











Haptoglobin concentrations in cows with history of natural bracken fern chronic ingestion

Elizabeth Moreira dos Santos Schmidt^{1*}  Carolina Dutra Minozzo²  João Henrique Perotta² 
Rosângela Locatelli-Dittrich²  Olair Carlos Beltrame²  Juliana Sperotto Brum² 
Peter David Eckersall³  Ivan Roque de Barros Filho² 

¹Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista (UNESP), 18618-681, Botucatu, SP, Brasil. E-mail: elizabeth.schmidt@unesp.br. *Corresponding author.

²Departamento de Medicina Veterinária, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brasil.

³Institute of Biodiversity, One Health, and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

ABSTRACT: Bracken ferns (*Pteridium* spp.) have been associated with intoxication in cattle and is the cause of bovine enzootic hematuria (BEH) characterized by intermittent hematuria, anemia, and poor body condition. Acute phase proteins have not previously been investigated in detail in BEH. Thus, this investigation determined serum concentrations of haptoglobin, selected hematological (complete blood count and fibrinogen), biochemical (total serum protein, albumin, urea, creatinine, aspartate aminotransferase, gamma glutamyl transferase, and creatine kinase), and urinary analytes, in 50 Nelore cows from farms with history of natural bracken fern chronic ingestion in the southern region of Brazil. Twenty-nine cows had macrohematuria and 21 microhematuria. From the 29 cows with macrohematuria, 13 had a countless number of erythrocytes per high power microscopic field and anemia. All cows had dipstick proteinuria. Median haptoglobin concentrations were elevated for 47 BEH cows with a 3.9-fold increase, suggesting a chronic inflammatory response while the number of total leukocytes and segmented neutrophils were above the reference intervals for 14 cows. Besides inflammation, there is a hypothesis that bracken fern could cause hemolysis in the circulation. Therefore, there could be a larger acute phase reaction but much of the haptoglobin was being removed as it was being produced. In our study, increases in haptoglobin, creatine kinase, hyperglobulinemia and anemia were found in cows with BEH, contributing to the understanding of the chronic inflammatory response which could be associated with neoplastic lesions in the urinary bladder caused by long-term and repeated ingestion of *Pteridium* species.

Key words: *Pteridium* spp., haptoglobin, cattle, hematuria, acute phase proteins.

Concentrações de haptoglobina em vacas com histórico de ingestão crônica de samambaia

RESUMO: Samambaias (*Pteridium* spp.) têm sido associadas à intoxicação em bovinos e causam a hematuria enzoótica bovina (BEH), caracterizada por hematuria intermitente, anemia e má condição corporal. As proteínas de fase aguda ainda não foram avaliadas em detalhes na BEH. Assim, o objetivo desta investigação foi determinar as concentrações séricas de haptoglobina, de parâmetros bioquímicos (proteína total, albumina, ureia, creatinina, aspartato aminotransferase, gama glutamyl transferase e creatina quinase), hemograma completo, fibrinogênio e urinálises em 50 vacas Nelore provenientes de fazendas com histórico de ingestão crônica de samambaia natural. Vinte e nove vacas apresentaram macrohematuria e 21 microhematuria. Das 29 vacas com macrohematuria, 13 apresentavam anemia e número incontável de eritrócitos por campo na urinálise. As vacas apresentaram proteinúria no exame químico da urina. As concentrações medianas de haptoglobina apresentaram aumento de 3,9 vezes em 47 vacas com BEH, sugerindo uma resposta inflamatória crônica. O número de leucócitos totais e neutrófilos segmentados estava acima dos intervalos de referência para oito e 23 vacas, respectivamente. Além da inflamação, existe a hipótese de que o princípio ativo da samambaia possa causar hemólise intravascular. Portanto, poderia haver uma reação de fase aguda maior nas vacas, mas possivelmente parte da haptoglobina estava sendo removida à medida que era produzida. As vacas com BEH deste estudo apresentaram elevação sérica de haptoglobina, creatina quinase, hiperglobulinemia e anemia, contribuindo para a compreensão da resposta inflamatória crônica que pode estar associada às lesões neoplásicas na bexiga urinária causadas pela ingestão prolongada e repetida de *Pteridium* spp.

Palavras-chave: *Pteridium* spp., haptoglobina, bovinos, hematuria, proteínas de fase aguda.

INTRODUCTION

Bracken ferns (*Pteridium* spp.) are plants with worldwide distribution that have been associated with animal health conditions (RAI et al., 2017). In South America, two species are associated with intoxication: *P. arachnoideum* and *P. caudatum* (RIBEIRO et al., 2020; ROCHA et al., 2022). In cattle, ingestion of *Pteridium* spp. causes three different clinical presentations: bovine enzootic

hematuria (BEH), upper alimentary tract neoplasia, and hemorrhagic diathesis (RIET-CORREA et al., 2023). Bovine enzootic hematuria is caused by chronic ingestion of *Pteridium* spp. in pastures infested by ferns and has been described in Brazil (GAVA et al., 2002; MARÇAL, 2003; FALBO et al., 2005; SOUTO et al., 2006; GABRIEL et al., 2009; SILVA et al., 2009, 2012; TOKARNIA et al., 2012; AZEVEDO et al., 2016). *Pteridium* spp. is the second most prevalent plant causing intoxication outbreaks

in beef cattle in southern Brazil, mostly in old cattle (GAVA et al., 2002; MOLOSSI et al., 2021).

