commonly affected include mediastinal lymph nodes, and lungs (Anderson & Sandinson 1968). In our study, metastasis was observed by the meat inspector veterinarian in a single case involving the mediastinal lymph node.

In the cholangiocarcinomas of the current study, only the multinodular pattern was observed, similar to other cases with the same gross pattern (OhFuji 2012, Azizi et al. 2016). In PHN, it is unclear whether the multiple nodules arise from intrahepatic vascular metastases or multiple origins in different foci (Wettimuny 1969, Barros 2016). A distinctive feature that can help differentiate cholangiocarcinomas from other liver tumors is that they are frequently umbilicated. This presentation was present in one of the cases in this study and is generally attributed to intratumoral necrosis and subsequent retraction by fibrosis (Head et al. 2003, Cullen 2017). Although this does not represent a particular characteristic of cholangiocarcinomas, it is a helpful criteria for gross differentiation. Another feature of cholangiocarcinomas is desmoplasia, which gives these neoplasms a firm consistency (Cullen 2017), as observed in this study in which all three cases had this characteristic. Metastases are common in cholangiocarcinomas; the main secondary sites are the lungs, lymph nodes, and peritoneum (Anderson & Sandinson 1968).

The cellular features of the hepatocellular carcinomas and cholangiocarcinomas vary according to the degree of differentiation (Head et al. 2003, Cullen 2017). The pattern of cell growth in hepatocellular carcinoma includes trabecular, solid, pseudoglandular, or squamous (Cullen 2017). We detected two patterns: trabecular and solid. As previously stated, the trabecular pattern was the most common in cattle (Wettimuny 1969, Bundza et al. 1984, Bettini & Marcato, 1992). Necrotic foci and cavities filled with red blood cells are frequent (Wettimuny 1969, Schlageter et al. 2014) and were observed respectively in 100% and 25% of the cases here reported. These histological characteristics, associated with the polygonal shape of the tumor cells, may help in the morphological diagnosis of these tumors. In one case, there was marked intracytoplasmic vacuolization; this is mentioned as frequent finding in hepatocellular carcinomas and mainly related to glycogen or lipids accumulation within the cytoplasm of the neoplastic cells (Cullen 2017).

Cholangiocarcinomas may have acinar or tubular histological patterns, which, when well-differentiated, resemble the normal biliary epithelium (Barros 2016), while in undifferentiated tumors a solid pattern may predominate (Head et al. 2003, Cullen 2017).

The solid pattern of cholangiocarcinoma was the most frequent in this study. Although the acinar patterns indicate fairly differentiation, in our study, this was not the case, as this pattern displayed marked cellular pleomorphism, high mitotic index, and vascular invasion. Fibrous connective tissue and mucin are classical histological findings of cholangiocarcinomas and valuable features for the recognition of this tumor (Head et al. 2003, Barros 2016, Cullen & Stalker 2016, Cullen 2017). In the present cases, a pronounced desmoplasia was confirmed by MT technique, and a slight amount of mucin in the PAS staining was found in two of cholangiocarcinoma cases.

Upon IHC technique, all hepatocellular carcinomas expressed Hep Par-1, while only one cholangiocarcinoma expressed CK7. The relative lack of immunolabeling in cholangiocarcinomas is probably due to the degree of differentiation. However, as there was also no labeling in the internal control (normal biliary epithelium), a prolonged fixation period in formaldehyde solution could be possible a factor involved. Previous studies have showed a decrease in the intensity of IHC-staining in some tissues after three days of exposure to formaldehyde and even an absence of immunostaining within seven days of fixation (Battifora & Kopinski 1986, Leong & Gilham 1989, Webster et al. 2009). However, good sensitivity and specificity of Hep Par-1 was detected as a marker of normal and neoplastic hepatocytes in cattle, and the combined employment of anti-Hep Par-1 and anti-CK7 may contribute to improve the accuracy of PHN diagnosis in cattle.

Other neoplasms and tumor-like lesions should be considered as macroscopic differential diagnoses of PHN. Among neoplasms, metastatic carcinomas of different primary sources (squamous epithelium, pulmonary, and endometrial cells) and lymphoma should be included. Infectious or parasitic diseases such as degenerate hydatid cysts and tuberculosis (Cullen & Stalker 2016, Kamelli et al. 2016, Bown et al. 2017, Faccin et al. 2018) may be considered as possible candidates for a gross dispute over a definitive diagnosis. In some cases, histological, histochemical, and immunohistochemical evaluation should be used for differentiation.

CONCLUSIONS

Hepatocellular carcinoma is the most common primary liver tumor in cattle.

Adult cows, as they are allowed to reach the “cancer age”, are more often affected than males.

Grossly, hepatocellular carcinomas were characterized by solitary masses or multifocal nodules, and their greenish color is an essential clue for tumor recognition.

Histologically, solid and trabecular patterns are the most common.

Cholangiocarcinomas appear macroscopically as multiple firm nodules, occasionally umbilicated, with a solid histologic arrangement.

Immunohistochemistry was an important tool to improve accuracy in the diagnosis of PHN. Hep par-1 is a useful immunohistochemical marker for hepatocellular carcinomas.

Acknowledgments.- The authors thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) for supporting this study.

Statement of conflict of interest. - The authors declare no conflict of interest with respect to the publication of this paper.

REFERENCES

Anderson L.J. & Sandinson A.T. 1968. Tumors of the liver in cattle, sheep and pigs. Cancer. 21(2):289-301. <https://doi.org/10.1002/1097-0142(196802)21:2<289::AID-CNCR2820210219>3.0.CO;2-C>

Azizi S., Kheirandiah R. & Sami M. 2016. Slaughterhouse report of intrahepatic cholangiocarcinoma in a Holstein cow. Comp. Clin. Pathol. 25(6):1321-1324. <http://dx.doi.org/10.1007/s00580-016-2324-z>

Barros C.S.L. 2016. Fígado, Vias Biliares e Pâncreas Exócrino, Cap 4, p. 222-265. In: Santos R.L. & Alessi A.C. (Eds). Patologia Veterinária. 2 ed. Roca, Rio de Janeiro.

Bastianello S.S. 1982. A survey on neoplasia in domestic species over a 40-year period from 1935 to 1947 in the Republic of South Africa. I. Tumors occurring in cattle. Onderstepoort J. Vet Res. 49(4):195-204. <PMid:7185036>

Battifora H. & Kopinski M. 1986. The influence of protease digestion and duration of fixation on the immunostaining of keratins. A comparison of formalin and ethanol fication. J. Histochem. Cytochem. 34(8):1095-1100. <http://dx.doi.org/10.1177/34.8.2426335>

Bettini G. & Marcato P.S.1992 Primary hepatic tumours in cattle. A classification of 66 cases. J. Comp. Path. 107(1):19-34. <https://doi.org/10.1016/0021-9975(92)90092-9>

Braun U., Nuss K., Soldati G. & Essent P. 2005. Clinical and ultrasonographic findings in four cows with liver tumors. Vet. Rec. 157(16):482-484. <https://doi.org/10.1136/vr.157.16.482>

Brown D.L., Van Wettere A.J., Cullen, J.M. 2017. Hepatobiliary system and exocrine pancreas, p.412-470. In: Zachary J.F. (Ed), Pathologic Basis of Veterinary Disease. 6th ed. Elsevier, St Louis.

