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

Doenças de bezerros no Sul do Brasil

Tese de Doutorado

Aluno executor: Claiton Ismael Schwertz
Orientador: Dr. David Driemeier
Coorientador: Dr. Saulo Petinatti Pavarini

Porto Alegre, Rio Grande do Sul
Setembro de 2022

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Tese apresentada como requisito parcial para obtenção do grau de Doutor em Ciências Veterinárias na área de concentração em Medicina Veterinária Preventiva e Patologia: Patologia Animal e Patologia Clínica.

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DOENÇAS DE BEZERROS NO SUL DO BRASIL

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RESUMO

As perdas de bezerros influenciam negativamente a lucratividade dos rebanhos bovinos e muitas propriedades trabalham com taxas acima das aceitáveis. O objetivo desta pesquisa foi determinar quais as principais doenças que causam mortalidade de bezerros no Sul do Brasil, mediante um estudo retrospectivo. Para tal, foram revisados os laudos de protocolos de necropsias de bezerros recebidos pelo Setor de Patologia Veterinária da Universidade Federal do Rio Grande do Sul (SPV-UFRGS) no período de 2005 a 2020, e elaborados dois artigos. No primeiro, foram compilados os dados obtidos do levantamento e discriminados quanto aos sistemas envolvidos, sexo, aptidão (corte ou leiteira) e faixa etária (0-3, 4-6, 7-9 e 10-12 meses de idade). 626 casos conclusivos foram considerados. Houve maior frequência de bovinos de corte (55,5%), raças leiteiras somaram 24,1% e em 20,4% essa informação era indisponível. As doenças do aparelho digestivo (24,9%) foram as mais frequentes, seguidas das doenças do sistema hemolinfático (18,5%). Doenças infecciosas e parasitárias predominaram (73%), seguidas de doenças tóxicas (9,5%) e transtornos do desenvolvimento (5,1%). Para o cálculo de frequência de diagnósticos por faixa etária e aptidões, apenas 499 casos dentre os 626 foram utilizados, pois nos 127 restantes essas informações eram indisponíveis. As condições mais frequentes foram enterites com 16% (80/499), que predominaram na faixa de 0-3 meses e em raças leiteiras; tristeza parasitária bovina com 15,6% (78/499) e presença constante nas faixas a partir de 4 meses, tanto em raças leiteiras quanto de corte; e pneumonias com 8,2% (41/499) e maior proporção de casos entre 0 e 6 meses, tanto em raças leiteiras quanto de corte. Esses dados mostram que medidas sanitárias que buscam prevenir doenças entéricas, respiratórias e hemoparasitárias podem ajudar a reduzir a mortalidade de bovinos no sul do Brasil. Já no segundo artigo, foi descrito um surto de condrodisplasia de origem nutricional em bovinos leiteiros. Nesse trabalho foram investigados casos de nascimento de bezerros com defeitos congênitos. Apenas bezerros nascidos de novilhas foram afetados e a doença ocorreu em animais mestiços e puros. Três necropsias foram realizadas, tecidos foram coletados para histopatologia e amostras de fígado de bezerros, soro sanguíneo e alimentos fornecidos para vacas e novilhas foram coletados para quantificar os níveis dos minerais: manganês, cobre e zinco. Os bezerros nasceram fracos, com nanismo desproporcional, deformidades nos membros e articulações aumentadas. As cabeças eram encurtadas e arredondas. Os ossos longos apresentavam diáfise encurtada e epífise de tamanho normal, quando comparados aos controles. A histopatologia das placas de crescimento revelou fechamento prematuro, desarranjo das colunas de condrócitos e colapso da esponjosa primária. Os níveis de manganês no fígado estavam abaixo dos valores de referência nos três bezerros. A análise dos alimentos revelou níveis insuficientes de manganês na dieta das novilhas, principalmente na silagem de sorgo, que foi fornecida como principal fonte de alimento para a categoria em alguns períodos. Nossos achados permitiram concluir o diagnóstico de condrodisplasia de origem nutricional e reforçar a tese de que o manganês é o mineral deficiente nesses casos.

Palavras-chave: Bovinocultura, bovinos neonatos e jovens, patologia animal, doenças infecciosas, doenças nutricionais.

ABSTRACT

Calf losses negatively influence the profitability of cattle herds and many farms work with rates above acceptable. The objective of this research was to determine which are the main diseases that cause calf mortality in Southern Brazil, through a retrospective study. For this purpose, the pathology reports of calf necropsies received by the Setor de Patologia Veterinária of the Universidade Federal do Rio Grande do Sul (SPV-UFRGS) from 2005 to 2020 were reviewed, and two manuscripts were written. In the first, the data obtained from the survey were compiled and discriminated according to the systems involved, sex, aptitude (beef or dairy), and age group (0-3, 4-6, 7-9 and 10-12 months of age). 626 conclusive cases were considered. There was a higher frequency of beef cattle (55.5%), dairy breeds totaling 24.1% and in 20.4% this information was unavailable. Diseases of the digestive system (24.9%) were the most frequent, followed by diseases of the hemolymphatic system (18.5%). Infectious and parasitic diseases predominated (73%), followed by toxic diseases (9.5%) and developmental disorders (5.1%). To calculate the frequency of diagnoses by age group and aptitude, only 499 cases out of the 626 were used, as in the remaining 127 these information was unavailable. The most frequent conditions were enteritis with 16% (80/499), which predominated in the range of 0-3 months and in dairy breeds; hemoprotozoal infection with 15.6% (78/499) and constant presence in the age ranges from 4 months on, both in dairy and beef breeds; and pneumonias with 8.2% (41/499) and higher proportion of cases between 0 and 6 months, both in dairy and beef breeds. These data show that sanitary measures that seek to prevent enteric, respiratory and hemoparasitic diseases can help to reduce the mortality of cattle in southern Brazil. In the second manuscript, an outbreak of chondrodysplasia of nutritional origin in dairy cattle was described. In this work, cases of birth of calves with congenital defects were investigated. Only calves born to heifers were affected and the disease occurred in both crossbred and purebred animals. Three necropsies were performed, tissues were collected for histopathology and liver samples from calves, blood serum and feed supplied to cows and heifers were collected to quantify the levels of minerals: manganese, copper and zinc. The calves were born weak, with disproportionate dwarfism, limb deformities and enlarged joints. The heads were shortened and domed. Long bones had a shortened diaphysis and normal-sized epiphysis when compared to controls. Histopathology of the growth plates revealed premature closure, disarrangement of the chondrocyte columns and collapse of the primary spongiosa. Liver manganese levels were below reference values in all three calves. Feed analysis revealed insufficient levels of manganese in the heifers diet, mainly in sorghum silage, which was provided as the main feed source for the category in some periods. Our findings allowed us to conclude the diagnosis of chondrodysplasia of nutritional origin and reinforce the thesis that manganese is the deficient mineral in these cases.

Key-words: Cattle, newborn and young cattle, animal pathology, infectious diseases, nutritional diseases.

SUMÁRIO

1 INTRODUÇÃO

Segundo os dados da Pesquisa da Pecuária Municipal e da Estatística da Produção Pecuária do IBGE (2020), em 2020 o Brasil contava com aproximadamente 218,2 milhões de bovinos, produziu cerca de 35,4 bilhões de litros de leite e exportou 1,7 milhão de toneladas de carne bovina in natura, uma alta de 10% em relação ao ano anterior. A região sul do país é destaque principalmente na produção de leite, sendo responsável por 34% da produção nacional, com 12,1 bilhões de litros produzidos. Em 2020, o Rio Grande do Sul contou com um rebanho bovino de aproximadamente 11,1 milhões de cabeças e foi responsável pela terceira maior produção de leite no país, com 4,3 bilhões de litros. Dessa forma, a bovinocultura é uma atividade de grande importância no estado, pois movimenta a economia e gera empregos diretos e indiretos.

As perdas perinatais e de animais jovens são comuns e uma taxa de até 5% de mortes do nascimento até os três primeiros meses de vida é considerada aceitável (Machado Neto *et al*., 2004). No entanto, altas taxas de mortalidade têm sido reportadas em alguns trabalhos ao redor do mundo, como 9,4% de mortalidade de bezerros leiteiros no pré-desmame nos Estados Unidos (Losinger & Henrichs 1997); 12,9% de mortalidade em bezerros até um ano de vida na China (Zhang *et al*., 2019); e 14,9% em Minas Gerais, Brasil (Frois & Viegas 1994). Em outro estudo, também realizado no Brasil, no Estado de Goiás, os autores acompanharam a mortalidade de bezerros em uma propriedade leiteira por quatro anos (1994-1997), sendo que, inicialmente, a mortalidade era de 18,65% e passou para 4,67% ao final do estudo, mediante a melhorias sucessivas no manejo dos animais (Silva *et al*., 2001).

No Rio Grande do Sul, um levantamento realizado no Laboratório Regional de Diagnóstico (LRD) da Faculdade de Veterinária da Universidade Federal de Pelotas (UFPel) apontou que entre 2000 e 2011, 15,4% dos materiais de bovinos recebidos para diagnóstico anatomopatológico eram de animais de até um ano de idade (Assis-Brasil *et al*., 2013a). Em outro estudo realizado na Embrapa gado de Corte, no Mato Grosso do Sul, que compilou dados produtivos do sistema de produção de bovinos de corte da instituição durante nove anos, constatou-se que a mortalidade de bezerros foi em média de 6% ao longo dos anos e representou 53,7% da mortalidade total de bovinos (Corrêa *et al*., 2001). Esses dados demonstram a

importância das doenças dos bovinos jovens para os sistemas de produção, bem como a expressividade dessa categoria na casuística de laboratórios de diagnóstico.