Clinically, the disease is characterized by intermittent hematuria, anemia, and poor body condition with progressive weight loss, and death (PRASAD, 1983; RAI et al., 2017). Intense monocytosis was reported as an early marker of the disease and hyperfibrinogenemia was detected in several cows chronically exposed to bracken fern (PEREZ-ALENZA et al., 2006; DI LORIA et al., 2012). Anemia, leucopenia, and neutropenia are common features due to chronic bone marrow suppression by the plant toxins (PRASAD, 1983; HOPKINS, 1990; PEREZ-ALENZA et al., 2006; TOKARNIA et al., 2012). In addition, bovine papillomaviruses (BVP types 1 and 2) have been implicated in carcinogenesis of the urinary bladder in cattle feeding on bracken fern (CAMPO et al., 1992; YUAN et al., 2007). The carcinogenicity of bracken fern is caused by the ptaquiloside, a compound that produces hyperplastic and neoplastic lesions on bladder epithelium (SOUTO et al., 2006; YAMADA et al., 2007; SILVA et al., 2012; AZEVEDO et al., 2016) intensifying the inflammatory response (FALBO et al., 2005).

A study with serum protein electrophoresis in cows with BEH reported increases in the gamma globulin fraction while the beta-globulin fraction was decreased in most animals (PEREZ-ALENZA et al., 2006). Haptoglobin is an alpha-globulin and is a major acute phase protein (APP) in ruminants (CECILIANI et al., 2012). Acute phase proteins are produced by hepatocytes and peripheral tissues stimulated by proinflammatory cytokines such as interleukins-1 β and -6 (IL-1 β and IL-6), and tumor necrosis factor alpha (TNF- α) in response to infection, inflammation, or trauma (SACO & BASSOLS, 2023). Haptoglobin is a biomarker for diagnosis and prognosis for many diseases in cattle, such as mastitis, metritis, pneumonia, fatty liver syndrome, peritonitis, enteritis, and endocarditis (CECILIANI et al., 2012; SACO & BASSOLS, 2023). Nevertheless, increased TNF- α levels have been found in rats given multiple doses of activated ptaquiloside (SHAHIN et al., 1998) while investigations regarding haptoglobin and toxins (mainly aflatoxins) in cows are contradictory (QUEIROZ et al., 2012; KINOSHITA et al., 2015).

Despite investigations about clinical-pathological analytes in cattle intoxicated by bracken fern worldwide (DAWRA et al., 1991; PEREZ-ALENZA et al., 2006; DI LORIA et al., 2012; RAI et al., 2017) and in Brazil (FALBO et al., 2005; GABRIEL et al., 2009; ROCHA et al., 2022), APP has not been previously investigated in

detail in cows with BEH. Thus, we hypothesized that cattle with BEH could have changes in haptoglobin concentration, selected hematological, biochemical, and urinary analytes involving systemic and urinary bladder inflammation. For this purpose, the serum concentrations of haptoglobin (Hp), total protein, albumin, urea, creatinine, the serum activities of aspartate aminotransferase (AST), creatine kinase (CK), and fibrinogen, urinalysis and the complete blood cell counts were determined.

MATERIALS AND METHODS

The study population comprised 50 Nelore cows (*Bos indicus*) from two to 15 years old. The animals belonged to small private farms in the municipalities of Ribeira (São Paulo state), Fazenda Rio Grande, Rio Branco do Sul, Bocaiúva do Sul, Doutor Ulysses and Adrianópolis (Paraná State) in the southern region of Brazil. All farms had the same extensive husbandry and similar health and nutritional management. These farms had previous reports of animals with BEH and the presence of bracken fern (*Pteridium* spp.) in the pasture (GROXCO et al., 2017).

Blood and urine samples were obtained from the cows from September 2016 to December 2017. The cows were monitored weekly by a veterinarian for body score, mucosae color, and the appearance of feces. The animals were vaccinated for foot and mouth disease, and brucellosis according to current legislation in the Animal Health National Programs in Brazil and had not been dewormed or vaccinated for a period of 30 days prior to sampling for this investigation. During the visits, hematuria was observed in several cows. The cows were included in the study if they fulfilled the following inclusion criteria: (1) older than 2 years; (2) at least 60 days after parturition; (3) history and/or clinical signs of hematuria.

Blood samples were collected from each animal from the coccygian blood vessel. One aliquot (10mL) was placed in plain tubes with gel separators, which were allowed to clot at room temperature for 30 minutes. After centrifugation (1500 x g for 5 min) sera were stored in Eppendorf microtubes at -80 °C. The other blood sample (5 mL) was placed in potassium ethylene diamine tetra acetic acid (EDTA) tubes (BD Vacutainer® K2 EDTA Blood Collection Tube; Becton, Dickinson and Company, USA), to perform the hemogram and fibrinogen for every animal.

Biochemical profile

Serum haptoglobin (Hp) concentrations were measured via a hemoglobin binding assay

(ECKERSALL et al., 1999) modified and validated for use in bovine (BRADY et al., 2019) with a commercial control Hp standard (Haptoglobin control Set, Tridelta Development Limited, Co. Kildare, Ireland). The reference range for haptoglobin was determined from a set of 10 banked serum samples from apparently clinical healthy (mixed breed and over 2 years old) cows and was 0.13 g/L (median), and 0.02-0.28 g/L (minimum-maximum).

The other aliquot of serum samples was used for biochemical analysis. Total serum protein, albumin, urea, creatinine, and AST were measured using Dialab® commercial kits (DIALAB GmbH, Vienna, Austria), and CK was measured using Kovalent® commercial kits (Biosys/Kovalent, Rio de Janeiro, Brazil). All assays were determined on an automated biochemistry analyzer (BS-200® automatic chemistry analyzer, Mindray, Shenzhen, China). All assays showed a within run imprecision of less than 10%.