Bundza A., Greig A.S. & Duke T.W. 1984. Primary hepatocellular tumors in animals killed at meat packing plants: report of 11 cases. Can Vet J. 25(2):82-85. <PMid:17422364>

Carvalho F.K.L., Dantas A.F.M., Riet-Correa F., Andrade R.L.F.S., Nóbrega Neto P.I., Miranda Neto E.G., Simões S.V.D. & Azevedo S.S. 2014. Estudo retrospectivo das neoplasias em ruminantes e equídeos no semiárido do Nordeste Brasileiro. Pesq. Vet. Bras. 34(3):211-216. <http://dx.doi.org/10.1590/S0100-736X2014000300003>

Chan E.S. & Yeh M.M. 2010. The use of immunohistochemistry in liver tumors. Clin Liver Dis. 14(4):687-703. <http://dx.doi.org/10.1016/j.cld.2010.10.001>

Chu P., Ishizawa S., Wu E. & Weiss L. 2002. Hepatocyte antigen as a marker of hepatocellular carcinoma: an immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. Am J Surg Pathol. 26(8):978-88. <http://dx.doi.org/10.1097/00000478-200208000-00002>

Cullen J.M. & Stalker M.J. 2016. Liver and biliary system, p.344-352. In: Maxie M.G. (Ed.), Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol. 2. 6th ed. Elsevier, St Louis.

Cullen J.M. 2017. Tumors of the liver and gallbladder, p. 602-631. In: Meuten D.J. (Ed). Tumors in domestic animals. 5th ed. John Wiley & Sons Inc., Iowa.

Faccin, T.C., Cargnelutti J.F., Rodrigues F.S., Menezes F.R., Piazer J.V.M., Melo S.M.P. Lautert B.F., Flores E.F. & Kommers G.D. 2018. Bovine upper alimentary squamous cell carcinoma associated with bracken fern poisoning: Clinical-pathological aspects and etiopathogenesis of 100 cases. PLoS One. 13(9): e0204656. <https://doi.org/10.1371/journal.pone.0204656>

Flores M.M., Bianchi R.M., Kommers G.D., Irigoyen L.F., Barros C.S.L. & Fighera R.A. 2013. Prevalência e achados epidemiológicos, anatomopatológicos e imuno-histoquímicos dos tumores hepáticos malignos primários de cães da Região Central do Rio Grande do Sul (1965-2012). Pesq. Vet. Bras. 33(4):497-511. <http://dx.doi.org/10.1590/S0100-736X2013000400014>

Food Safety Inspection Service. 2013. Using dentition to age cattle. Food Safety Inspection Service, United States Department of Agriculture, Washington, D.C. Available at <http://www.fsis.usda.gov/ofo/tsc/bse_information.htm> Accessed on February 23, 2020.

Freitas M.R. 1999. Caracterização anatomopatológica de bursites cervicais de bovinos abatidos sob Inspeção Federal no Estado de Goiás. Master's Thesis in Animal Science, Escola de Veterinária, Universidade Federal de Goiás, Goiânia. 65p.

Head K.W., Cullen J.M., Dubielzig R.R., Else R.W., Misdorp W., Patnaik A.K., Tateyama S. & Van der Gaag I. 2003. Histological classification of tumors of the alimentary system of domestic animals. Vol. 5. 2nd series. Armed Forces Institute of Pathology, Washington, 257p.

Jeong W.I., Do S.H., Sohn M.H., Yun H.S., Kwon O.D., Kim T.H., Jeong D.H., Williams B.H. & Jeong K.S. 2005. Hepatocellular carcinoma with metastasis to the spleen in a Holstein cow. Vet Pathol. 42(2):230-232. <http://dx.doi.org/10.1354/vp.42-2-230>

Kamelli M., Borji H. & Naghibi A. 2016. Genetic identification of cattle hydatid cyst isolates from northeast and southwest of Iran. Ann. Parasitol. 62(4):301–305. <http://dx.doi.org/10.17420/ap6204.65>

Lawrence H.J., Erb H.N. & Harvey H.J. 1994. Nonlymphomatous hepatobiliary masses in cats: 41 cases (1972 to 1991). Vet. Surg. 23(5):365-368. <http://dx.doi.org/10.1111/j.1532-950x.1994.tb00496.x>

Leong A. S-Y. &. Gilham P. N. 1989. The effects of progressive formaldehyde fixation on the preservation of tissue antigens. Pathology 21(4):266-268. <http://dx.doi.org 10.3109/00313028909061071>

Liptak J.M., Dernell W.S., Monnet E., Powers B.E., Bachand A.M., Kenney J.G. & Withrow S.J. 2004. Massive hepatocellular carcinoma in dogs: 48 cases (1992–2002). J. Am. Vet. Med. Assoc. 225(8):1225-1230. <http://dx.doi.org/10.2460/javma.2004.225.1225>

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 tumours in Brazilian cattle. J. Comp. Path. 145(1):20-24. <http://dx.doi.org/10.1016/j.jcpa.2010.11.002>

Mello L.S., Bianchi M.V., Sonne L., Driemeier D. & Pavarini S.P. 2017. Causas de morte em vacas leiteiras no Rio Grande do Sul. Pesq. Vet. Bras. 37(9):916-920. <http://dx.doi.org/10.1590/s0100-736x2017000900003>

Ministério da Agricultura 2017. Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (R.I.I.S.P.O.A). Aprovado pelo decreto no. 9013/2017. Ministério da Agricultura, Brasília, DF. 66p.

Ohfuji S. 2012. Cholangiocarcinoma of possibly hepatic progenitor cell origin in a cow. Vet. Q. 32(3/4):183-186. <http://dx.doi.org/10.1080/01652176.2012.749363>

Patnaik A.K., Hurvitz A.I., Lieberman P.H. & Johnson G.F. 1981. Canine bile duct carcinoma. Vet. Pathol. 18(4):439-444. <http://dx.doi.org/10.1177/030098588101800403>

Reis M. De O., Slaviero M., Lorenzett 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). Pesq. Vet. Bras. 37(2):105-109. <http://dx.doi.org/10.1590/s0100-736x2017000200002>

Rosa F.B., Kommers G.D., Lucena R.B., Galiza G.J.N., Tochetto C., Silva T.M. & Silveira I.P. 2012. Aspectos epidemiológicos, clinicopatológicos e imuno-histoquímicos de carcinoma de células escamosas vulvares em 33 vacas. Pesq. Vet. Bras. 32(11):1127-1132. <http://dx.doi.org/10.1590/S0100-736X2012001100009>

Schlageter M., Terracciano L.M., Angelo S. & Sorrentino P. 2014. Histopathology of hepatocellular carcinoma. World J Gastoenterol. 20(43):15955-15964. <http://dx.doi.org/10.3748/wjg.v20.i43.15955>

Tessele B. & Barros C.S.L. 2016. Tumores em bovinos encontrados em abatedouros frigoríficos. Pesq. Vet. Bras. 36(3):145-160. <http://dx.doi.org/10.1590/S0100-736X2016000300002>

Trigo F.J., Thompson H., Breeze R.G. & Nash A.S. 1982. The pathology of liver tumours in the dog. J Comp. Pathol. 92(1):21-39. <http://dx.doi.org/10.1016/0021-9975(82)90040-8>

Van Sprundel R.G.H.M, van den Ingh T.S.G.A.M., Guscetti F., Kershaw O., van Wolferen M.E., Rothuizen J, & Spee B. 2014. Classification of primary hepatic tumours in the cat. Vet. J. 202(2):255-266. <http://dx.doi.org/10.1016/j.tvjl.2014.07.002>

Webster J.D., Miller M.A., DuSold D. & Ramos-Vara J. 2009. Effects of prolonged formalin fixation on diagnostic immunohistochemistry in domestic animals. J. Histochem. Cytochem. 57(8):753-761. <http://dx.doi.org/10.1369/jhc.2009.953877>

Wettimuni S.G. 1969. Primary liver tumours of cattle in Ceylon. J. Comp. Path. 79(3):355-362. <http://dx.doi.org/10.1016/0021-9975(69)90050-4>