Se os índices zootécnicos de um rebanho bovino forem satisfatórios, mas a taxa de mortalidade no primeiro ano de vida for alta, há um grande prejuízo em todo o trabalho previamente realizado. Em um sistema bem conduzido, a mortalidade é um fator muito importante, porque é influenciada por aspectos sanitários que elevam os custos de produção (Lopes *et al*., 2009).

A mortalidade pós-natal de bezerros de rebanhos leiteiros pode ser influenciada por vários fatores, dentre eles o tamanho do rebanho (rebanhos maiores têm maior mortalidade), pelo manejo do colostro e do aleitamento, pelo local onde os bezerros são criados, pela idade de acesso ao concentrado (quanto mais cedo melhor) e pelo período de desmame (mortalidade menor quando desmamados antes de completar três meses de idade) (Machado Neto *et al*., 2004). Um estudo com rebanhos leiteiros na China demonstrou alguns fatores de risco para uma maior mortalidade ou descarte de animais jovens. A estação do ano influenciou significativamente, sendo que os bezerros nascidos na primavera tiveram menor mortalidade, ao contrário dos nascidos no outono. A ordem de parto da mãe também teve influência na taxa de sobrevivência, sendo que os bezerros jovens nascidos de vacas primíparas tiveram 1,16 vezes mais chances de morrer do que bezerros nascidos de vacas de segunda ordem de parto. Os animais nascidos mediante assistência ao parto ou com necessidade de intervenção cirúrgica tiveram o maior risco de mortalidade, o que não foi estatisticamente significativo se comparado aos bezerros que nasceram sem necessidade de assistência (Zhang *et al*., 2019).

A adequada colostragem é de extrema importância para a saúde e sobrevivência dos bezerros. A falha de transferência de imunidade passiva colostral (FTIP) em bezerras leiteiras de reposição tem sido associada a morbidade e mortalidade neonatal e redução na produtividade a longo prazo (Beam *et al.*, 2009). A FTIP é frequente e em um estudo realizado no Brasil, acometeu de 10% a 20% dos bezerros avaliados, dependendo do parâmetro considerado (Feitosa *et al*., 2010). No entanto, os mesmos autores inferem que nem sempre a constatação da ausência de concentrações séricas adequadas de imunoglobulinas em bezerros neonatos implica diretamente na ocorrência de doenças, mas a ocorrência de FTIP deve ser considerada como indicador de maior probabilidade de morbidade e mortalidade, o que reforça a importância de algumas medidas de segurança, tais como o fornecimento de colostro de boa qualidade, no

momento preciso e em quantidade suficiente para manter a higidez do plantel e favorecer a sobrevivência dos bezerros recém-nascidos. Beam et al. (2009) estimaram uma prevalência de 19,2% de FTIP em bezerros de fazendas leiteiras nos EUA, tendo como critério de diagnóstico uma concentração de IgG menor que 10 mg/mL no sangue coletado entre o primeiro e o sétimo dia de vida. Com base nos resultados do trabalho, esses autores sugerem que os produtores forneçam colostro manualmente aos bezerros dentro das primeiras quatro horas de vida, e que os bezerros nascidos em partos complicados devem receber atenção extra para garantir uma adequada transferência de imunidade passiva.

Dentre as principais doenças que acometem os bezerros, vários estudos destacam os distúrbios digestivos e respiratórios (USDA 2010; Assis-Brasil *et al*., 2013a; Zhang *et al*., 2019). Nos EUA, relata-se que os problemas digestivos (p. ex. enterites) são a principal causa de morte de bezerros lactentes, enquanto que os problemas respiratórios (p. ex. pneumonias) são a principal causa de morte de bezerros desmamados (USDA 2010). No sul do Rio Grande do Sul, foi relatado que as doenças neurológicas, digestivas e respiratórias foram as mais frequentes em bovinos de até um ano de idade. Os autores relatam que a elevada proporção de doenças neurológicas é pontual, uma vez que no período do estudo foram diagnosticados vários surtos de raiva e tétano (Assis-Brasil *et al*., 2013a). As doenças respiratórias, por sua vez, foram frequentes em bovinos desde as primeiras semanas de vida até um ano de idade, e foram associadas principalmente à pneumonia enzoótica por infecção pelo vírus sincicial respiratório bovino (BRSV) (Assis-Brasil *et al*., 2013a; Assis-Brasil *et al*., 2013b).

Dentre os distúrbios digestivos associados a doença clínica e mortalidade de bezerros destacam-se as diarreias, que levam a queda de desempenho, desidratação e, nos casos mais severos, a morte (Langoni *et al*., 2004; Schuch 2007). Os agentes etiológicos mais encontrados são *Escherichia coli*, Rotavírus, Coronavírus e *Clostridium perfringens* em bezerros de até um mês de vida, enquanto em animais com um a seis meses, *Eimeria* spp., *Cryptosporidium* spp. e *Salmonella* spp., juntamente com os parasitos gastrointestinais, são os principais agentes. O aparecimento de diarreia nos animais está sempre associado a fatores predisponentes que envolvem condições de manejo, higiênico-sanitárias, imunidade e nutrição dos bezerros (Schuch 2007). Associações entre agentes também ocorrem, podem agravar o quadro clínico e tornam necessário o emprego de diferentes técnicas de diagnóstico para a identificação dos diferentes microrganismos envolvidos (Langoni *et al*., 2004; Schuch 2007).

A inflamação do umbigo e das suas estruturas associadas ocorrem comumente nos bezerros recém-nascidos. Pode ocorrer logo após o nascimento e resultar em onfalite, onfaloflebite, onfaloarterite e/ou infecção do úraco, sendo os quadros mais graves associados com a FTIP (Naik S. *et al*., 2011). Uma consequência comum de infecções umbilicais é a disseminação hematógena de bactérias (sepse), que culmina em processos inflamatórios diversos, como meningite/meningoencefalite, poliartrite, pericardite e abscessos hepáticos (Assis-Brasil *et al*., 2013; Konradt *et al*., 2017).

Além das doenças mais comuns, que foram previamente mencionadas, há relatos da ocorrência de surtos de várias outras doenças que acometem bezerros no Rio Grande do Sul. Pierezan *et al*. (2010) descreveram um surto de aflatoxicose em bezerros de quatro meses de idade alimentados com milho moído contaminado. A análise do milho, nessa oportunidade, revelou 5.136 ppb de aflatoxina B_1. Cunha *et al*. (2010) relataram um surto de polioencefalomalacia por ingestão excessiva de enxofre na dieta de bezerros com 10 a 12 meses de idade. Os autores concluíram o diagnóstico mediante dosagens do enxofre nos componentes da dieta, além de mensuração de sulfeto de hidrogênio ruminal e exclusão de diagnósticos diferenciais. Também foi descrita, em bezerros no Rio Grande do Sul, a ocorrência da síndrome do abscesso pituitário associada ao uso de tabuleta nasal para desmame (Loretti *et al*., 2003). Segundo os autores, o uso da tabuleta para desmame interrompido pode ocasionar lesões na mucosa nasal, a partir das quais a bactéria pode chegar até a pituitária através da circulação sanguínea ou linfática, e causar abscessos. Outra doença neurológica descrita em bovinos no Rio Grande do Sul e que acomete principalmente animais jovens, é a meningoencefalite por herpesvírus bovino, que tem sido diagnosticada em bezerros submetidos a situações estressantes como desmame e agrupamento de animais (Rissi *et al*., 2008; Wronski *et al*., 2018). A raiva, que pode acometer animais de qualquer idade, foi responsável por 29,6% das enfermidades de bezerros que apresentavam sinais nervosos em um estudo no Sul do Rio Grande do Sul (Santos *et al*., 2018). Os autores chamam a atenção para a prática de vacinação contra a doença, que deve ser realizada antes dos três meses de idade.

Os defeitos congênitos também geram perdas de bovinos jovens e representaram 0,88% da casuística de necropsia de bovinos em um estudo (Marcolongo-Pereira *et al*., 2010). Nesse mesmo trabalho, dentre os diversos defeitos congênitos diagnosticados, destacaram-se os que afetaram o sistema esquelético (43,75%), sistema nervoso central (18,75%) e o sistema muscular

(18,75%). Os defeitos congênitos esporádicos representaram 45,83%, e os hereditários (ou provavelmente hereditários) representaram 35,41% dos casos. Por outro lado, as malformações associadas a causas ambientais, embora em menor proporção (16,6%), causaram prejuízos econômicos mais significativos, devido ao maior número de animais acometidos. Em outro estudo no Rio Grande do Sul realizado por Macêdo *et al*. (2011), os defeitos congênitos que acometeram o sistema nervoso central foram os mais frequentes (28,3%), seguidos pelos defeitos do aparelho urogenital (17%) e musculoesquelético (15,1%). Nessa pesquisa, os bovinos com defeitos congênitos representaram 0,4% da casuística de bovinos de um laboratório de diagnóstico.

Visto um pouco da grande variedade de doenças que podem acometer os bezerros, cada qual com suas particularidades, tornam-se necessários estudos que agreguem conhecimento geral sobre a frequência dessas doenças, bem como os principais aspectos etiológicos, epidemiológicos, clínicos e patológicos relacionados à sua ocorrência. O conhecimento agregado a esse tema pode auxiliar profissionais da área e produtores a prevenir, diagnosticar, controlar e tratar adequadamente as enfermidades de bezerros.

2 OBJETIVOS

2.1 Objetivo geral

Determinar quais são as principais doenças que causam mortalidade de bovinos do nascimento até um ano de idade no Rio Grande do Sul.