Hemogram and fibrinogen

The complete blood count (CBC) was performed in each animal using an automated Hematology Analyzer (BC-2800Vet®, Mindray, Shenzhen, China). The hematocrit was determined by the microhematocrit method. Differential leukocyte counts in 100 cells were manually performed in Diff Quick® stained blood smears with optical microscopy (1000x magnification) evaluation (JAIN, 1986). Plasma fibrinogen was determined by the heat precipitation method (56 °C) (KANEKO & SMITH, 1967).

Urinalysis

Sixty milliliters of urine were sampled from 50 cows during spontaneous voiding in plastic vials by perineal stimulation after cleaning with disposable paper towel.

The fifty urine samples underwent immediate routine urinalysis. Physical examination was performed by visual inspection of color and clarity/turbidity, including measurement of urine specific gravity (SG) by a hand-held refractometer (Goldberg-type) immediately after collection.

Color scores were: 1 = pale yellow; 2 = yellow; 3 = light brown; 4 = red; and for turbidity, the scores were: 1 = clear; 2 = mild to moderate turbidity; 3 = turbid; 4 = with blood clots.

Chemical examination (urinary protein and occult blood in urine) was carried out by semiquantitative dipstick test (Urofit® 10 DL, Urinalysis Dipstick, Alere Inc., USA) and the scores were categorized as: 1 = presence; 2 = absence.

Urine samples were kept at 4 °C and taken to the Veterinary Clinical Pathology Laboratory of UFPR, Curitiba, Brazil within 6 hours for the microscopic sediment analysis. Twenty milliliters of each urine were centrifuged at 1,200 x g for 5 minutes. The microscopic sediment analysis was performed by resuspending 20 µL of urine sediment and subsequent placement on microscope glass slides and covered by a glass coverslip. The urine sediment was examined under high-power (400x) field for evaluation of epithelial cells, red blood cells (RBCs), and leukocytes (WBCs). The sediment findings were quantified by the number of structures seen per microscopic field (hpf = high power field) (STOCKHAM & SCOTT, 2013; HERMAN et al., 2019).

Statistics were performed using statistical software (GraphPad Version 9.5.1 for Windows, GraphPad Software Inc., San Diego, CA, USA). Mann-Whitney *U* test was used to compare the BEH group (n = 50) with the results from the set of 10 banked serum samples from clinically healthy cows for haptoglobin concentrations as the data was not normally distributed. Statistical significance was set at $P < 0.05$. Haptoglobin data is reported as median and minimum and maximum values unless otherwise stated. Hematological and biochemical analytes data are reported as mean, standard deviation (SD), and minimum and maximum.

RESULTS

Haptoglobin concentrations and biochemical analytes in BEH cows are presented in table 1. Statistically significantly ($P < 0.05$) increased concentrations of haptoglobin (3.9-fold) were observed in BEH cows when comparing with the set of 10 serum samples from apparently healthy cows. Fold values represent fold differences between the median values of each group. The BEH cows also had higher globulins and significantly higher activity of CK than the reference interval (RI) values. Total protein, albumin, urea, creatinine, and the activity of AST results were within RI (Table 1).

Urine samples of twenty-nine cows with history of bracken fern ingestion had a countless number of erythrocytes per hpf, appeared light brown to red and were turbid. Twenty-one cows had pale to dark yellow urine samples, mild-to-moderate turbidity, and three or more erythrocytes per hpf. In all analyzed samples, the mean SG value obtained was 1.018 ± 0.008 (1.005 to 1.032). All cows had score 1 for urinary protein. The microscopic examination of the urine sediment in all 50 BEH cows revealed the

Table 1 - Results of haptoglobin and biochemical analytes concentrations of cows with Bovine Enzootic Haematuria (BEH) with history of bracken fern ingestion. Mean (SD) [minimum-maximum].

Analytes	Bovine Enzootic Haematuria (BEH)	
	Mean (SD) [min-max]	Reference Intervals**
Haptoglobin (g/L) ^a	0.56 [0.26-1.56]	0.13 [0.02-0.28] [*]
	----- (P value < 0.0001) -----	
Total protein (g/dL)	6.9 (1.3) [4.2-9.5]	6.7-7.5
Albumin (g/dL)	3.5 (0.7) [2.0-5.2]	2.6-3.7
Globulins (g/dL)	4.3 (0.9) [2.8-6.8]	3.0-3.5
CK (UI/L)	207 (111.4) [48.6-597.4]	31.2-143.8 ^{***}
AST (UI/L)	97.3 (30.8) [53.4-146.9]	78-132
Urea (mg/dL)	19.6 (7.2) [8.8-43.1]	20-30
Creatinine (mg/dL)	1.3 (0.4) [0.5-2.8]	1.0-2.0

^aMann-Whitney tests. P values less than 0.05 are considered statistically significant; ^{*}Significantly different between haematuria (n = 50) and set of 10 banked serum samples from clinically healthy cows.

^{**}Kaneko et al. (2008).

^{***}Conceição et al., 2019.

presence of WBCs (less than 5 cells per hpf), and few desquamated urothelial cells.

Hematological analytes for BEH cows are presented in table 2. Several cows had decreased RBC, hematocrit, and hemoglobin (Hb) concentration (9, 13, and 22 animals, respectively). Fourteen cows had leukocytosis, 14 had neutrophilia, 15 had lymphocytosis, six had neutropenia, and two had lymphopenia when compared to RI. Plasma fibrinogen, platelets, monocytes, and eosinophils were within the RI for cattle (Table 2).