Yabushita K., Yamamoto K., Ibuki N., Okano N., Matsumura S., Okamoto R., Shimada N. & Tsuji T. 2001. Aberrant expression of cytokeratin 7 as a histological marker of progression in primary biliary cirrhosis. Liver Int. 21(1):50–55. <https://doi.org/10.1034/j.1600-0676.2001.210108.x>

Table 1. Histological features observed in 16 hepatocellular carcinomas

Feature	Intensity			
	None	**Mild**	**Moderate**	**Marked**
Pleomorphism	-	-	75% (12/16)	25% (4/16)
Mitotic index	-	75% (12/16)	18.75% (3/16)	6.25% (1/16)
Desmoplasia	25% (4/16)	43.75% (7/16)	31.25% (5/16)	-
Mucin	100% (16/16)	-	-	-
Necrosis	12.5% (2/16)	56.25% (9/16)	31.25% (5/16)	-

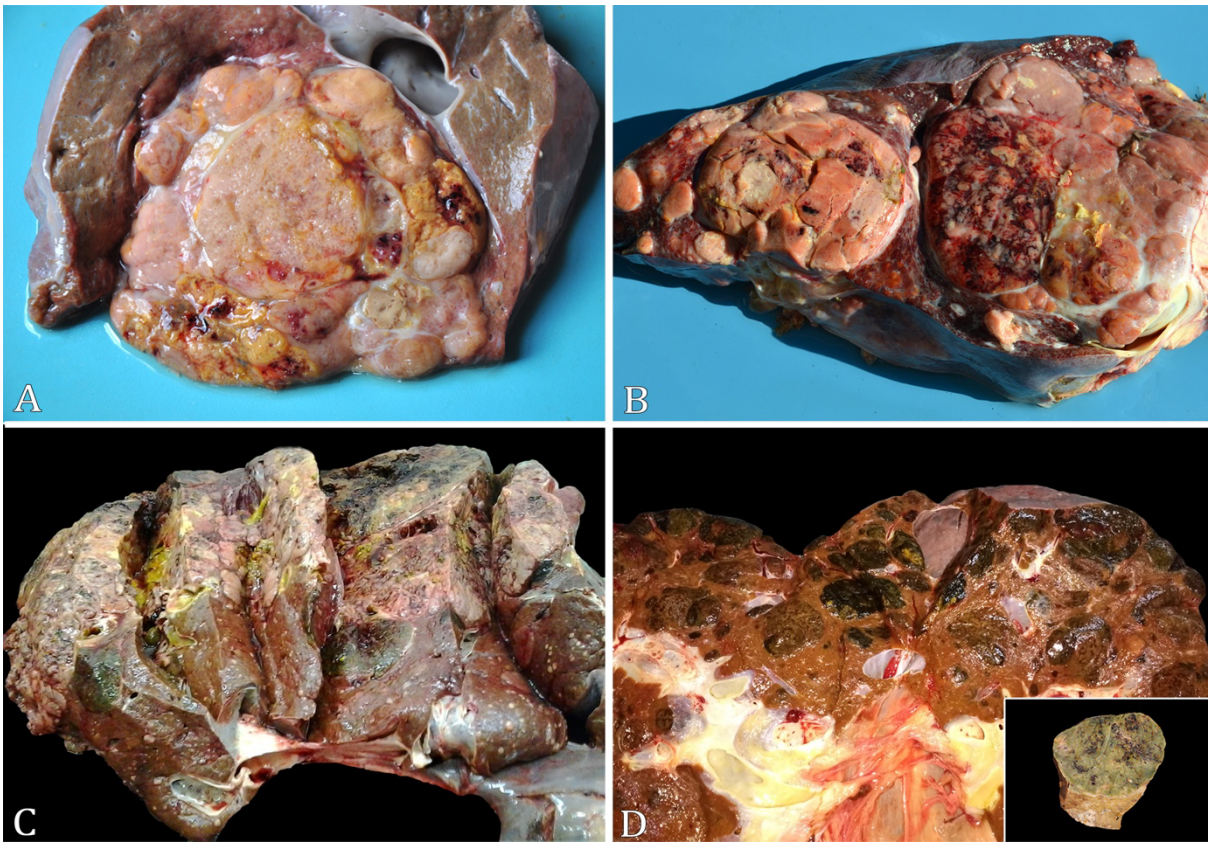

Fig. 1. Gross aspects of hepatocellular carcinomas in cattle. (**A**) Focal pattern. A nodular area partially effaces the liver parenchyma. The cut surface has a predominance of white areas interspersed with yellow to red foci. (**B**) Multifocal pattern. Multiple nodular structures of different sizes are observed. The cut surface is similar to that of Figure 1A, with wide red areas. (**C**) Diffuse pattern. The neoplasm almost completely replaces the liver parenchyma. Within the tumor parenchyma, there are friable green areas. (**D**) Cut surface. Dark-green nodular multifocal areas are distributed randomly across the parenchyma. Inset: a formalin-fixed liver fragment with a green nodular area.