2.2 Objetivos específicos

- Determinar a frequência das doenças que cursam com mortalidade de bezerros;
- Caracterizar a etiologia, sistemas orgânicos envolvidos e alguns aspectos epidemiológicos dos principais diagnósticos;
- Correlacionar os diagnósticos a aspectos epidemiológicos, tais como: sexo, grupos etários e aptidão produtiva (corte ou leiteira);
- Descrever um surto de condrodisplasia de origem nutricional em bezerros, descrevendo os aspectos epidemiológicos, clínicos e patológicos desta condição ainda não descrita no Brasil.

3 RESULTADOS

Neste item serão apresentados os dois artigos que foram elaborados a partir da pesquisa realizada no doutorado do estudante. O artigo 1 trata-se de um estudo retrospectivo da casuística do Setor de Patologia Veterinária da UFRGS, que compila dados de 626 casos de necropsias de bezerros, com diagnóstico conclusivo. Já o artigo 2 trata-se do relato de um surto de condrodisplasia de origem nutricional em bovinos leiteiros.

3.1 Artigo 01 - Causes of death of calves up to one year old in Southern Brazil

Este artigo será submetido em breve ao periódico Pesquisa Veterinária Brasileira.

Causes of death of calves up to one year old in Southern Brazil

Claiton I. Schwertz, Ronaldo M. Bianchi, Manoela M. Piva, Anderson Gris, Andréia Vielmo, Luciana Sonne, Saulo Petinatti Pavarini, and David Driemeier.

Abstract

Perinatal and young animal losses negatively influence the productive efficiency and profitability of cattle herds. Thus, this study aimed to determine which are the main diseases that cause mortality in cattle from birth to one year of life in the state of Rio Grande do Sul, Brazil, correlating with some epidemiological aspects, such as age group and aptitude (beef or dairy). A retrospective study of necropsy protocols of cattle between 0 and 12 months old was carried out. Data regarding sex, breed, aptitude (dairy or beef), and age range of calves were also obtained. Age groups were defined as: 0-3 months, 4-6 months, 7-9 months, and 10-12 months old. The diagnoses were categorized according to the organic system involved and divided according to disease etiology into six major groups. The most frequent diseases were further discriminated considering age group and aptitude of calves. In total, 626 conclusive cases were considered. There was a higher frequency of beef cattle (55.5%), dairy breeds totaling 24.1% and in the remaining 20.4% there was no information about the breed in the records. Diseases affecting the digestive system (24.9%) were the most frequent, especially in the 0-3 months old group, followed by diseases of the hemolymphatic system (18.5%), which were frequent in all age groups. Infectious and parasitic diseases were the predominant etiology (73%), followed by toxic diseases (9.5%), and developmental disorders (5.1%). To calculate the distribution of specific diagnoses by age group and comparison of aptitude (beef or dairy), only 499 cases out of 626 were used, as the remaining 127 did not include this information in the records. The most frequent conditions were enteritis with 16% (80/499), which predominated in the range of 0-3 months and in dairy breeds; hemoprotozoal infection with 15.6% (78/499) and constant presence in groups from 4 months on, both in dairy and beef breeds; and pneumonias with 8.2% (41/499) and higher proportion of cases between 0 and 6 months, both in dairy and beef breeds. These data

show that sanitary measures that seek to prevent enteric, respiratory and hemoparasitic diseases can greatly help to reduce cattle mortality in southern Brazil.

Key-words: Calf mortality; bovine pathology; infectious diseases; enteritis.

INTRODUCTION

Perinatal and young animal losses are common and a rate of up to 5% of deaths from birth to the first three months of life is considered acceptable (Machado Neto et al. 2004). However, high mortality rates have been reported in some studies around the world, such as 9.4% pre-weaning dairy calf mortality in the United States (Losinger & Henrichs 1997); 12.9% mortality in calves up to one year old in China (Zhang et al., 2019); 14.9% in Minas Gerais, Brazil (Frois & Viegas 1994); and 18.65% in Goiás (Silva et al., 2001). In a study carried out at Embrapa Gado de Corte, in Mato Grosso do Sul, it was found that calf mortality averaged 6% over nine years and represented 53.7% of total cattle mortality (Corrêa et al., 2001). These data demonstrate the importance of diseases in young cattle for production systems, as well as the expressiveness of this category in the series of diagnostic laboratories. Furthermore, high rates of morbidity, specially due to infectious diseases, are related to increased use of antibiotics and a marked rise in antimicrobial resistance (World Health Organization, 2014).

If the zootechnical indices of a bovine herd are satisfactory, but the mortality rate in the first year of life is high, there is a great loss in all the work previously carried out. In a well-managed system, mortality is a very important factor, because it is influenced by health aspects that increase production costs (Lopes et al., 2009). In this way, the knowledge about the main diseases involved in the death of calves is extremely important. Thus, this study aimed to determine which are the main diseases that cause mortality in cattle from birth to one year of life in the state of Rio Grande do Sul, Brazil, correlating with some epidemiological aspects, such as age group and aptitude (beef or dairy).

MATERIAL AND METHODS

A retrospective study was carried out in which the records of necropsies of cattle from the Setor de Patologia Veterinária of the Universidade Federal do Rio Grande do Sul (SPV-UFRGS) were reviewed. Records of samples mailed in to the laboratory for analysis were also included. The records accessed included the clinical history, pathology report, and ancillary tests, as bacteriology, dosage of minerals in tissues, parasitological, virological and molecular (PCR) tests, which allowed to conclude the diagnosis.

Data were compiled for the period from January 2005 to December 2020, related to newborn cattle up to one year of age. The diagnoses of the reports were firstly divided into conclusive and inconclusive cases. Data regarding sex, breed, aptitude (dairy or beef), and age range of calves were also obtained. Age groups were defined based on a previous study (Assis-Brasil et al. 2013); 0-3 months, 4-6 months, 7-9 months, and 10-12 months of age. The conclusive diagnoses were categorized according to the organic system involved and then divided according to disease etiology into six major groups: infectious and parasitic diseases, toxic diseases, developmental disorders, metabolic and nutritional diseases, neoplasms and tumoriform lesions, and others. The most frequent diseases were further discriminated considering age group and aptitude of calves.

The area covered by the laboratory mainly includes the metropolitan region of Porto Alegre, southeast, central-east, and northeast regions of the state of Rio Grande do Sul, although the study also included samples received from other regions of the state and also from other states in southern Brazil, such as Santa Catarina and Paraná.

RESULTS

At all, 797 records were selected and reviewed. Of these, 626 had a conclusive diagnosis (78,5%) and 171 had an inconclusive diagnosis (21,5%). Females were 377 (47.3%), males 333 (41,8%), and in 87 cases the sex was not informed (10,9%). Details regarding the aptitude and breed of the cattle are described in Table 1. There was a greater frequency of beef cattle, mainly

represented by taurine breeds: Angus and Hereford. As for dairy cattle, the Holstein and Jersey breeds stood out.

Table 1: Aptitude and breed of calves sent for diagnosis

	N. of cases and %	Aptitude			Breed				
		D	B	NI	MB	BTT*	BTI**	SB***	NI
Total %	797 (100%)	192 (24.1%)	442 (55.5%)	163 (20.4%)	111 (13.9%)	525 (65.9%)	23 (2.9%)	39 (4.9%)	99 (12.4%)

NI: not informed; MB: mixed breed; D: dairy; B: beef.

*_Bos taurus taurus_, with predominance of Angus, Holstein, Jersey, and Hereford cattle.

** _Bos taurus_ indicus, with predominance of Nelore cattle.

*** Synthetic breed, with predominance of Brangus and Braford cattle.

As for the system involved in the final diagnosis, there was a predominance of the digestive system (24.9% [154/626]), followed by the hemolymphatic (18.5% [116/626]), and nervous systems (15.3% [96/626]). The detailed classification can be seen in Figure 1. As for the etiological classification, there was a wide predominance of infectious and parasitic diseases (73% [457/626]), followed by toxic diseases (9.5% [59/626]), developmental disorders (5.1% [32/626], nutritional and metabolic diseases (4.4% [28/626]), neoplasms and tumoriform lesions (1.1% [7/626]), and other disorders 6.9% [43/626]) (Figure 2).

To access the frequency of diagnosis of the most frequent diseases within each age group, and also with comparison of aptitude (beef or dairy), only 499 cases among the 626 conclusive ones were used (Table 2). The remaining 127 cases were not considered for these analyzes because they lacked information about productive aptitude (beef or dairy) and specific age (months or days old) in the pathology report.

Within age groups, enteritis, malformations, and pneumonias were the most frequent diseases in calves from 0 to 3 months of age, totaling 48,8% of all presumed causes of death. The “pneumonia” diagnosis included bacterial pneumonias and pleuropneumonias, mostly by *Pasteurella multocida* and *Mannheimia haemolytica*, viral broncointerstitial pneumonia, and fungal pneumonia, with respect to the often mixed etiology of cases of respiratory disease in cattle.