DISCUSSION

The main changes observed in cows with history of bracken fern ingestion were increases in serum haptoglobin, globulins, and CK, alterations in urine color and turbidity, and abundance of RBCs in the urinary sediment. Forty-seven cows had increased haptoglobin concentrations and several cows had anemia, leukocytosis, neutrophilia, and lymphocytosis.

Hematuria is a term derived from the Greek words *haima* (blood) and *ouron* (urine), which refers to the presence of blood in the urine and can be either grossly visible (macrohematuria) or only detectable under a microscope (microhematuria). Microhematuria is said to be present when urine microscopy reveals three or more erythrocytes per hpf (BOLENZ et al., 2018). In our study, there were 29 cows with macrohematuria and 21 with microhematuria. A light to dark yellow urine color,

such as those presented by the 21 microhematuric cows, made it difficult and/or impossible to detect hematuria on physical examination, suggesting that the absence of changes in urinary color cannot be used alone for the diagnosis of BEH. Additionally, in healthy cows, urine is straw yellow, most frequently clear and in occasionally mildly turbid, with a threshold of < 3 elements/microscopic field or cells, hyaline casts, and crystals (HERMAN et al., 2019). The very high number of RBC, the presence of WBCs per hpf, and desquamated urothelial cells in the urinary sediment are common findings in urinary bladder inflammation, neoplasia, and hyperplasia reported in cattle with BEH (SÁNCHEZ-VILLALOBOS et al., 2004; PEREZ-ALENZA et al., 2006).

Conversely, the presence of blood in urine of cattle with BEH could be related to the source of the neoplastic cells as reported by PEREZ-ALENZA et al. (2006) associated or not with BPV types 1 and 2 (CAMPO et al., 1992; FALBO et al., 2005; YUAN et al., 2007). A previous investigation of laboratory analytes in cattle with history of natural ingestion of bracken fern suggested difficulties in the clinical-laboratory evaluation of animals with microhematuria or in remission periods of the disease, as in these situations the blood analytes did not show significant changes (FALBO et al., 2005).

The presence of dipstick proteinuria was demonstrated for BEH cows. PEREZ-ALENZA et al. (2006) reported similar results of proteinuria at different intensities in 100% of cattle chronically exposed to *Pteridium* spp. HOPKINS (1990) found

Table 2 - Total red blood cells, hematocrit, hemoglobin concentration, total white blood cells, segmented neutrophils, lymphocytes, eosinophils, monocytes, and fibrinogen of cows with Bovine Enzootic Haematuria and history of bracken fern ingestion (n = 50). Mean [standard deviation], (minimum-maximum).

Analytes	BEH [SD] (min-max)	Reference intervals*	Number of cows above RI (A) or below RI (B)
RBCs ($\times 10^6/\text{mm}^3$)	5.56 [1.3] (3.3-9.4)	4.8 - 7.6	4A; 9B
Hematocrit (%)	27.4 [6.2] (13-42)	24 - 39	1A; 13B
Hemoglobin (g/dL)	8.2 [1.8] (3.5-12.9)	8.2 - 13	22B
WBCs ($/\text{mm}^3$)	9,200 [3,412] (3,100-16,300)	4,400 - 10,800	14A; 6B
Segmented neutrophils ($/\mu\text{L}$)	3,889 [2,090] (930-10,595)	800 - 5,000	14A
Lymphocytes ($/\mu\text{L}$)	4,386 [1,723] (1,518-9,548)	1,800 - 4,900	15A; 2B
Eosinophils ($/\mu\text{L}$)	819.2 [489.7] (47-2,438)	100 - 2,100	1A
Monocytes ($/\mu\text{L}$)	130.8 [205.2] (0-960)	300 - 1,200	41B
Fibrinogen (g/dL)	0.23 [0.1] (0-0.6)	0.3 - 0.7**	0

Bovine Enzootic Haematuria (BEH); Red blood cells (RBC); White blood cells (WBCs); *WOOD (2022); **KANeko et al. (2008). RI (Reference interval).

8% of hematuria and 33% of proteinuria in urine samples from apparently healthy cattle raised in regions where HEB and the presence of *Pteridium* spp. in pastures were common, suggesting that this finding could indicate a preclinical state of BEH. In this study, all cows grazed in pastures where a large amount of bracken fern was found, mainly sprouts of *Pteridium* spp. and a previous study of the prevalence of bovine enzootic hematuria in the municipalities of Adrianópolis and Ribeira, Brazil (GROXCO et al., 2017) was carried out on the same small private farms where part of the samples were obtained for this investigation.

Acute phase proteins play major roles in various phases of the systemic reaction to inflammation (CECILIANI et al., 2012). The elevated haptoglobin and globulin results obtained in our study for BEH cows could indicate a chronic systemic inflammatory response which could be associated with the urinary bladder mucous lesions caused by the metabolites of ptaquiloside carcinogenic toxin and the possibility of urinary bladder neoplasia development (PEREZ-ALENZA et al., 2006; WEISS, 2022). In our study, median haptoglobin concentrations for BEH cows had a 3.9-fold increase suggesting a chronic inflammatory response; however, lower than the major magnitude increase (10 to 100 fold) stimulated by an acute

inflammatory response in ruminants (SACO & BASSOLS, 2023). The possibility of urinary bladder neoplasia could not be ruled out; however, a limitation of our study is that cystoscopy was not available.