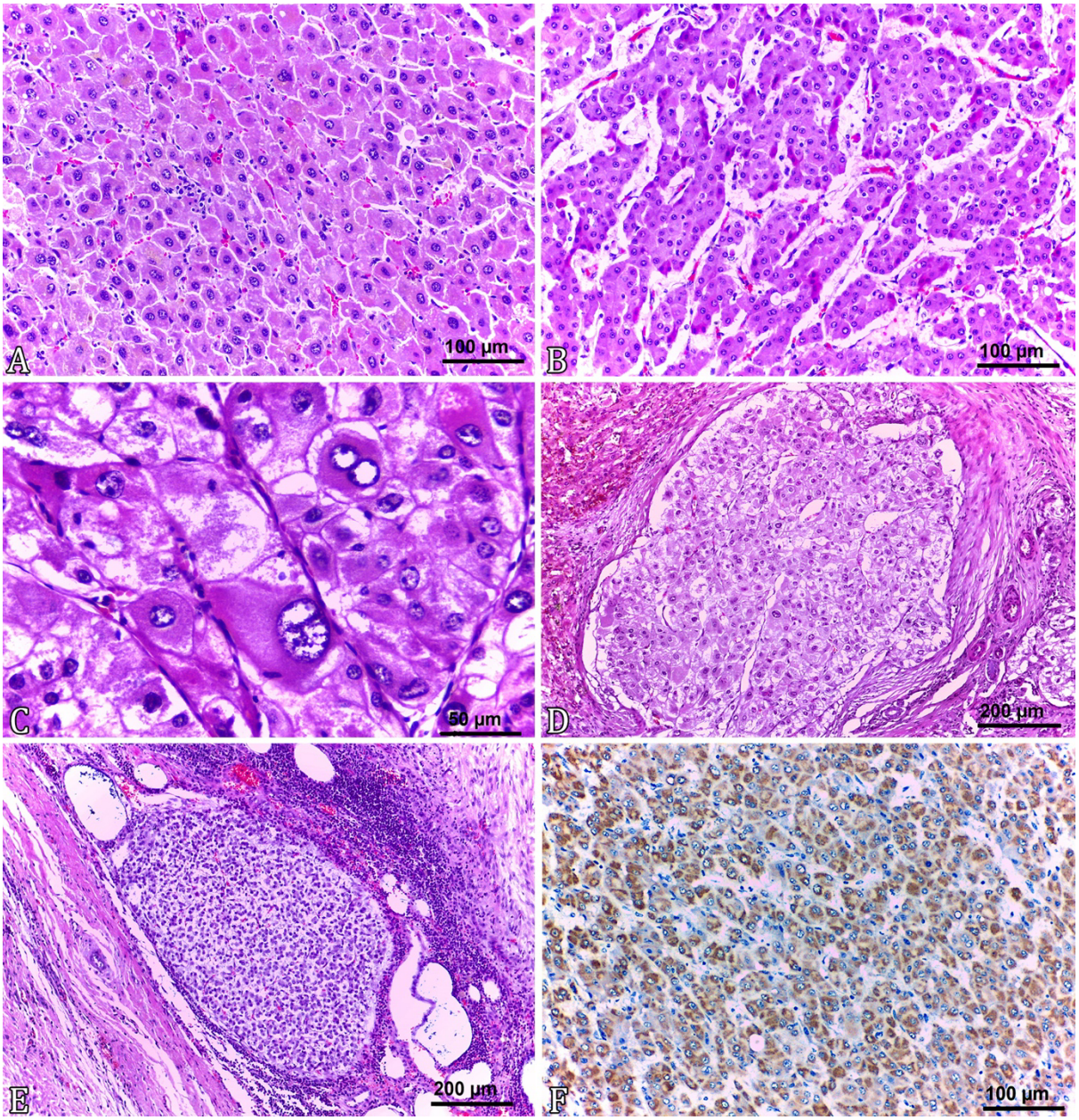

Fig. 2. Histological and immunohistochemical aspects of hepatocellular carcinomas in cattle. (**A**) Solid pattern. The parenchyma is replaced by a mantle of neoplastic hepatocytes displaying marked nuclear pleomorphism. HE, bar=100µm. (**B)** Trabecular pattern. The neoplastic hepatocytes are arranged in trabeculae of varying thickness. Unlike figure 2A, the tumor cells are well differentiated. HE, bar=100µm. (**C)** Cellular pleomorphism characterized by marked anisocytosis and binucleated and vacuolated neoplastic hepatocytes HE, bar=200µm. **(D)** Embolus of neoplastic cells occluding the lumen of a blood vessel within the liver parenchyma. HE, bar=200µm. **(E)** Metastasis of hepatocellular carcinoma in a lymph node. In the medullary sinuses, a blood vessel is obliterated by tumor cells. HE, bar=200µm. **(F)** There is a marked diffuse granular immunolabeling in the cytoplasm of neoplastic cells. Anti-Hep Par-1 IHC, AEC chromogen, bar=100µm.

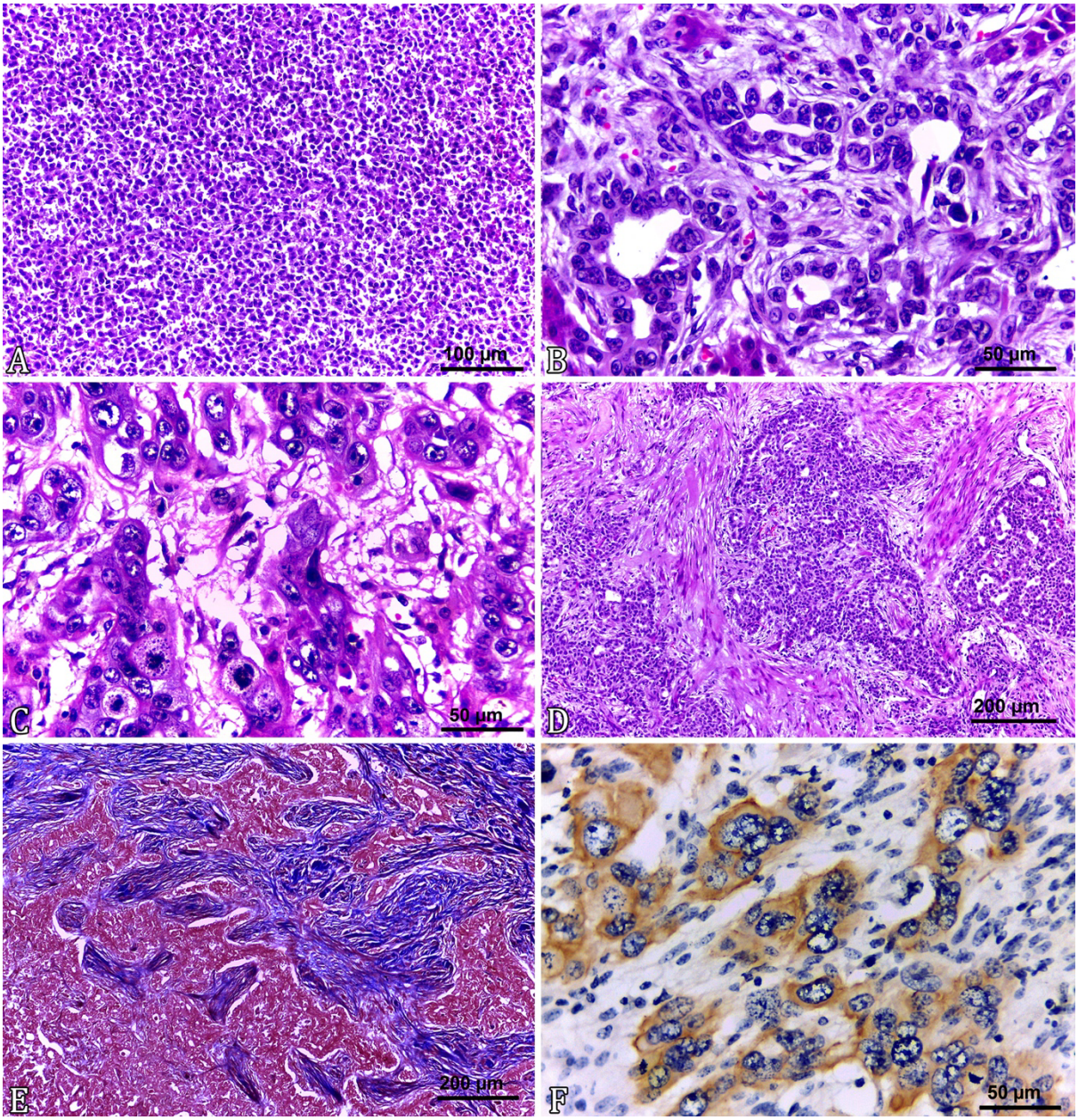

Fig. 3. Histological and immunohistochemical aspects of cholangiocarcinomas in cattle. **(A)** Solid pattern. Neoplastic cells homogeneously replace the liver parenchyma. HE, bar=100µm. **(B)** Acinar pattern characterized by ductules immersed in an abundant fibrous stroma. The cells are cuboidal to rounded, with predominantly oval nuclei and one or more prominent nucleoli. HE, bar=50µm. **(C)** Cellular pleomorphism is characterized by marked anisocytosis, anisokaryosis, and occasional megalocytosis. Mitosis and occasional bi or multinucleated cells are evident. HE, bar=50µm. **(D)** A marked proliferation of fibrous connective tissue dissects and isolates groups of neoplastic cells. HE, bar=200um. **(E)** Abundant fibrous connective tissue. MT, bar=200µm. **(F)** There is marked immunolabeling in the cytoplasm of neoplastic epithelial cells. Anti-CK-7 IHC, AEC chromogen, bar=50µm.