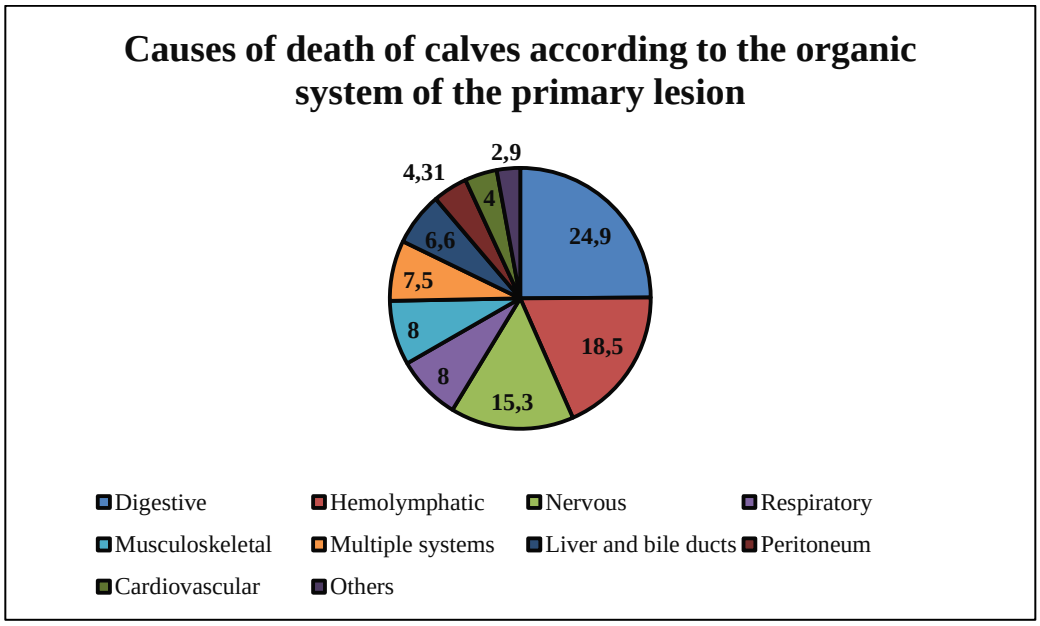

Figure 1. Causes of death of calves according to the organic system of the primary lesion.

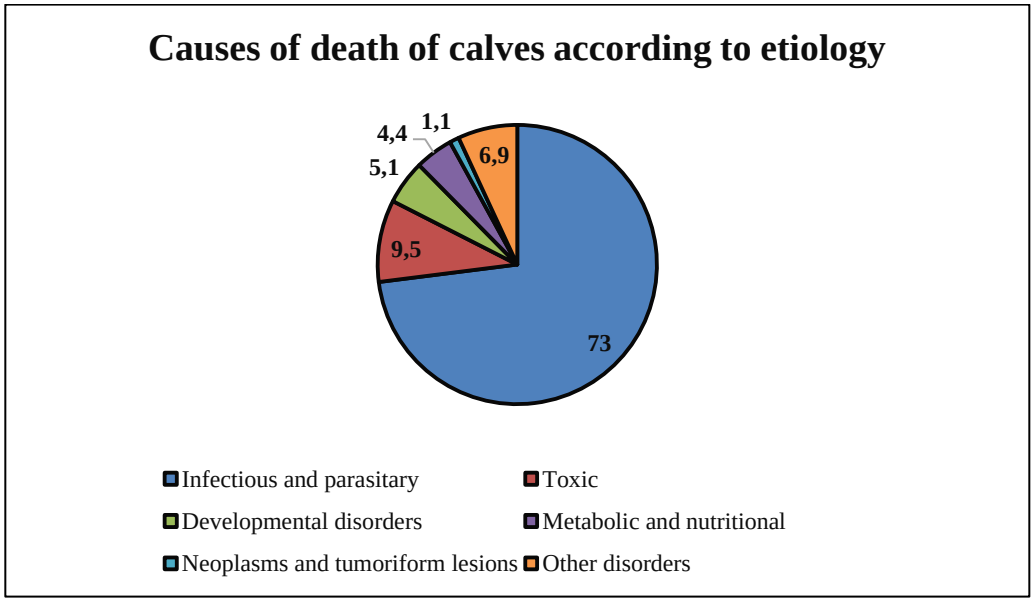

Figure 2. Causes of death of calves according to etiology.

The enteritis diagnosis included bacterial causes (mainly *Escherichia coli* and *Salmonella* spp.), parasitic enteritis caused by *Eimeria* sp. and suspected viral enteritis (mainly coronavirus and rotavirus). For beef calves, hemoprotozoal infection was also important for this age group. Hemoprotozoal infection (tick fever/ infection by *Anaplasma marginale*, *Babesia bovis* and *B.*

bigemina) was among the most frequent diagnosis in all the other age groups, and ranked second in the general survey.

Table 2. Causes of death of calves ranked by age group (intervals of three months) and aptitude (beef or dairy).

Category	Placing	Beef + dairy		Beef		Dairy	
		Disease	**Frequency**	**Disease**	**Frequency**	**Disease**	**Frequency**
0 to 12 months	1st	Enteritis	16,0% (80/499*)	Hemoprotozoal infection	19.5% (68/348)	Enteritis	20.5% (31/151)
	2nd	Hemoprotozoal infection	15.6% (78/499)	Enteritis	14.1% (49/348)	Pneumonias	8.0% (12/151)
	3rd	Pneumonias	8,2% (41/499)	Pneumonias	8.3% (29/348)	Hemoprotozoal infection	6.6% (10/151)
0 to 3 months	1st	Enteritis	30.8% (58/188)	Enteritis	29.6% (34/115)	Enteritis	32.9% (24/73)
	2nd	Malformations	9.0% (17/188)	Hemoprotozoal infection	10.4% (12/115)	Pneumonias	8.2% (6/73)
	3rd	Pneumonias	9.0% (17/188)	Malformations and Pneumonias	9.6% each (11/115)	Septicemia	8.2% (6/73)
4 to 6 months	1st	Hemoprotozoal infection	25.5% (25/98)	Hemoprotozoal infection	27.8% (20/72)	Hemoprotozoal infection	19.2% (5/26)
	2nd	Rabies	11.2% (11/98)	Rabies	11.1% (8/72)	Pneumonias	19.2% (5/26)
	3rd	Pneumonias	11.2% (11/98)	Pneumonias	8.3% (6/72)	Rabies	11.5% (3/26)
7 to 9 months	1st	Hemoprotozoal infection	24.3% (18/74)	Hemoprotozoal infection	27.6% (16/58)	*Cestrum intermedium* poisoning	18.8% (3/16)
	2nd	Worm infections	6.8% (5/74)	Worm infections	8.6% (5/58)	Hemoprotozoal infection	12.5% (2/16)
	3rd	*Senecio* spp. poisoning	6.8% (5/74)	*Senecio* spp. Poisoning	8.6% (5/58)		
10 to 12 months	1st	Hemoprotozoal infection	12.5% (12/96)	Hemoprotozoal infection	14.7% (11/75)	*Senecio* spp. Poisoning, malignant catarrhal fever, Herpesvirus meningoencephalitis and Black leg	10.5% each (2/19)
	2nd	Rabies	9.4% (9/96)	Rabies	10.7% (8/75)		
	3rd	Bovine viral diarrhea	7.3% (7/96)	Bovine viral diarrhea	9.3% (7/75)		

* Out of 626 conclusive cases, 499 cases that included more complete information were used for this table. The remaining 127 cases were not considered for these analyzes because they lacked information about productive aptitude (beef or dairy) and specific age (months or days old) in the pathology report.

In 4-6 months group, pneumonias were also frequent causes of death, accompanied by rabies, which was also highlighted in the 10-12 months category. Toxic causes, such as poisoning by *Senecio* spp., became more prominent in the 7-9- and 10-12-months age groups. Details can be seen in table 2.

DISCUSSION

Our study presents a survey of presumed causes of death in calves (newborn cattle up to 12 months of age), compiled in 16 years of community service and rural extension work carried out by a laboratory within a public university. These data can help in decision making aiming at the diagnosis, control and prevention of diseases in the bovine population. Some of the biases of our work include those typical of retrospective studies of the casuistic from diagnostic laboratories, such as the underrepresentation of easily diagnosable causes on the farm itself, such as trauma, in addition to inconclusive cases due to autolysis or the absence of lesions or information that allow to stablish the diagnosis.

From the 626 cases in which a conclusive diagnosis was obtained, a large proportion (73%) corresponded to infectious causes. This high proportion of infectious diseases was expected, since this animal category is susceptible due to the immaturity of the immune system, when compared to adults (Feitosa et al. 1999). Factors that affect the serum immunoglobulins (Ig) concentration are especially important for development of infectious diseases. Calves that lack adequate passive immunity have increased mortality rate and are more susceptible to most calfhood infectious diseases than are calves with high Ig concentrations (Virtala et al., 1999; Svensson et al. 2003). A successful colostrum management program requires producers to consistently provide calves with a sufficient volume of clean, high-quality colostrum within the first few hours of life (Godden 2008). Failure of transfer of passive colostral immunity (FTIP) in replacement dairy calves has been associated with neonatal morbidity and mortality and reduced long-term productivity (Beam et al., 2009). FTIP is frequent, and in a study carried out in Brazil, it affected 10% to 20% of the calves evaluated, depending on the parameter considered (Feitosa et al., 2010).

Infectious diarrhea is considered the most significant cause of morbidity and mortality in neonatal dairy calves throughout the world (Uhde et al. 2008), and in our study the enteritis was the most frequent condition both among dairy and beef cattle. The pathogens most commonly incriminated in neonatal calf enteritis include viral (rotavirus and coronavirus), protozoal (*Cryptosporidium parvum* and *Eimeria* spp.) and bacterial pathogens (enterotoxigenic *Escherichia coli* K99 and *Salmonella* spp.) (Izzo et al. 2011). Although this group of enteric diseases ranked first in frequency in our study, the losses of calves associated with diarrhea may

be even greater than those estimated in this study, since it is a common condition and not always the farm staff and veterinarians send material for diagnosis. Environmental and management factors, such as cleanliness of calving facilities and calving areas, can be important for the control of diarrhea in calves (Frank; Kaneene 1993), and immunoprophylaxis by vaccinating pregnant cows can also decrease the incidence and severity of the disease (Pourjafar et al. 2010).