Guinea pigs, like cattle, have been reported to be most susceptible to bracken fern poisoning with hematuria, edema, and hemorrhage in the urinary bladder (USHIJIMA et al., 1983). Guinea pigs experimentally injected with bracken extract developed vascular hemolysis and hemoglobinuria, suggesting that the mechanism of hemolysis was caused by hemolysin, a bracken toxin fraction (TJATUR RASA et al., 1999). Assuming the *Pteridium* spp. metabolites causes intravascular hemolysis, released Hb will react with haptoglobin and be removed from the circulation. Thus, the small rise in haptoglobin is a balance between increased production and increased removal after Hb binding (ECKERSALL & CONNER, 1990; CECILIANI et al., 2012). Therefore, there could be a larger acute phase reaction but much of the haptoglobin was being removed as it was being produced.

Forty-seven cows had elevated haptoglobin concentrations while the number of total WBC and segmented neutrophils were above the reference intervals for 14 cows. Although, leukopenia and neutropenia are common findings in bovines with

BEH (PRASAD, 1983; XU, 1992; SÁNCHEZ-VILLALOBOS et al., 2004; FALBO et al., 2005), the main findings in our study were leukocytosis and neutrophilia. Thus, haptoglobin was useful for assessing the inflammatory response which could be associated with urinary bladder lesions when comparing to hematological analytes. Acute phase proteins have been reported to have higher diagnostic value than that of the hemogram, and haptoglobin and serum amyloid A (SAA) may be used to discriminate between acute and chronic inflammatory conditions in ruminants (HORADAGODA et al., 1999). Moreover, the assessment of APP, especially haptoglobin in bovids, is frequently used, because in addition to being an indicator of inflammation, it is the most sensitive marker of systemic inflammation (CECILIANI et al., 2012; WOOD, 2022).

In Brazil, previous investigations reported that the cytopathological examination of bovine urinary bladder washing with BEH allowed the identification of predominantly inflammatory non-neoplastic lesions (AZEVEDO et al., 2016) and urinary bladders of cattle with BEH were found to have non-neoplastic and neoplastic lesions, isolated or associated, with a higher frequency of dysplasia, clear cell metaplasia, inflammation, and vascular thickening in bladders with neoplasm (FALBO et al., 2005; SILVA et al., 2012). Further investigations concerning measuring SAA or even alpha-1 acid glycoprotein would be of interest to understand if large increases would demonstrate an acute or moderate inflammatory reaction.

From the 29 cows with macrohematuria, 13 had countless erythrocytes per hpf and the total RBC, hematocrit, and Hb concentrations were below the reference intervals for bovine (WOOD, 2022), suggesting anemia that could be caused by the urinary blood loss as these are common changes during hematuria which is the main clinical sign in animals with BEH (PAMUKCU et al., 1967; TOKARNIA et al., 1967). In these cases, anemia is proportional to the degree of hematuria (DÖBEREINER et al., 1969; SÁNCHEZ-VILLALOBOS et al., 2004; GABRIEL et al., 2009; SILVA et al., 2009). Although, the bone marrow of the BEH cows of this study were not evaluated, RIBEIRO et al. (2020) and WEISS (2022) suggested that anemia could reflect the chronic bone marrow suppression after repeated exposure to the ptaquiloside and pterosin B toxins which are present in high levels in *Pteridium aquilinum* species complex in Brazil. Although, bracken fern intoxication was reported to be a major production issue in the area where

this study was carried out (GROXCO et al., 2017), another limitation of this investigation was the unavailability of ptaquiloside levels determination and the daily ingestion of the plant for each animal.

The higher serum activities of CK observed in 33 BEH cows could indicate muscle injury in some of the animals as the reactive behavior of the Nelore breed must be considered as well as the physical effort required to group the cows, the uneven topography of the area, and the intensification of the animals' muscular exercise during handling for sample collection (CONÇEIÇÃO et al., 2019). Additionally, in domestic species, CK activity is mainly used as a marker of skeletal muscle injury associated with trauma, nutritional myopathies, exercise induced muscle injury, or congenital myopathies (VALBERG, 2008).

Despite being a chronic disease, BEH presents continuous, intermittent clinical signs and periods of remission and exacerbation of the disease are common (FRANÇA et al., 2002; TOKARNIA et al., 2012). Ideally a combination of two or three APP could be determined to properly characterize the acute phase reaction of cows with BEH. However, this paper provides an overview of the chronic inflammatory response produced by BEH in cows in field conditions. In addition, those findings are useful for bovine practitioners to understanding of pathophysiological symptoms of an intoxication caused by *Pteridium* species especially in areas where BEH is endemic.

CONCLUSION

In our study, elevations in haptoglobin, creatine kinase (CK), hyperglobulinemia, neutrophilia, lymphocytosis, and anemia were detected in cows with bovine enzootic hematuria (BEH), contributing towards an understanding of the chronic inflammatory response which could be associated with the lesions in the urinary bladder caused by long-term and repeated ingestion of *Pteridium* species.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

ACKNOWLEDGMENTS

This research was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001. - Master's degree grant for Carolina Dutra Minozzo.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

Approval was granted and approved by the Ethics Committee of the Faculty's Animal Experimentation Ethics Committee of the Universidade Federal do Paraná - UFPR, Curitiba, Brazil (Number 076/2016 - CEUA).