3. ARTIGO 2

Nesse item é apresentado o artigo intitulado:

Penile tuberculosis in a Bull

Andréia Vielmo, Bruna C. Lopes, Welden Panziera, Ronaldo M. Bianchi, Fabiana Q. Mayer, Luís A. Vielmo, Claudio S.L. Barros and David Driemeier

(Artigo publicado na revista *Journal of Comparative Pathology* 180:5-8, 2020)

INFECTIOUS DISEASE

Short title: Penile Tuberculosis in a Bull

Short Paper

Penile Tuberculosis in a Bull

A. Vielmo*, B.C. Lopes*, W. Panziera*, R.M. Bianchi*, F.Q. Mayer†, L.A. Vielmo§, C.S.L. Barros‡, D. Driemeier*

** Setor de Patologia Veterinária (SPV), Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária (FAVET), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, † Centro de Pesquisa em Saúde Animal, Instituto de Pesquisas Veterinárias Desidério Finamor, Eldorado do Sul, § Ministério da Agricultura, Pecuária e Abastecimento (MAPA), Santa Maria, and ‡ Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil*

Correspondence to A. Vielmo (e-mail: andreiavielmo@yahoo.com.br).

Summary

We describe a case of penile tuberculosis in a bull. The prepuce was thickened, firm and pale with multifocal to coalescent caseous nodules, and the inguinal and mesenteric lymph nodes were moderately enlarged. Lesions in the prepuce, penis and lymph nodes were characterized by multifocal to coalescent areas of caseous necrosis and marked granulomatous inflammation. Acid-fast bacilli were seen within necrotic foci and Langhans giant cells. *Mycobacterium tuberculosis* var. bovis was identified in inguinal lymph nodes by the polymerase chain reaction technique and by bacterial isolation. Although rare, tuberculosis should be considered in the differential diagnosis of granulomatous lesions in the genital tract of bulls.

Keywords: *Mycobacterium tuberculosis* var. bovis; penis; prepuce; tuberculous balanoposthitis

Bovine tuberculosis (bTB) is a chronic infectious disease with a worldwide distribution and is mainly caused by *Mycobacterium tuberculosis* var. bovis (Riojas *et al*., 2018), a Gram-positive, slow-growing aerobic bacillus (OIE, 2018). Tuberculosis is an important zoonosis and causes direct and indirect economic losses to livestock farming due to decreased production and condemnation of carcasses and animal by-products (Azami and Zinsstag, 2018).

Lesions of tuberculosis are characterized by granulomatous inflammation and caseous necrosis and can involve one or more organs (Cousins, 2001). Lesion location depends mainly on the route of infection (Domingo *et al.*, 2014). When exposure occurs via the respiratory route, lesions may occur in the lungs and regional lymph nodes whereas gastrointestinal infection may result in lesions in the mesenteric lymph nodes with subsequent systemic dissemination (Neill *et al*., 1994; Neill *et al*., 2001). Tuberculous lesions of the male reproductive tract have been rarely described in cattle (Trotter, 1903; Thoen *et al.*, 1977; Foster, 2016) but those reports lack information on the gross, histopathological and molecular features of the disease. Bovine tuberculosis is one of the most common infectious and zoonotic diseases in developing countries, including Brazil (Carvalho *et al.*, 2016; Bica *et al.*, 2018; Carneiro *et al.*, 2020), where it is endemic (MAPA, 2020). We now report a case of penile tuberculosis caused by *M. tuberculosis* var. bovis in a bull in Brazil. Our finding of bTB in this unusual anatomical location is relevant to the diagnosis and control of bovine tuberculosis.

Gross lesions, morphologically compatible with tuberculosis, were seen in the penis, prepuce and inguinal and mediastinal lymph nodes but not in any other organ, at meat inspection of a 7-year-old Aberdeen Angus bull. The animal had been used for natural mating in a farm located in the county of Santa Vitória do Palmar (33° 32′2” S,

53° 20´59” W), in Southern Brazil, and sent for routine slaughter without any indication of bTB infection. Samples of penis, prepuce and inguinal and mediastinal lymph nodes were collected in the abattoir and sent to the Veterinary Pathology Laboratory of the Universidade Federal do Rio Grande do Sul for pathological examination. The prepuce was thickened, firm, and its internal surface was pale. The prepuce also had multifocal to coalescent nodules, 0.5-5.0 cm in diameter, that contained slightly granular, centrally located caseous yellowish material. Similar lesions, 1.0-4.0 cm in diameter, were seen along the penis close to the sigmoid flexure and its distal extremity (Fig. 1). The penile lesions involved the corpus cavernosum and corpus spongiosum. The lymph nodes were moderately enlarged with multifocal areas of caseous necrosis, which obliterated the parenchyma (Fig. 2). Tissue samples were fixed in 10% neutral buffered formalin, processed routinely for histopathology, embedded in paraffin-wax and stained with haematoxylin and eosin (HE) and by the Ziehl-Neelsen (ZN) method. Duplicate tissue samples were frozen at -20°C for bacterial culture and molecular analysis.

For molecular analysis, tissue fragments (0.5 cm^3) were subjected to DNA extraction followed by a polymerase chain reaction (PCR) technique, specific to *M. tuberculosis* var. bovis, as described (Mayer *et al.*, 2012; Maciel *et al*., 2018). PCR was performed on a ProFlex™ thermal cycler (Thermo Fischer Scientific, Waltham, Massachussets, USA) with positive and negative controls. For bacterial culture, the samples were decontaminated with 2% sodium hydroxide and inoculated in Stonebrink-Leslie and Löwenstein-Jensen culture media following the procedures recommended by the World Organisation for Animal Health (OIE, 2018). The inoculated samples were incubated at 37 °C aerobically and observed periodically until the appearance of suspicious colonies. After 100 days, one bacterial colony was observed in a sample of inguinal lymph node sample and confirmed as *M. tuberculosis* var. bovis by PCR.

Histopathological examination revealed multifocal to coalescent, often mineralized, areas of caseous necrosis in the penis, prepuce and lymph nodes. Necrotic areas were surrounded by a marked inflammatory cell infiltrate comprising epithelioid macrophages, lymphocytes, plasma cells, and Langhans giant cells. Fibrous connective tissue surrounded the granulomatous nodules (Fig. 3). The penile lesions were moderate and involved the corpus cavernosum, corpus spongiosum and tunica albuginea. Extensive areas of caseous necrosis and granulomatous inflammation were observed predominantly in the mucosal aspect of the prepuce and on the mucosa of the penile shaft. Lymph node lesions were severe and obliterated the architecture of the cortex and medulla. Occasional acid-fast bacilli were observed within necrotic foci and in the cytoplasm of Langhans giant cells (Fig. 4).

Although negative by PCR, the histopathological changes in the penis and prepuce, including the presence of acid-fast bacilli, were characteristic of bTB which was confirmed by molecular analysis of an inguinal lymph node sample. Despite its low sensitivity, the histological identification of acid-fast bacilli is highly specific when associated with the characteristic gross and histological lesions of tuberculosis (Varello *et al.*, 2008).

Bovine tuberculosis lesions are frequently observed in the lungs, liver and tracheobronchial, mediastinal, retropharyngeal, mandibular, mesenteric, prescapular and prefemoral lymph nodes of affected animals (Terefe, 2014). Tuberculosis involving the male genital tract of cattle is rarely reported (Trotter, 1903; Thoen *et al.*, 1977; Foster, 2016) although uterine tuberculosis is often described (Schlafer and Foster, 2016). Unlike in cattle, the genitourinary tract is one of the most common sites of human extrapulmonary tuberculosis, although penile lesions are rare in both cattle and humans (Trotter, 1903; Thoen *et al.*, 1977; Angus *et al.*, 2001). *Mycobacterium tuberculosis* var.

tuberculosis is the most consistent isolate from human cases of tuberculosis (Rossi *et al.*, 1999; Angus *et al.*, 2001; Ryang *et al.*, 2015), although mycobacteriosis of the human genital tract, resembling tuberculosis, can also be caused by *Mycobacterium avium-intracellulare* (De Caprariis *et al.*, 1984) and *Mycobacterium celatum* (Dahl *et al.*, 1996).