One of the main infectious diseases that affected the population studied was hemoprotozoal infections, which have already been identified as one of the main causes of death in cattle in southern Brazil (Lucena et al. 2010; Molossi et al. 2021), and find in this region a favorable environment for the manifestation of clinical disease, mainly due to the enzootic instability of its main vector, the *Rhipicephalus microplus* tick (Gonçalves 2000). Hemoprotozoal infection was more frequent in calves aged 4 to 9 months, mainly in beef breeds. This predominance can be explained by the difference between the production systems, in which the breeding phase of beef cattle is usually carried out in pasture systems, and it makes less likely that farm staff detect clinical cases at the beginning, in time for medicate the animals, contrary to what happens with dairy cattle, in which the more intensive management allows earlier detection of clinical cases.

Pneumonias were frequently incriminated as causes of death of calves in this study, mainly in individuals up to 6 months old, similar to other study with Southern Brazil casuistic (Assis-Brasil et al 2013a). The etiologic agents of bovine respiratory complex include viruses (Bovine herpesvirus 1 [BHV-1], Bovine parainfluenza virus 3 [BPIV-3], Bovine viral diarrhea virus 1 and 2 [BVDV-1–2], Bovine respiratory syncytial virus [BRSV], Bovine adenovirus A–D [BAdV-A–D], and Bovine coronavirus [BCoV]), bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*), and *Mycoplasma* spp., often in associations (Fulton et al. 2009). In the pathology reports of the cases reviewed in this study, viral agents were rarely detected, but bacteria were frequently isolated, especially *P. multocida* and *M. haemolytica*, although we can not exclude mixed infections with viral involvement. Assis-Brasil et al. (2013b) detected BRSV and the main infectious agent in enzootic pneumonia in calves in their study, sometimes with concurrent bacterial infections.

Rabies, a lethal viral disease caused by a Lyssavirus, was also frequent in this survey. Santos et al. (2018) highlighted the occurrence of the disease in young cattle in Rio Grande do Sul, with 11 out of 48 outbreaks affecting calves less than 90 days old. In our study, this disease

was more frequent in the age groups of 4 to 6 and 10 to 12 months. Thus, we emphasize the importance of vaccinating calves in endemic areas for the disease.

The data generated in this work can help producers, veterinarians and other professionals to establish diagnoses and prevention measures to control calf mortality. Specific sanitary measures that seek to prevent enteric, respiratory, and hemoparasitic diseases may have a greater positive impact, in addition to good management practices in general, such as an efficient colostrum management.

CONCLUSIONS

In this study, the main causes of calf losses in southern Brazil were infectious diseases, mainly enteritis, hemoprotozoal infections, and pneumonias. The enteritis was more frequent in 0-3 months cattle, hemoprotozoal infections were important for all subgroups, and pneumonias were more frequent in 4-6 months calves.

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Statement of animal rights

The project that gave rise to the present data was approved to the Research Committee (COMPESQ) of the Universidade Federal do Rio Grande do Sul (UFRGS) (Project number 38659). The manuscript does not contain clinical studies or patient data.

Conflicts of interest

The authors declare no conflicts of interest.

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3.2 Artigo 02 - Nutritional chondrodysplasia in cattle in Brazil

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REGULAR ARTICLES

Nutritional chondrodysplasia in cattle in Brazil

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Abstract
In this work, we investigated cases of birth of calves with congenital defects in a farm in Southern Brazil. Only calves born from heifers were affected, and the disease occurred in both crossbred and purebred calves. Three necropsies were performed, tissues were collected for histopathology, and samples of liver of calves, blood serum, and food provided for cows and heifers were collected to quantify the levels of the minerals: manganese, copper, and zinc. The calves were born weak, with disproportionate dwarfism, limb deformities, and enlarged joints. Heads were shortened and domed. Long bones had a shortened diaphysis and a normal-sized epiphysis, when compared to the control. In one of the cases, there were white-yellowish lines on the metaphyseal surface of the epiphyseal plate. Histopathology of growth plates revealed premature closure, disarrangement of chondrocyte columns, and collapse of primary spongiosa. These findings supported a diagnosis of chondrodysplasia. Liver manganese levels were under the reference values in the three calves. Food analysis revealed insufficient levels of manganese in the diet of heifers, especially in sorghum silage, which was provided as the main source of food for the category in some periods. Approximately 6 months after the diet was changed, the problem ceased and only normal calves continued to be born. Our findings allowed to conclude the diagnosis of chondrodysplasia of nutritional origin and reinforce the thesis that manganese is the mineral deficient in these cases.

Keywords Bone pathology · Nutritional disease · Congenital defects · Manganese deficiency

Introduction

Outbreaks of chondrodysplasia of suspected nutritional origin have been described in Europe, North America, South Africa, New Zealand, and Australia, both in confined and pasture herds (Hart et al. 1947; Ribble et al., 1989; Valero et al. 1990; Peet and Creeper 1994; McLaren et al. 2007; Mee and O'Kiely 2013; Craig et al. 2017). Several designations have been used to identify affected calves including acorn calves, bulldog calves, congenital joint laxity and dwarfism, congenital spinal stenosis, and more recently congenital chondrodystrophy of unknown origin (CCUO) (White and Windsor 2012). The condition is related to disturbances in the endochondral ossification of affected bones (White and Windsor 2012; Craig et al. 2017) and is characterized by the birth of calves with disproportionate dwarfism, often associated with superior brachygnathia, shortened limbs, joint enlargement, and spinal deformities (McLaren et al. 2007; White and Windsor 2012; Craig et al. 2017).

Morphologically, nutritional or hereditary cases of chondrodysplasia are indistinguishable. For this reason, etiological, epidemiological, and pathological analyzes are essential to stablish a definitive diagnosis and control measures (Craig et al. 2017). Cases of nutritional congenital chondrodysplasia have been mainly associated with calves born from females that during pregnancy were fed only with one type of food (for example, corn silage or hay) or that were kept on pastures degraded by the drought. Thus, a common hypothesis among the different authors of the reports would be maternal mineral deficiency, mainly manganese (White and Windsor 2012; Craig et al. 2017).

Therefore, this work aims to report the clinical, pathological, and epidemiological aspects of the occurrence of congenital chondrodysplasia of nutritional origin in calves in Brazil.

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Materials and methods

A diagnostic investigation was conducted in a farm in the municipality of Nova Bassano, Rio Grande do Sul state, Southern Brazil (28°43′56.4″S, 51°42′17.8″W). It was a dairy farm with approximately 40 lactating cows and around 80 cattle in total, with a history of birth of calves with congenital defects, which were often born premature and weak. Two visits were carried out in the farm, and three necropsies were performed, being two in the first and one in the second visit.

Fragments of the tissues from the abdominal and thoracic cavities, as well as the brain, spinal cord, and bones, were collected and fixed in 10% buffered formalin. For bone sampling, a standard collection was established, which included the proximal and distal epiphysis of the femur and humerus, in addition to the rib at the fifth costochondral joint, and a vertebra from the lumbar region. Bones were always sampled from the right side of the body, and the same bones were also collected from three calves born morphologically normal, which served as controls. They were the same age as the affected calves and have died due to other causes, like omphalitis and trauma. Bone fragments were decalcified in 8% nitric acid and routinely processed for histopathology with other organs. Sections were stained with hematoxylin and eosin (HE) and alcian blue pH 2.5 (AB) stains. Samples of lymphoid organs and liver were collected from the three calves and kept under refrigeration. Blood serum was also collected from calves 1 and 2. The serum and lymphoid organs (pool of lymph nodes and spleen) were sent for RT-PCR for bovine viral diarrhea virus (BVDV), under a previously described methodology (Vilcek et al. 1994). The liver samples (200 g) from three necropsied calves were sent to quantify the levels of the manganese, cooper, and zinc through atomic absorption spectrometry.

During the second visit, some additional samples were collected. Blood was collected from seven heifers, (three pregnant and four not), and from nine lactating cows of the property, to measure serum levels of manganese and zinc. For this analysis, specific tubes for the analysis of trace minerals were used and the levels were measured through graphite furnace atomic absorption spectrometry (GFAAS) (Rukgauer et al. 1997). Additionally, samples of ingredients used in heifer's feed (corn silage, sorghum silage, and corn on the cob ground) were also collected and sent to determine the levels of manganese, zinc, and copper, through atomic absorption spectrometry.

Results

History

The complaint had occurred for at least 2 years and affected only calves born from heifers (all around 2 years old at parturition), corresponding to about 90% of births from this category. Some of these heifers came from another farm just before insemination, and yet, the calves were born with the problem. Some calves were born prematurely, very weak, and ended up dying within the first few days of life. Those who were born more easily and at term used to survive, had limited development, and remained dwarfs throughout their lives. The herd on the property was mostly Holstein cattle; however, the problem also affected calves born from Holstein heifers with bulls of other breeds, such as Angus, Jersey, and Gir cattle. The cattle were vaccinated with reproductive vaccines (infectious bovine rhinotracheitis (IBR), BVDV, and leptospirosis), and mating had been carried out by phenotype evaluation since few years ago. The same semen was used for heifers and lactating cows, as well as in other properties in the region, which did not report the problem. Calves born on the farm, right after weaning, were kept in paddocks until insemination and the initial third to mid-gestation, and in those paddocks, they were fed almost exclusively with corn or sorghum silage, sometimes added with corn on the cob ground (corn roll), soybean shell, mineral salt, and common salt (NaCl). Corn silage and sorghum silage were used interchangeably, that is, when a sorghum silage silo was opened, the heifers consumed that food until the silo was finished, and after that, they started receiving corn silage. At the end of gestation, heifers were transferred to another paddock, where they were kept with dry cows. However, nutrition remained the same until the parturition. The lactating cows received balanced feed three times a day, consisting of corn silage, hay, concentrate for lactating cattle (commercial formulation), mineral salt, mycotoxin adsorbents, and sodium bicarbonate. In some periods, they also had access to cultivated pasture.