REFERENCES

- AZEVEDO, M. A. S. et al. Bovine urinary bladder washing with enzootic hematuria: standardization of harvesting technique, sample collection and cytopathological evaluation. **Arquivos do Instituto Biológico**, v.82, p.1–8, 2016. Available from: <<https://doi.org/10.1590/1808-1657001002013>>. Accessed: Jun. 11, 2023. doi: 10.1590/1808-1657001002013.
- BOLENZ, C. et al. The investigation of hematuria. **Deutsches Arzteblatt International**, v.115, p.801–807, 2018. Available from: <<https://doi.org/10.3238/2Farztebl.2018.0801>>. Accessed: May, 31, 2023. doi: 10.3238/2Farztebl.2018.0801.
- BRADY, N. et al. An immunoturbidimetric assay for bovine haptoglobin. **Comparative Clinical Pathology**, v.28, p.21–27, 2019. Available from: <<https://doi.org/10.03.239/s00580-018-2863-6>>. Accessed: May, 12, 2023. doi: 10.1007/s00580-018-2863-6.
- CAMPO, M. S. et al. Association of Bovine Papillomavirus Type 2 and Bracken Fern with Bladder Cancer in Cattle. **Cancer Research**, v.52, p.6898–6904, 1992. Available from: <<https://aacrjournals.org/cancerres/article/52/24/6898/498403>>. Accessed: Apr. 13, 2023.
- CECILIANI, F. et al. Acute phase proteins in ruminants. **Journal of Proteomics**, v.75, p.4207–4231, 2012. Available from: <<https://doi.org/10.1016/j.jprot.2012.04.004>>. Accessed: Apr. 22, 2023. doi: 10.1016/j.jprot.2012.04.004.
- CONÇEIÇÃO, W. L. F. et al. Serum biochemical profile of Nellore and Girolando cows raised in state of Maranhão. **Ciência Animal Brasileira**, v.20, p.1–7, 2019. Available from: <<https://doi.org/10.1590/1089-6891v20e-33796>>. Accessed: Aug. 13, 2023. doi: 10.1590/1089-6891v20e-33796.
- DAWRA, R. K. et al. The enzymatic profile of urine and plasma in bovine urinary bladder cancer (enzootic bovine haematuria). **Veterinary Research Communications**, v.15, p.421–426, 1991. Available from: <<https://doi.org/10.1007/BF00346537>>. Accessed: Aug. 30, 2023. doi: 10.1007/BF00346537.
- DI LORIA, A. et al. Clotting profile in cattle showing chronic enzootic haematuria (CEH) and bladder neoplasms. **Research in Veterinary Science**, v.93, p.331–335, 2012. Available from: <<https://doi.org/10.1016/j.rvsc.2011.07.011>>. Accessed: Aug. 18, 2023. doi: 10.1016/j.rvsc.2011.07.011.
- DÖBEREINER, J. et al. Ocorrência da Hematúria Enzoótica e de Carcinomas Epidermóides No Trato Digestivo Superior Em Bovinos No Brasil. II. Estudos complementares **Pesquisa Agropecuária Brasileira**, v.4, p.209–224, 1969. Available from: <<https://seer.sct.embrapa.br/index.php/pab/article/view/17847/11961>>. Accessed: Jul. 21, 2023.
- ECKERSALL, P. D.; CONNER, J. G. Plasma haptoglobin in cattle (*Bos taurus*) exists as polymers in association with albumin. **Comparative Biochemistry and Physiology B**, v.96, p.309–314, 1990. Available from: <[https://doi.org/10.1016/0305-0491\(90\)90379-8](https://doi.org/10.1016/0305-0491(90)90379-8)>. Accessed: Mar. 10, 2023. doi: 10.1016/0305-0491(90)90379-8.
- ECKERSALL, P. D. et al. An automated biochemical assay for haptoglobin: Prevention of interference from albumin. **Comparative Haematology International**, v.9, p.117–124, 1999. Available from: <<https://doi.org/10.1007/BF02600369>>. Accessed: Jun. 10, 2023. doi: 10.1007/BF02600369.
- FALBO, M. K. et al. Hematological, biochemical, Urinary and histopathological changes in natural intoxication in bovine by bracken fern (*Pteridium aquilinum*) (L.) Kühn. **Semina: Ciências Agrárias**, v.26, p.547–558, 2005. Available from: <<https://doi.org/10.5433/1679-0359.2005v26n4p547>>. Accessed: Aug. 13, 2023. doi: 10.5433/1679-0359.2005v26n4p547.
- FRANÇA, T. do N. et al. Diseases caused by the radiomimetic principle of *Pteridium aquilinum* (Polypodiaceae). A review. **Pesquisa Veterinária Brasileira**, v.22, p.85–96, 2002. Available from: <<https://www.scielo.br/j/pvb/a/WNVB9SK6MQ4yS4HYGqCf7Zw/>>. Accessed Jun. 12, 2023. doi: 10.1590/S0100-736X2002000300001.
- GABRIEL, A. L. et al. Clinical and hematological aspects and urinary bladder lesions in chronic spontaneous poisoning by *Pteridium aquilinum* in cattle. **Pesquisa Veterinária Brasileira**, v.29, p.515–525, 2009. Available from: <<https://doi.org/10.1590/S0100-736X2009000700005>>. Accessed: Sept. 10, 2023. doi: 10.1590/S0100-736X2009000700005.
- GAVA, A. et al. Bracken fern (*Pteridium aquilinum*) poisoning in cattle in southern Brazil. **Veterinary and Human Toxicology**, v.44, p.362–365, 2002. Available from: <https://www.researchgate.net/publication/11011025_Bracken_fern_Pteridium_aquilinum_poisoning_in_cattle_in_Southern_Brazil>. Accessed: Sept. 18, 2023. doi: 10.1590/S0100-736X2009000700005.
- GROXCO, R. B. et al. Prevalência de hematúria enzoótica bovina nos municípios de Adrianópolis, PR e Ribeira, SP-Brasil. **Archives of Veterinary Science**, v.22, p.67–72, 2017. Available from: <<http://dx.doi.org/10.5380/avs.v22i4.56895>>. Accessed: Jan. 15, 2024. doi: 10.5380/avs.v22i4.56895.
- HERMAN, N. et al. Urinalysis and determination of the urine protein-to-creatinine ratio reference interval in healthy cows. **Journal Veterinary Internal Medicine**, v.33, p.999–1008, 2019. Available from: <<https://onlinelibrary.wiley.com/doi/10.1111/jvim.15452>>. Accessed: Jan. 17, 2024. doi: 10.1111/jvim.15452.
- HOPKINS, A. Bracken (*Pteridium aquilinum*): Its distribution and animal health implications. **British Veterinary Journal**, v.146, p.316–326, 1990. Available from: <[https://doi.org/10.1016/S0007-1935\(11\)80024-2](https://doi.org/10.1016/S0007-1935(11)80024-2)>. Accessed: Aug. 19, 2023. doi: 10.1016/S0007-1935(11)80024-2.
- JAIN, N.C. **Schalm's Veterinary Hematology**, 4th ed., (Lea & Febiger: Philadelphia), 1986.