Tuberculosis of the male genital tract in cattle can be acquired from mating with a female that has genital tuberculosis (Domingo *et al.*, 2014) and occasionally is the only site of the disease (Foster, 2016). Although not confirmed, and the bTB status of this herd was unknown, a similar route of transmission may have occurred in this bull with subsequent haematogenous or lymphatic dissemination to lymph nodes. However, the presence of lesions in the mediastinal lymph nodes suggests that infection may have been by the respiratory route even though lesions were not observed in the lungs.

The main differential diagnoses of bTB include *Actinomyces bovis* (Basile and Diniz, 1979) and *Actinobacillus lignieresii* (Rycroft and Garside, 2000) infections, which can cause similar gross lesions. Histopathological lesions of actinomycosis and actinobacillosis differ from those of bTB in that they comprise pyogranulomatous inflammation associated with the presence of Splendore-Hoeppli bodies (Tessele *et al.*, 2014). Tuberculosis should be considered as a potential cause of caseocalcareous lesions in the reproductive tract of bulls.

Funding

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for supporting this study.

Conflict of Interest Statement

The authors declare no conflicts of interest with respect to the publication of this manuscript.

References

Angus BJ, Yates M, Conlon C, Byren I (2001) Cutaneous tuberculosis of the penis and sexual transmission of tuberculosis confirmed by molecular typing. *Clinical Infectious Diseases*, **33**, e132-e134.

Azami HY, Zinsstag J (2018) Economics of bovine tuberculosis: a one health issue. In: *Bovine tuberculosis*, Chambers M, Gordon S, Olea-Popelka F, Barrow P, CABI Internacional, Boston, pp.31-42.

Basile JR, Diniz JMF (1979) Actinomicose no pênis de bovino. *Semina,* **1**, 45-46.

Bica RFP, Copetti MV, Brum MCS (2018) Hydatidosis, cysticercosis, and tuberculosis rates in bovine slaughtered under state sanitary inspection in Rio Grande do Sul, Brazil. *Ciência Rural*, **48**, e20170811.R2.

Carneiro PAM, Pasquatti TN, Takatani H, Zumárragas MJ, Marfil MJ *et al*. (2020) Molecular characterization of *Mycobacterium bovis* infection in cattle and buffalo in Amazon region, Brazil. *Veterinary Medicine and Science*, **6**, 133–141.

Carvalho RCT, Vasconcellos SEG, Issa MDA, Soares FPM, Mota PMPC *et al*. (2016) Molecular typing of *Mycobacterium bovis* from cattle reared in midwest Brazil. *PLoS One,* **11**, e0162459.

Cousins DV (2001) *Mycobacterium bovis* infection and control in domestic livestock. *Revue Scientifique et Technique de l'OIE,* **20**, 71-85.

Dahl DM, Klein D, Morgentaler A (1996) Penile mass caused by the newly described organism *Mycobacterium celatum*. *Urology,* **47**, 266-268.

De Caprariis PJ, Giron JA, Goldstein JA, LaBombardi VJ, Guarneri JJ *et al*. (1984) *Mycobacterium* avium-intracellulare infection and possibly venereal transmission. *Annals of Internal Medicine,* **101**, 721.

Domingo M, Vidal E, Marco A (2014) Pathology of bovine tuberculosis. *Research in Veterinary Science,* **97**, S20-S29.

Foster RA (2016) Male genital system. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 6th Edit., Vol. 3, MG Maxie, Ed., Elsevier, St. Louis, pp. 465-510.

Maciel ALG, Loiko MR, Bueno TS, Moreira JG, Coppola M *et al*. (2018) Tuberculosis in Southern Brazilian wild boars (*Sus Scrofa*): first epidemiological findings. *Transboundary and Emerging Diseases*, **65**, 518-526.

MAPA (Ministério da Agricultura, Pecuária e Abastecimento) Coordenação de Informação e Epidemiologia – Saúde Animal (2020). http://indicadores.agricultura.gov.br/saudeanimal/index.htm Last accessed 21 May 2020

Mayer FQ, Cerva C, Driemeier D, Da Cruz CEF, Loiko MR *et al*. (2012) *Mycobacterium bovis* infection in a collared peccary (*Tayassu tajacu*): insights on tuberculosis wild reservoirs. *Veterinary Microbiology,* **160**, 549-551.

Neill SD, Pollock JM, Bryson DB, Hanna J (1994) Pathogenesis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology*, **40**, 41-52.

Neill SD, Bryson DG, Pollock JM (2001) Pathogenesis of tuberculosis in cattle. *Tuberculosis*, **81**, 79-86.

OIE (World Organization for Animal Health) *Bovine tuberculosis* (2018) https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.04.06_BOVINE_TB.pdf Accessed 21 May 2020

Riojas MA, McGough KJ, Rider-Riojas CJ, Rastogi N, Hazbón MH (2018) Phylogenomic analysis of the species of the *Mycobacterium tuberculosis* complex demonstrates that *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium microti* and *Mycobacterium pinnipedii* are later heterotypic synonyms of *Mycobacterium tuberculosis*. *International Journal of Systemic and Evolutionary Microbiology*, **68**, 324-332.

Rossi R, Urbano F, Tortoli E, Trotta M, Zuccati G *et al*. (1999) Primary tuberculosis of the penis. *Journal of the European Academy of Dermatology and Venereology,* **12**, 174-176.

Ryang SH, Eom M, Kang TW, Lee CM, Chung HC *et al*. (2015) Penile mass caused by *Mycobacterium tuberculosis*. *Urogenital Tract Infection,* **10**, 126-129.

Rycroft AN, Garside LH (2000) *Actinobacillus* species and their role in animal disease. *The Veterinary Journal,* **159**, 18-36.

Schlafer DH, Foster RA (2016) Female genital system. In: *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*, 6th Edit., MG Maxie, Ed., WB Saunders, St. Louis, Missouri, pp. 358-464. e351.

Terefe D (2014) Gross pathological lesions of bovine tuberculosis and efficiency of meat inspection procedure to detect-infected cattle in Adama municipal abattoir. *Journal of Veterinary Medicine and Animal Health,* **6**, 48-53.

Tessele B, Martins TB, Vielmo A, Barros CSL (2014) Granulomatous lesions found in cattle slaughtered for meat production. *Pesquisa Veterinária Brasileira,* **34**, 763-769.

Thoen CO, Himes EM, Stumpff CD, Parks TW, Sturkie HN (1977) Isolation of *Mycobacterium bovis* from the prepuce of a herd bull. *American Journal of Veterinary Research,* **38**, 877-878.

Trotter AM (1903) Tuberculosis of the penis of a bull. *Journal of Comparative Pathology and Therapeutics,* **16**, 252-253, IN5.

Varello K, Pezzolato M, Mascarino D, Ingravalle F, Caramelli M *et al*. (2008) Comparison of histologic techniques for the diagnosis of bovine tuberculosis in the framework of eradication programs. *Journal of Veterinary Diagnostic Investigation,* **20**, 164-169.