Clinical evaluation and gross pathology

Three calves were necropsied on the property (calf 1, 2, and 3). Calves 1 and 2 were euthanized due to poor prognosis, and calf 3 had a spontaneous perinatal death. Calf 1 was a 2-day-old male Holstein, born 10 days before the expected. It was smaller than usual and had limited mobility, with marked hyperextension of the limbs, being the hindlimbs more affected. The joints were slightly enlarged, without excessive fixation (arthrogryposis), and the sacrum was displaced ventrally, as if the animal was crouching (Fig. 1a).

Calf 2 was a 4-day-old female Holstein, which had disproportionate dwarfism, shortened limbs, and enlarged joints. The limbs were over-extended and rotated around its longitudinal axis; forelimbs displayed supinatory rotation, and the hindlimbs displayed slight pronation (Fig. 1b, c). Calf 3 was a Holstein female, who was born weak and died a few hours later. It presented a phenotype similar to bovine 2, with disproportionate dwarfism, enlarged joints, tortuous and

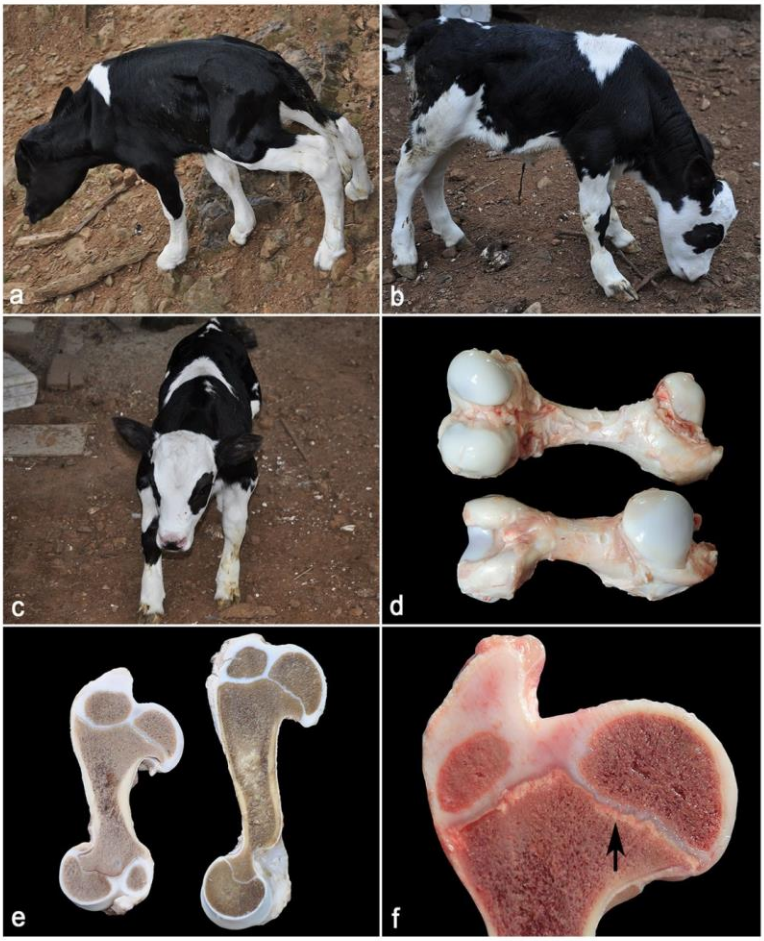

Fig. 1 Gross changes in chondrodysplastic calves. **a** Chondrodysplastic calf 1. The calf is small, has over-extended limbs, enlarged joints, domed and shortened head, and the posterior train is displaced ventrally, as if the animal was crouching. **b, c** Chondrodysplastic calf 2. The calf is a disproportionate dwarf, has over-extended limbs, enlarged joints, domed and shortened head, and the limbs are rotated around its longitudinal axis; forelimbs display supinatory rotation, and the hindlimbs display a slight pronation. **d** Femur and humerus from calf 3. The bone diaphysis is shorter in length. **e** Cut section of the humerus from calf 3 (left) and a control (right) for comparison purposes. The bone diaphysis is shorter in length, although the epiphyses are similar in size. **f** Humerus from calf 3. There is a diffuse white-yellowish line on the metaphyseal face of the physis (arrow)

hyperextended limbs, and kyphosis. In all calves, the heads were shortened and domed, with superior brachygnathism.

At necropsy of the three cattle, the changes were restricted to bones and joints. Necropsy evidenced and clarified the aforementioned changes. In the skull, there was a conformational deformity, which was characterized by a distortion of the basal bones of the skull, which justifies the skull doming. In the femur and humerus, there was marked shortening of the bone diaphysis, with epiphyses similar in size to the control (Fig. 1d, e). In bovine 3, white-yellowish lines were also observed on the metaphyseal face of the growth plates of the femur, humerus, and ribs (Fig. 1f). The articular cartilages of all cases showed irregular thickness, with some areas of erosion in the distal femur of calf 3. Calf 3 had a slight kyphosis, and at cut surface, there were cartilage cores visible amid the spongy bone of the vertebral bodies. The spinal cord was not compressed, and tracheal rings, skeletal muscles, and internal viscera were also apparently normal in all calves.

Three other animals born with the problem were also clinically evaluated. Two of them were crosses of Holstein with Angus or Gir cattle, were born strong, and were kept on the property for raising and fattening. A third animal was a 4-year-old Holstein cow, which reached the third lactation until that moment. This cow had disproportionate dwarfism, with a shortened and domed head, and shortened limbs (Fig. 2a, b), but it was a good milk producer, and its calves were always born normal.

Histopathology

All sick calves showed physeal and metaphyseal abnormalities. These abnormalities were of varying degrees of severity when compared to controls, with some morphological differences among the affected ones (Fig. 3a–f). The growth plates displayed a highly irregular and usually increased thickness. In the epiphyseal aspect of the physis, there was multifocal to coalescing areas of deposition of osteoid matrix, interpreted as an evidence of premature growth plate closure. The cellularity of the plate was usually reduced, and the extracellular matrix had a fibrillar to granular and faintly stained appearance, that was better evidenced through AB stain (Fig. 3e, f). There was marked disorganization of the chondrocyte columns, with variations in the number, size, and alignment of cells. The hypertrophic layer presented foci of variable thickness decrease, sometimes with only 2 to 4 chondrocytes per row, against 5 to 8 chondrocytes per row in the control sections. Calf number 2 had an increase in the proliferative layer and an apparently normal hypertrophic layer in all bones evaluated. Chondrocyte cellular scaffolds were frequently observed amid the columns, interpreted as "ghost cells" (Fig. 3c, arrowhead).

In the metaphyseal aspect of physis, there was irregular formation and mineralization of trabeculae in a truncated primary spongiosa. In some sections, there was accumulation of an eosinophilic material suggestive of degenerated matrix, surrounded by osteoclasts. These areas were frequently accompanied by microfractures, that were more severe and grossly visible in animal 3, but also present in calf 1. Horizontal metaphyseal trabeculae of bone (growth arrest lines) were just occasionally found, more specifically in the distal femur and humerus of calf 1 and proximal femur of animal 3, but these changes were poorly defined. All sections also displayed a slight to moderate osteopenia, characterized by thin and rarefied trabeculae, when compared to controls. In the vertebrae, in addition to all the changes described, cartilage islands were present within the vertebral body. In animal 3, there was still a solution of continuity (erosion) of the articular cartilage of the trochlea of femur, which was grossly evident. The tracheal rings, central nervous system, skeletal muscles, and abdominal and thoracic organs displayed no histological changes.

Complementary exams and mineral analysis

The analysis of the microelements in the liver can be seen in Table 1. In all three calves, the hepatic levels of manganese were below the minimum acceptable values for neonates, which is 8 µg/g (Constable et al. 2017). It should be noted that in calf 3, manganese levels were below to the minimum limit of detection, which is 2.90 µg/g.

Levels of manganese were accessed in the main ingredient of the diet and were especially low in the sorghum silage, which was exclusively supplied to the heifers. Detailed dosages are shown in Table 2. As for the serum levels of zinc and manganese, the values were near the reference values for bovine species and did not reveal any disturbance. It is worth mentioning that these serum samples were collected on the second visit, when the suspicion of nutritional disorder had already been established and the farmer had established some management changes, under the guidance of the field veterinarian. Reference values considered for this analysis were manganese: 0.18 to 0.19 mg/L and zinc: 0.8 to 1.2 mg/L (Constable et al. 2017).

The RT-PCR for BVDV on serum and lymphoid organs resulted negative for the three necropsied cattle. About 1 year after the diagnosis and the nutritional adjustment of the feed provided to the heifers, the producers reported that there was no birth of calves with the malformations described above.