- KANEKO, J. J.; SMITH, R. The estimation of plasma fibrinogen and its clinical significance in the dog. **California Veterinarian**, v.21, p.21–24, 1967. Available from: <<https://www.cabidigitallibrary.org/doi/full/10.5555/19682200361>>. Accessed: Jun. 8, 2023. CABI Record Number: 19682200361.
- KANEKO, J. J. et al. **Clinical Biochemistry of Domestic Animals**, 6th ed., (Academic Press, London), 2008.
- KINOSHITA, A. et al. Effects of Fusarium mycotoxins in rations with different concentrate proportions on serum haptoglobin and hepatocellular integrity in lactating dairy cows. **Journal of Animal Physiology and Animal Nutrition**, v.99, p.887–892, 2015. Available from: <<https://doi.org/10.1111/jpn.12293>>. Accessed: Aug. 17, 2023. doi: 10.1111/jpn.12293.
- MOLOSSI, F. A. et al. Causes of death in beef cattle in southern Brazil. **Journal of Veterinary Diagnostic Investigation**, v.33, p.677–683, 2021. Available from: <<https://doi.org/10.1177/10406387211007952>>. Accessed: Aug. 21, 2023. doi: 10.1177/10406387211007952.
- HORADAGODA, N. U. et al. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. **Veterinary Record**, v.144, p.437–441, 1999. Available from: <<https://doi.org/10.1136/vr.144.16.437>>. Accessed: Jun. 09, 2023. doi: 10.1136/vr.144.16.437.
- PAMUKCU, A. M. et al. Urinary Bladder Neoplasms Induced by Feeding Bracken Fern (*Pteris aquilina*) to Cows. **Cancer Research**, v.27, p.917–924, 1967. Available from: <https://aacrjournals.org/cancerres/article/27/5_Part_1/917/476516>. Accessed: Aug. 17, 2023.
- PEREZ-ALENZA, M. D. et al. Clinico-pathological findings in cattle exposed to chronic bracken fern toxicity. **New Zealand Veterinary Journal**, v.54, p.185–192, 2006. Available from: <<https://doi.org/10.1080/00480169.2006.36693>>. Accessed: Aug. 17, 2023. doi: 10.1080/00480169.2006.36693.
- PRASAD, M. C. Haematological and biochemical alterations in enzootic bovine haematuria. **Acta Veterinaria Brno**, v.52, p.157–161, 1983. Available from: <<https://doi.org/10.2754/avb198352030157>>. Accessed: Jun. 22, 2023. doi: 10.2754/avb198352030157.
- QUEIROZ, O. C. M. et al. Effect of adding a mycotoxin-sequestering agent on milk aflatoxin M1 concentration and the performance and immune response of dairy cattle fed an aflatoxin B1-contaminated diet. **Journal of Dairy Science**, v.95, p.5901–5908, 2012. Available from: <<https://doi.org/10.3168/jds.2011-5287>>. Accessed: Jun. 20, 2023. doi: 10.3168/jds.2011-5287.
- RAI, S. K. et al. Survey of ferns and clinico-pathological studies on the field cases of Enzootic bovine haematuria in Himachal Pradesh, a north-western Himalayan state of India. **Toxicon**, v.138, p.31–36, 2017. Available from: <<https://doi.org/10.1016/j.toxicon.2017.08.010>>. Accessed: Jun. 28, 2023. doi: 10.1016/j.toxicon.2017.08.010.
- RIBEIRO, D. D. S. F. et al. Ptaquiloside and pterisin B levels in mature green fronds and sprouts of *Pteridium arachnoideum*. **Toxins**, v.12, p.1–8, 2020. Available from: <<https://doi.org/10.3390/toxins12050288>>. Accessed: Apr. 20, 2023. doi: 10.3390/toxins12050288.
- RIET-CORREA, F. et al. Plants causing poisoning outbreaks of livestock in South America: A review. **Toxicon**, v.17, p.100150, 2023. Available from: <<https://doi.org/10.1016/j.toxicon.2023.100150>>. Accessed: Jun. 20, 2023. doi: 10.1016/j.toxicon.2023.100150.
- ROCHA, J. F. et al. Occurrence enzootic hematuria in buffaloes in Brazil: epidemiological, clinical, and pathological aspects. **Pesquisa Veterinária Brasileira**, v.42, p.1–13, 2022. Available from: <<https://doi.org/10.1590/1678-5150-PVB-6875>>. Accessed: Jun. 20, 2023. doi: 10.1590/1678-5150-PVB-6875.
- SACO, Y.; BASSOLS, A. Acute phase proteins in cattle and swine: A review. **Veterinary Clinical Pathology**, v.52, p.50–63, 2023. Available from: <<https://doi.org/10.1111/vcp.13220>>. Accessed: Jun. 20, 2023. doi: 10.1111/vcp.13220.
- SÁNCHEZ-VILLALOBOS, A. et al. Observaciones Clínico Patológicas En Vacas Con Hematuria Enzootica Bovina. **Revista Científica**, v.14, p.317–323, 2004. Available from: <https://www.researchgate.net/profile/Julio-Boscan-Ocando/publication/242663129_>. Accessed: Aug. 13, 2023.
- SHAHIN, M. et al. Bracken fern carcinogenesis: Multiple intravenous doses of activated ptaquiloside induce DNA adducts, monocytosis, increased TNF α levels, and mammary gland carcinoma in rats. **Biochemical and Biophysical Research Communications**, v.244, p.192–197, 1998. Available from: <<https://doi.org/10.1006/bbrc.1998.8240>>. Accessed: Sept. 13, 2023. doi: 10.1006/bbrc.1998.8240.
- SILVA, M. A. et al. Prevalence of bovine enzootic haematuria in dairy cattle in the Caparaó microregion, southern Espírito Santo, between 2007 and 2008. **Ciência Rural**, v.39, p.1847–1850, 2009. Available from: <<https://doi.org/10.1590/S0103-84782009005000121>>. Accessed: Jun. 29, 2023. doi: 10.1590/S0103-84782009005000121.
- SILVA, M. A. et al. Histopathologic characterization of urinary bladders associated with Bovine Enzootic Hematuria. **Revista Brasileira de Medicina Veterinária**, v.34, p.319–326, 2012. Available from: <<https://bjvm.org.br/BJVM/article/view/732>>. Accessed: Jul. 11, 2023.
- SOUTO, M. de A. M. et al. Urinary bladder neoplasms associated with bovine enzootic hematuria. **Ciência Rural**, v.36, p.1647–1650, 2006. Available from: <<https://doi.org/10.1590/S0103-84782006000500050>>. Accessed: Jun. 20, 2023. doi: 10.1590/S0103-84782006000500050.
- STOCKHAM, S. L.; SCOTT, M. A. **Fundamentals of Veterinary Clinical Pathology**. (Blackwell Publishing, Oxford), 2013.
- TJATUR RASA, F. S. et al. The Hemolytic Activity of Bracken Extracts in Guinea Pigs. **Journal of Veterinary Medical Science**, v.61, p.129–133, 1999. Available from: <<https://doi.org/10.1292/jvms.61.129>>. Accessed: Oct. 20, 2023. doi: 10.1292/jvms.61.129.
- TOKARNIA, C. et al. Acute bracken fern (*Pteridium aquilinum*) (L.) Kuhn poisoning of cattle in Brazil. **Pesquisa Agropecuária Brasileira**, v.2, p.329–336, 1967. Available from: <<https://ainfo.cnptia.embrapa.br/digital/bitstream/item/191832/1>>. Accessed: May, 18, 2023.
- TOKARNIA, C. H. et al. **Plantas tóxicas do Brasil para animais de produção**, 2^a ed., (Helianthus: Rio de Janeiro, Brazil), 2012.
- USHIJIMA, J. et al. Toxicities of bracken fern in guinea pigs. **The Japanese Journal of Veterinary Science**, v.45, p.593–602, 1983.