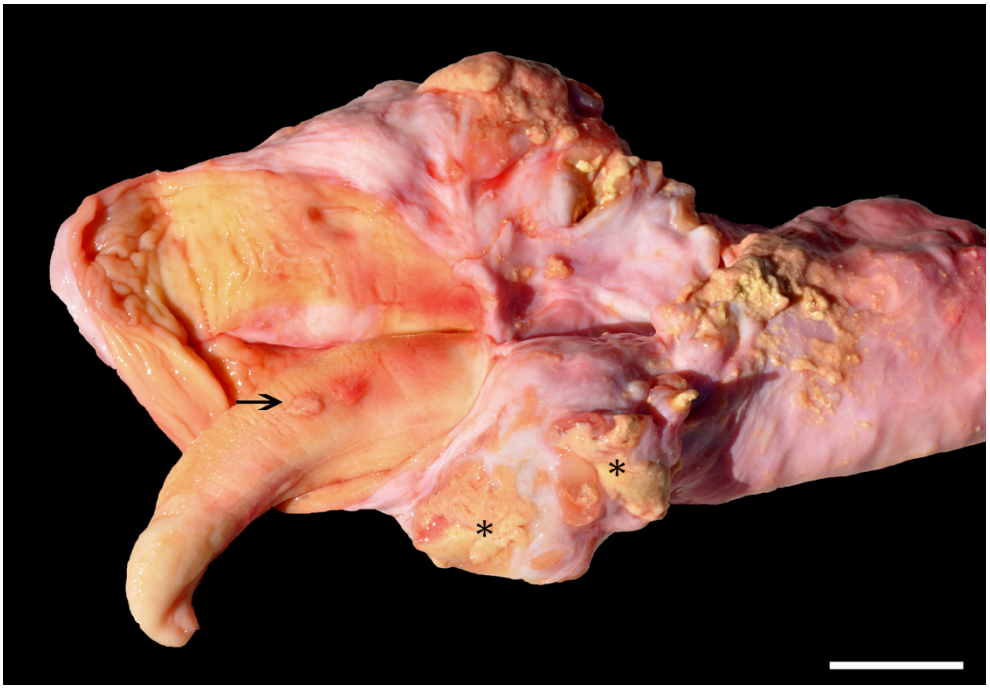

Fig. 1. Bull, prepuce. Multifocal nodular to coalescent foci of yellow caseous material in thickened, pale preputial mucosa (asterisks) and distal penis (arrow). Bar, 4.5 cm.

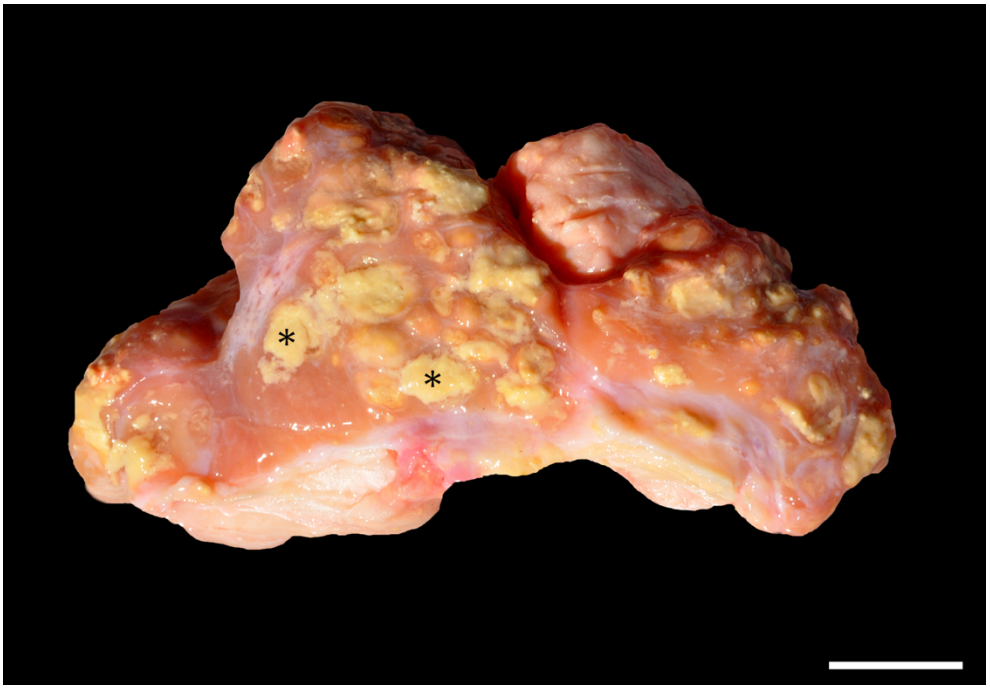

Fig. 2. Bull, inguinal lymph node. Yellow multifocal to coalescent foci throughout nodal parenchyma (asterisks). Bar, 3.1 cm.

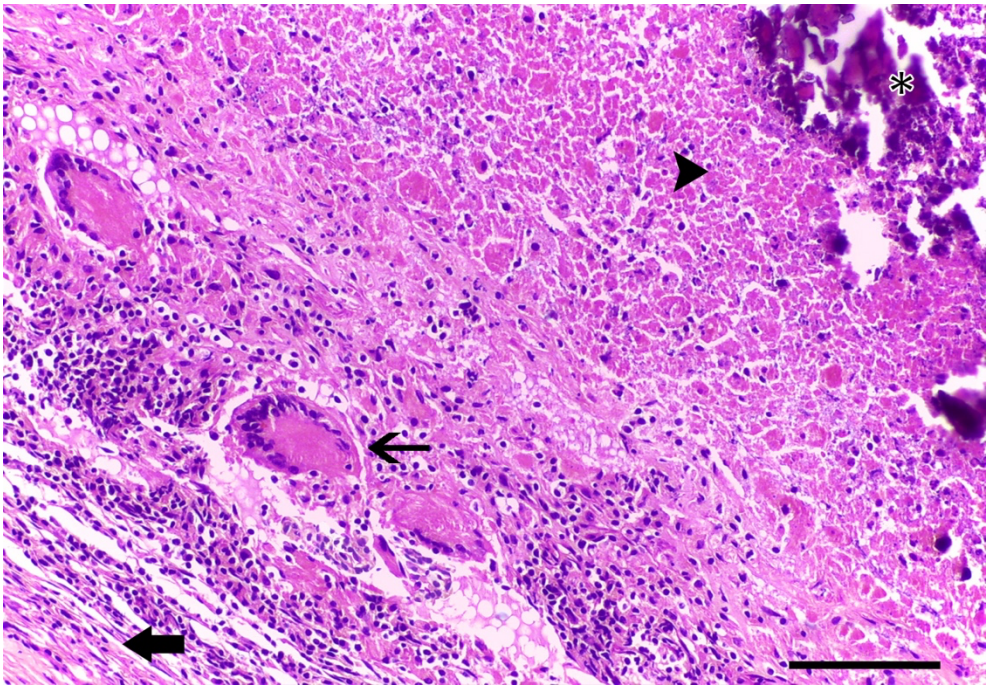

Fig. 3. Bull, prepuce. Granuloma comprising caseous necrosis (arrowhead) with central mineralization (asterisk) bordered by dense infiltrate of epithelioid macrophages, lymphocytes, plasma cells and Langhans giant cells (thin arrow), is surrounded by fibrous tissue (thick arrow). HE. Bar, 100 µm.