Discussion

The diagnosis of chondrodysplasia of nutritional origin was established through the association of epidemiological, clinical, and pathological findings and analysis of the food supplied to the heifers and also of the levels of minerals in the liver of calves. The exclusive involvement of calves born

Fig. 2 Adult 4-year-old dwarf cow. **a** On the left, a normal contemporary dairy cow. The chondrodysplastic cow has disproportionate dwarfism, characterized mainly by shorter stature. **b** Same cow of **a**, inside profile of face, which highlights the shortened head (superior brachygnathism)

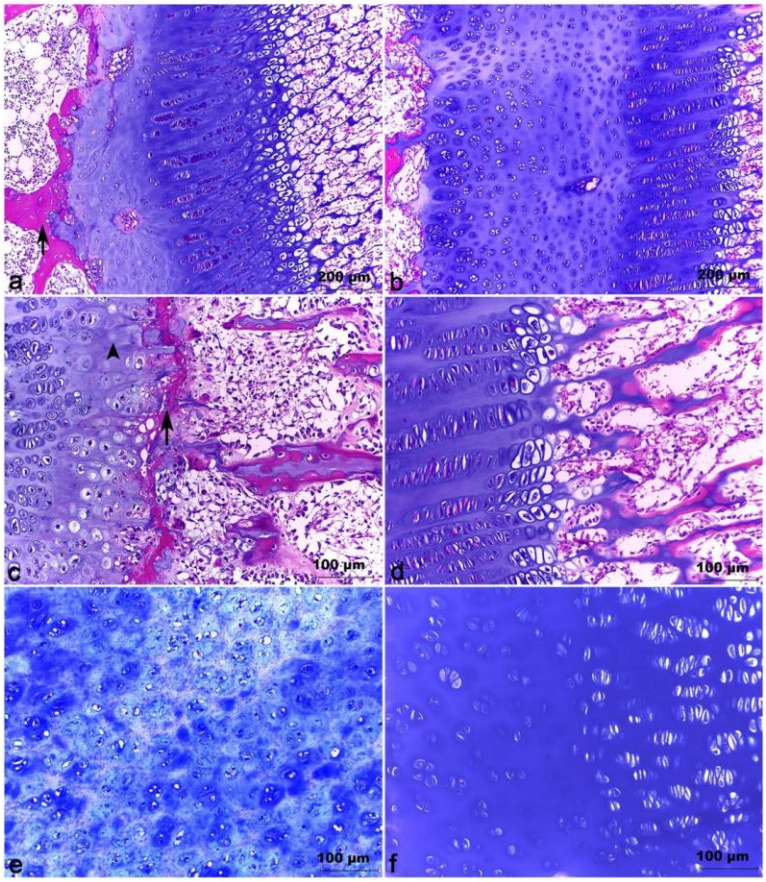

Fig. 3 Histological and histochemical changes in chondrodysplastic calves, compared to controls. **a** Chondrodysplastic calf, distal femur. In the epiphyseal aspect of the physis, there are multifocal to coalescing areas of deposition of osteoid matrix that mean the premature growth plate closure (arrow). There is marked disorganization of the chondrocyte columns, with variations in the number, size, and alignment of cells. H&E 100×. **b** Control calf, distal femur. The growth plate is opened, as expected in a neonatal bovine. The cellularity of the plaque is uniform, and the rows of chondrocytes are aligned. H&E 100×. **c** Chondrodysplastic calf, proximal humerus. There is collapse of the primary spongiosa; the bone trabeculae in the mineralization zone are rarefied, and there is proliferation of osteoclasts. There is also the formation of a cleft associated with the deposition of amorphous eosinophilic material (interpreted as degenerated matrix—arrow). The chondrocytes of the hypertrophic layer are irregular, rarefied, and misaligned, and there are also cellular scaffolds (ghost cells—arrowheads). H&E 200×. **d** Control calf, proximal humerus. The chondrocyte layers are aligned and contiguous to the mineralization zone. H&E 200×. **e** Chondrodysplastic calf, proximal humerus. The cartilaginous extracellular matrix displays a fibrillar to granular and faintly stained appearance. H&E 200×. **f** Control calf, proximal humerus. The cartilaginous extracellular matrix displays a strong and homogeneous blue staining. H&E 200×

from heifers has led to the suspicion of an environmental etiology. Since the feeding scheme for the heifers was what differed in the management of the cattle on the farm, the suspicion of nutritional deficiency stood out.

The inadequate feeding management to which heifers were subjected and the morphological aspects of affected calves resemble the reports described in the international literature of outbreaks of CCUO, also designated as acorn calves, bulldog calves, and congenital joint laxity and dwarfism (Ribble et al. 1989; Valero et al. 1990; Cutler 2006; McLaren et al 2007; White and Windsor 2012). The main epidemiological aspect common to the cases described in the literature was the feeding stress to which pregnant cows were subjected. The circumstances of

Table 1 Levels of copper, manganese, and zinc in the liver of three cattle with chondrodysplasia (the values are expressed on the basis of dry matter)

Element	Unit	Result		
		Calf 1	Calf 2	Calf 3
Copper	µg/g	214	238	588
Manganese	µg/g	6.24	7.71	ND
Zinc	µg/g	1017	656	1031

Minimum detection limit of the technique: 2.90 µg/g. Reference values for bovine species, according to Constable et al. (2017)—copper: deficiency is considered with levels < 50 µg/g, manganese: 12 mg/kg in adults and a minimum of 8 mg/kg in neonates, and zinc: 30 and 150 µg/g

ND not detectable

Table 2 Levels of copper, manganese, and zinc in ingredients used to feed cattle on the farm with a history of chondrodysplasia in newborn calves (the values are expressed on the basis of dry matter)

Element	Unity	Result		
		Corn silage	Sorghum silage	Ground corn cob
Copper	µg/g	2.73	5.95	10.1
Manganese	µg/g	63.5	23.7	95.5
Zinc	µg/g	20.2	21	40.7

nutritional stress reported include raising pregnant cows in poor pastures in times of drought (Peet & Creeper 1994), and periods of confinement of pregnant cows, mainly in winter, if they are fed exclusively with one type of food, such as silage or hay (Cutler 2006). CCUO is more reported in beef cattle, but dairy cattle can also be affected when subjected to similar situations (White and Windsor 2012), as seen in this outbreak. In our cases, the feed provided to heifers, composed almost exclusively of corn or sorghum silage, generated the necessary condition to trigger the problem. Approximately 6 months after the diet was changed, the problem ceased and only normal calves continued to be born. Dietary changes included access to cultivated pasture and ad libitum supply of mineral supplement specific for dairy cattle on pasture. The composition of this supplement was as follows (values of minimum concentration per kg of product): phosphorus 80 gm; calcium 141 gm; sodium 133 gm; sulfur 10 gm; cobalt 50 gm; copper 1.2 gm; chromium 0.03 gm; iodine 0.075 gm; manganese 1 gm; selenium 0.015 gm; and zinc 3.6 gm.

In Brazil, cases of congenital defects in cattle, morphologically similar to those described here, were associated with the consumption of apple pulp by pregnant cows. In this case, however, the authors associated the malformations with the presence of pesticide residues in the diet and not with the deficiency of any nutrient (Wicpolt & Gava 2019).

Although the exact etiology of CCUO has not been defined, some studies indicate the lack of manganese (Mn) as responsible (Valero et al. 1990; Cutler 2006; White and Windsor 2012). Manganese is known to interfere with fetal development. It is a necessary microelement for the activation of glycosyltransferases involved in the biosynthesis of glycosaminoglycan. Manganese deficiency causes decreased production and increased degradation of glycosaminoglycan of the cartilage, which potentially affect the development of bones that undergo endochondral ossification (McLaren et al. 2007; Craig et al. 2017). In a study on the controlled supply of the mineral to pregnant heifers, calves were born with superior brachygnathism, disproportionate dwarfism, and weakness from a group of heifers that received a diet with 16.6 mg/kg of Mn on dry matter. In another group of heifers fed a diet with 66.6 mg/kg of Mn on dry matter, normal calves were born (Hansen et al. 2006). According to the analyzes carried out on the food collected on the farm where the outbreak described here occurred, without additional supplementation, both corn silage (63.5 mg/kg) and sorghum silage (23.7 mg/kg) were unable to supply the amount of the mineral (66.6 mg/kg) that was able to prevent the birth of malformed calves in the aforementioned study, although the deficit with sorghum silage was greater. As mentioned before, sorghum silage feeding was the predominant feeding scheme during some periods of the heifer's gestation. The levels of Mn detected in the liver of necropsied calves also indicated a deficiency, more pronounced in bovine 3, which did not consume colostrum, that usually tends to rapidly increase the levels of the element in the body (Craig et al. 2017), as observed in calves 1 and 2. However, even though they had received colostrum, they still had hepatic levels of manganese below the minimum value for neonates, which is 8 µg/g (Constable et al. 2017).

Another important factor to be highlighted is that the cows of the farm did not produce malformed calves after the first calving, even if being submitted to the heifer's diet during the dry period. This fact can be justified, as fetal skeletal changes tend to occur during the middle third of pregnancy, especially between the third and sixth month (Hart et al. 1947; White and Windsor 2012). In this way, when cows are submitted to the dry period after reaching the seventh gestational month, the fetal bone structure is already formed.

The possibility of a genetic origin for the cases of chondrodysplasia described here was ruled due to the presence of affected crossbred calves, in addition to the fact that semen from the same bulls was also used in cows and in neighboring properties that did not present the problem. Additionally, the occurrence of several cases of congenital defect on the same property, as an outbreak, is more compatible with an environmental than a hereditary cause

(Marcolongo-Pereira et al. 2010; Dittmer and Thompson, 2015). Among the infectious causes, the animals in this study were negative for BVDV, which is identified as an infectious agent responsible for malformations in cattle in Brazil (Pavarini et al. 2008). Furthermore, chondrodysplasia is not usually identified as a malformation induced by viral agents, unlike arthrogryposis and brain, eye, and spinal defects (Grooms 2004, Agerholm et al., 2015). The producers did not apply vermifuges to the pregnant heifers, and no potentially toxic plants were found in the paddock where the heifers were managed.

The reports on literature bring several morphological differences between the cases of CCUO described, both macroscopically and microscopically. These differences were reviewed by White and Windsor (2012). Histologically, the most constant aspects among the cases were the disorganization and shortening of columns of chondrocytes and premature closure of the growth plate demonstrated by transphyseal bone trabeculae. It is possible that the origin of chondrodystrophy differs for each of the cases described. However, consideration must be given to the differences that would be expected from interpretation of histopathology slides by different histopathologists, using different bones subjected to different preparation techniques and stains, with specimens from calves of different ages and locations (White and Windsor 2012). Also, we must consider that the cows that gave birth to the defective calves may have been subjected to nutritional stresses of different origins, durations, intensities, and periods of gestation, which may explain not only the differences between the alterations reported in different papers, but also the phenotypic differences between the calves affected in each outbreak and differences in the severity of clinical signs.