Available from: <<https://doi.org/10.1292/jvms1939.45.593>>.
Accessed: Jun. 20, 2023. doi: 10.1292/jvms1939.45.593.

VALBERG, S. J. Skeletal muscle function In: KANEKO, J. J., et al.(eds) **Clinical Biochemistry of Domestic Animals**, 6th ed. (Academic Press, London), p.459–484, 2008.

WEISS, D. J. Blood and Bone Marrow Toxicity Induced by Drugs, Heavy Metals, Chemicals, and Toxic Plants In: BROOKS, M. B. et al. (eds) **Schalm's Veterinary Hematology**, 7th ed., (Wiley-Blackwell, Ames), p.122–132, 2022.

WOOD, R. D. Hematology of Bovids. In: BROOKS, M. B. et al. (eds) **Schalm's Veterinary Hematology**, 7th ed., (Wiley-Blackwell, Ames), p.2.865–2.891, 2022.

XU, L. R. Bracken poisoning and enzootic haematuria in cattle in China. **Research in Veterinary Science**, v.53, p.116–121, 1992. Available from: <[https://doi.org/10.1016/0034-5288\(92\)90095-J](https://doi.org/10.1016/0034-5288(92)90095-J)>. Accessed: Jun. 27, 2023. doi: 10.1016/0034-5288(92)90095-j.

YAMADA, K. et al. Ptaquiloside, the major toxin of bracken, and related terpene glycosides: chemistry, biology and ecology. **Natural Product Reports**, v.24, p.798–813, 2007. Available from: <<https://doi.org/10.1039/B614160A>>. Accessed: Jun. 20, 2023. doi: 10.1039/b614160a.

YUAN, Z. et al. Bovine papillomavirus infection in equine sarcoids and in bovine bladder cancers. **Veterinary Journal**, v.174, p.599–604, 2007. Available from: <<https://doi.org/10.1016/j.tvjl.2006.10.012>>. Accessed: Jun. 24, 2023. doi: 10.1016/j.tvjl.2006.10.012.