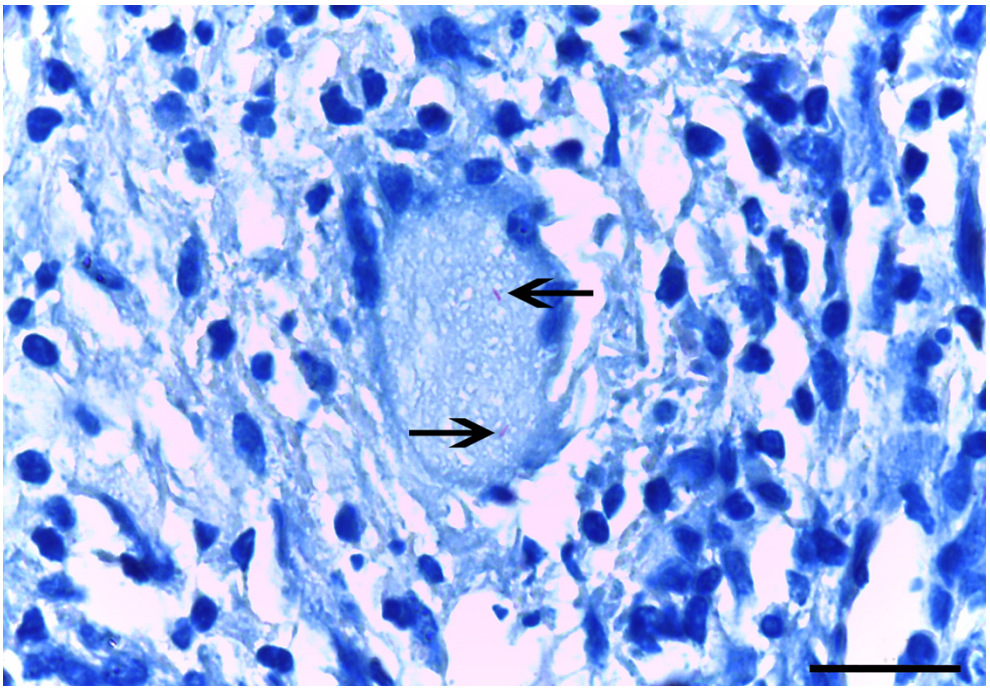

Fig. 4. Bull, prepuce. Acid-fast bacilli (arrows) in cytoplasm of a Langhans giant cell. ZN. Bar, 20 µm.

4. ARTIGO 3

Nesse item é apresentado o artigo intitulado:

Histological and immunohistochemical features of carcinomas with pulmonary involvement in cattle

Andréia Vielmo, Igor R. Santos, Manoela M. Piva, Marcele B. Bandinelli, Saulo P. Pavarini, Welden Panziera and David Driemeier

(Artigo a ser submetido para a revista *Veterinary Pathology*)

5. CONSIDERAÇÕES FINAIS

- Os resultados aqui apresentados permitem concluir que carcinoma hepatocelular é o tumor hepático mais frequentemente diagnosticado em bovinos.
- Vacas adultas são as mais acometidas por neoplasias neste estudo.
- Adenocarcinomas foram os tumores primários pulmonares mais frequentes em bovinos.
- TTF-1 demonstrou ser um bom imunomarcador para confirmação da origem de tumores primários pulmonares.
- Imuno-histoquímica foi uma excelente ferramenta para determinar o tipo celular de tumores hepáticos e pulmonares.
- Tuberculose deve ser considerada um importante diagnóstico para lesões caseocalcáreas no trato reprodutivo de touros.

REFERÊNCIAS BIBLIOGRÁFICAS

ABIEC. Associação Brasileira das Indústrias Exportadoras de Carne. Beef Report – Perfil da Pecuária no Brasil, 2021. Disponível em: http://abiec.com.br/publicacoes/beef-report-2021/

BIANCHI, R.M. et al. Pathological and microbiological characterization of mastitis in dairy cows. Tropical Animal Health and Production, v.51, n.7, p.2057-2066, 2019.

BIDONE, N.B. et al. Slaughter condemnation in bovine due to parasitic lesions and their economic impact in Federal Inspection System establishments in Brazil and in State inspection System in Rio Grande do Sul State. Brazilian Journal of Veterinary Parasitology, v.30, n.1, e022720, 2021.

CARVALHO, R. C. T. Molecular typing of Mycobacterium bovis from cattle reared in Midwest Brazil. Plos One, v.11, p.1-16, 2016.

DOMINGO, M. et al. Pathology of bovine tuberculosis. Research in Veterinary Science, v.97, S20–S29, 2014.

DUPUY, C. et al. Factors associated with offal, partial and whole carcass condemnation in ten french cattle slaughterhouses. Meat Science, v. 97, p. 262–269, 2014.

DUTRA, L. H. et al. Mapping risk of bovine fasciolosis in the south of Brazil using Geographic Information Systems. Veterinary Parasitology, v.169, p. 76-81, 2010.

FRUET, A.P.B. et al. Perdas econômicas oriundas das condenações de vísceras bovinas em matadouros de Santa Maria, Rio Grande do Sul. Revista Brasileira de Ciência Veterinária, v.20, n.2, p.99-103, 2013

FURLANETTO, L. V. et al. Prevalência de tuberculose bovina em animais e rebanhos abatidos em 2009 no estado de Mato Grosso, Brasil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v.64, n.2, p.274-280, 2012.

JEDREJEK, D. et al. Animal by-products for feed: characteristics, European regulatory framework, and potential impacts on human and animal health and the environment. Journal of Animal and Feed Sciences, v.25, p.189-202, 2016.

MARQUES, G. M. et al. Avaliação dos registros de condenação por cisticercose em bovinos abatidos em frigoríficos da região centro oeste do estado de São Paulo – 1996 a 2000. Veterinária e Zootecnia, v.15, n.1, p.114-120, 2008.

MINISTÉRIO DA AGRICULTURA - Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (R.I.I.S.P.O.A). Aprovado pelo decreto no. 9013/2017 Brasília, 2017, 66p

PANZIERA, W. et al. Aspectos macroscópicos e histológicos da cisticercose bovina. Pesquisa Veterinária Brasileira, v. 37, p. 1220-1228, 2017.

PANZIERA, W. et al. Atypical parasitic lesions in slaughtered cattle in Southern Brazil. Brazilian Journal of Veterinary Parasitology, v.29, n.3, e001720, 2020.

QUEIROZ, M. R. et al. Epidemiological status of bovine tuberculosis in the state of Rio Grande do Sul, Brazil. Semina: Ciências Agrárias, v.37, p.3647-3658, 2016.

SANTOS, D.V. et al. Análise das principais lesões encontradas nos abatedouros registrados na CISPOA. Informativo Técnico, n.4, Ano 1, 2010. Disponível em: <http://www2.agricultura.rs.gov.br/uploads/1284486430Informativo_Tecnico_DPA_N.__04.pdf>. Acesso em: 21 fev. 2022.

SCHENK, M. A. M.; SCHENK, J. A. P. Prevalência de tuberculose, cisticercose e hidatidose em bovinos abatidos nos matadouros-frigoríficos do estado de Mato Grosso do Sul, Brasil (1974/1979). Comunicado Técnico – EMBRAPA. 1982.

TESSELE, B. et al. Lesões parasitárias encontradas em bovinos abatidos para consumo humano. Pesquisa Veterinária Brasileira, Rio de Janeiro, v. 33, n. 7, p. 873-889, 2013a.

TESSELE, B. et al. Miosite eosinofílica em bovinos abatidos para consumo humano. Pesquisa Veterinária Brasileira, v.33, n.11, p.1345-1348, 2013b.

TESSELE, B. et al. Lesões granulomatosas encontradas em bovinos abatidos para consumo. Pesquisa Veterinária Brasileira, v.34, n.8, p.763-769, 2014.

TESSELE, B. & BARROS, C. S. L. Tumores em bovinos encontrados em abatedouros frigoríficos. Pesquisa Veterinária Brasileira, v. 36, p. 145-160, 2016.

VIAL, F. et al. Risk factors for whole carcass condemnations in the swiss slaughter cattle population. Plos One, v.10, n.4, p. 1-17, 2015.

VIDAL, E. et al. Six-Year Follow-up of slaughterhouse surveillance (2008–2013): the Catalan slaughterhouse support network (SESC). Veterinary Pathology, p. 1-13, 2015.

UNGAR, M. C. et al. O valor dos registros de matadouros para Saúde Pública. Revista científica da Faculdade de Veterinária da USP. v. 14, p. 91-97, 1990.