To the knowledge of the authors, this is the first report of CCUO, possibly associated with manganese deficiency, described in Brazil. The conjunct analysis of the epidemiological, clinical, and pathological aspects, together with the dosages of microelements in the diet and liver of the affected calves, was fundamental for the elucidation of the case. The incorrect nutritional management of heifers from this farm can be extrapolated to several other farms in the country and, therefore, reinforce the importance of correct management in all categories, in order to have adequate productive results, as observed in the farm after nutritional adjustment.

Author contribution CIS and RMB conceived and designed research. CIS, RMB, and AV conducted data collection. AG and MMP contributed to the analysis of the results. CIS and RMB wrote the manuscript. SPP and DD reviewed data and manuscript writing. All authors read and approved the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Statement of animal rights The project that gave rise to the present data was approved to the Research Committee (COMPESQ) of the Universidade Federal do Rio Grande do Sul (UFRGS) (project number 38659). The manuscript does not contain clinical studies or patient data.

Conflict of interest The authors declare no competing interests.

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3.2 Resultados adicionais

Neste item são apresentadas as tabelas completas resultantes do levantamento de dados que gerou o artigo 02. Essas tabelas são extensas e comtemplam doenças diagnosticadas com pouca frequência, por isso, a princípio, não foram adicionadas na íntegra ao artigo, o qual contou apenas com a apresentação dos dados mais robustos.

Tabela 01. Doenças infecciosas diagnosticadas em bezerros no sul do Brasil

Doenças	N. de casos	% da categoria	% Total	Doenças	N. de casos	% da categoria	% Total
Tristeza parasitária bovina*	97	21,2	15,5	Febre catarral maligna	4	0,9	0,7
Enterites**	45	9,9	7,2	Poliartrite	3	0,7	0,5
Raiva	44	9,7	7,0	Endocardite valvar	3	0,7	0,5
Pneumonias (pneumonia enzoótica bovina)	38	8,3	6,1	Pneumonia bacteriana embólica	2	0,4	0,3
Peritonite****	24	5,3	3,8	Yersinose	2	0,4	0,3
Septicemia	22	4,8	3,5	Infecção fúngica sistêmica*****	2	0,4	0,3
Diarreia viral bovina – doença das mucosas	21	4,6	3,4	Meningoencefalite não supurativa	2	0,4	0,3
Colibacilose***	16	3,5	2,6	Empiema basilar	2	0,4	0,3
Eimeriose	14	3,1	2,2	Botulismo	1	0,2	0,15
Salmonelose***	14	3,1	2,2	Pneumonia fúngica	1	0,2	0,15
Carbúnculo sintomático	13	2,9	2,1	Rumenite Fúngica	1	0,2	0,15
Leptospirose	10	2,2	1,6	Fasciolose	1	0,2	0,15
Meningoencefalite herpética	10	2,2	1,6	Pododermatite ulcerativa	1	0,2	0,15
Parasitoses gastrointestinais	10	2,2	1,6	Necrobacilose	1	0,2	0,15
Hemoglobinúria bacilar	7	1,6	1,2	Abscesso mandibular por *T. pyogenes*	1	0,2	0,15
Onfaloflebite e hepatite abscedativa	7	1,6	1,2	Infestação massiva por carrapatos	1	0,2	0,15
Espondilite com compressão medular	7	1,6	1,2	Neurite necrótica periférica bilateral em MPs	1	0,2	0,15
Encefalites/meningoencefalites bacterianas	6	1,1	0,8	Actinobacilose	1	0,2	0,15
Tétano	5	1,1	0,8	Abscesso de laringe	1	0,2	0,15
Tuberculose	5	1,1	0,8	Pericardite bacteriana	1	0,2	0,15
Dictiocaulose	5	1,1	0,8	Outros	5	1,1	0,8
Total					**457**	**100%**	**73,0%**

*Inclui 46 casos exclusivos de Anaplasmose, 32 casos exclusivos de Babesiose (sendo sete de babesiose cerebral) e dois casos de infecção mista. Nos demais casos os agentes não foram especificados.

**Inclui 22 casos de enterites bacterianas, 16 enterites virais e sete de origem não determinada.

***Casos principalmente relacionados a casos de enterites e, ocasionalmente, infecções sistêmicas.

****Principalmente associada a casos de onfaloflebite, perfuração de úlcera de abomaso e castração.

*****Inclui um caso de infecção sistêmica por *Mortierella Wolfii*.

Tabela 2. Intoxicações e distúrbios do desenvolvimento diagnosticados em bezerros no sul do Brasil

Doenças	N. de casos	% da categoria	% Total	Doenças	N. de casos	% da categoria	% total
Tóxicas				**Distúrbios do desenvolvimento**			
Intoxicação por *Senecio* sp.	16	27,1	2,6	Malformações cardíacas*	8	25,0	1,3
Intoxicação aguda por samambaia	14	23,7	2,2	Condrodisplasia	5	15,7	0,8
Intoxicação por *Perreyia flavipes*	6	10,1	1,0	Artrogripose	3	9,4	0,5
Intoxicação por *Amorimia exotropica*	5	8,5	0,8	Malformações do SNC**	3	9,4	0,5
Miopatias tóxicas	5	8,5	0,8	Quielosquise com pneumonia aspirativa	2	6,2	0,3
Intoxicação por *Cestrum intermedium*	4	6,8	0,7	Hipoplasia de omaso	2	6,2	0,3
Intoxicação por fluorsilicato de sódio	3	5,1	0,5	Craniosquise com coristoma pulmonar	1	3,1	0,15
Intoxicação por carbamato/organofosforado	2	3,4	0,3	Amelia de membros pélvicos	1	3,1	0,15
Intoxicação por estricnina	1	1,7	0,15	Hemimelia e hipoplasia renal	1	3,1	0,15
Intoxicação por nitrato	1	1,7	0,15	Displasia renal	1	3,1	0,15
Intoxicação por polpa cítrica	1	1,7	0,15	Outras malformações	5	15,7	0,8
Hepatopatia tóxica aguda	1	1,7	0,15	**Total**	**32**	**100%**	**5,1%**
Total	**59**	**100%**	**9,5%**				

*Inclui malformações como Tetralogia de Fallot, Persistência de forame oval e Defeito de septo interventricular.

**Inclui malformações como hidrocefalia e agenesia parcial de verme cerebelar.

Tabela 3. Doenças metabólicas e nutricionais, neoplasmas e lesões tumoriformes e outros distúrbios diagnosticados em bezerros no sul do Brasil

Doenças	N. de casos	% da categoria	% Total	Doenças	N. de casos	% da categoria	% Total
Metabólicas e nutricionais				**Outros distúrbios**			
Polioencefalomalacia	14	50,0	2,2	Ruminite necrótica aguda	6	14,0	1,0
Timpanismo agudo por sobrecarga alimentar	3	10,7	0,5	Reticulite traumática	4	9,4	0,7
Deficiência de cobre	3	10,7	0,5	Timpanismo gasoso por obstrução esofágica	4	9,4	0,7
Acidose ruminal	2	7,1	0,3	Pneumonia aspirativa	4	9,4	0,7
Raquitismo	2	7,1	0,3	Politraumatismo	2	4,6	0,3
Deficiência de vitamina E e selênio	2	7,1	0,3	Uroperitônio por ruptura de bexiga	1	2,3	0,15
Timpanismo espumoso (trevo)	1	3,6	0,15	Hidronefrose	1	2,3	0,15
Urolitíase	1	3,6	0,15	Torção de raiz mesentérica	1	2,3	0,15
Total	**28**	**100%**	**4,4%**	Esofagite traumática por corpo estranho	1	2,3	0,15
				Evisceração em hérnia umbilical	1	2,3	0,15
Neoplasmas e lesões tumoriformes				Rumenite química	1	2,3	0,15
Papilomatose cutânea	3	42,8	0,5	Displasia e subluxação coxofemoral	1	2,3	0,15
Linfoma juvenil	2	28,6	0,3	Luxação escápulo-umeral	1	2,3	0,15
Nefroblastoma metastático	1	14,3	0,15	Doença do armazenamento lisossomal	1	2,3	0,15
Osteoma	1	14,3	0,15	Hemoperitônio - ruptura da artéria umbilical	1	2,3	0,15
Total	**7**	**100%**	**1,1%**	Lipomatose	1	2,3	0,15
				Outros	12	27,9	1,9
				Total	**43**	**100%**	**6,9%**

4 CONSIDERAÇÕES FINAIS

Os dados levantados neste estudo permitiram elencar quais doenças afetam bezerros no sul do Brasil e permitiram também elencar alguns aspectos epidemiológicos das principais condições. Quanto à etiologia, as doenças infecciosas e parasitárias foram predominantes (73%), seguidas de doenças tóxicas (9,5%) e transtornos do desenvolvimento (5,1%). Quanto aos sistemas envolvidos, as doenças do aparelho digestivo (24,9%) foram as mais frequentes, principalmente na faixa de 0 a 3 meses, seguidas das doenças do sistema hemolinfático (18,5%), frequentes em todas as faixas etárias. De forma geral, as condições mais frequentes foram enterites com 16% (80/499), tristeza parasitária bovina com 15.6% (78/499) e pneumonias com 8,2% (41/499). Esses dados mostram que medidas sanitárias que buscam prevenir doenças entéricas, respiratórias e hemoparasitárias podem ajudar muito a reduzir a mortalidade de bovinos no sul do Brasil. Ainda, através do relato de um surto de condrodisplasia de origem nutricional, foi possível ressaltar que condições nutricionais também podem estar associadas a perdas e desta forma faz-se necessária a atenção à adequada nutrição dos rebanhos.

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