



Ocurrence enzootic hematuria in buffaloes in Brazil: epidemiological, clinical, and pathological aspects¹

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It is reported the occurrence of enzootic hematuria (EH) in buffaloes in Brazil after performing an epidemiological survey and clinicopathological analyses. To date, EH caused by ingestion of *Pteridium esculentum* subsp. *arachnoideum*, a radiomimetic plant popularly known as “bracken fern”, has not been described in this species in Brazil. Bovine EH is responsible for high economic losses in Brazil’s Southeast Region not only because of the deaths it causes, but also owing to its negative effect on productivity. In São José do Barreiro County, São Paulo, some farmers in areas with a high incidence of bovine EH have been replacing cattle with buffaloes, based on the premise that the latter would be more resistant to poisoning by ingestion of *Pteridium* spp. However, even though initial observations indicated that buffaloes are indeed less sensitive than cattle to the toxic principle of *Pteridium* spp., cases of hematuria in this species have been reported. According to preliminary data, EH only occurs in buffaloes over six years of age. Macroscopic examination revealed a thickened urinary vesicle mucosa, along with multiple foci of ulcerated, exophytic, verrucous, and pedunculated lesions. In one of the buffaloes studied, the bladder wall was ruptured and exhibited marked secondary inflammation. Histologically, neoplastic and non-neoplastic changes similar to those described in cattle poisoned by *Pteridium* spp. were observed. The neoplasms found included papilloma, carcinoma *in situ*, urothelial carcinoma (low and high grade), inverted, microcystic, and trabecular variants, urothelial carcinoma with divergent differentiation (squamous and glandular), squamous cell carcinoma, lymphangioma, hemangioma, and hemangiosarcoma. There was also coexistence of epithelial and mesenchymal neoplasms. Bovine papillomavirus particles were not detected by polymerase chain reaction in the bladder samples analyzed.

INDEX TERMS: Hematuria, buffaloes, Brazil, *Pteridium* spp., bracken fern, bladder neoplasm.

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RESUMO.- [Ocorrência de hematúria enzoótica em búfalos no Brasil: aspectos epidemiológicos, clínicos e patológicos.]

Descreve-se, através de levantamento epidemiológico e avaliação clínico-patológica, a ocorrência de hematúria enzoótica (HE) em búfalos no Brasil. Essa condição, causada pela ingestão da planta radiomimética *Pteridium esculentum* subsp. *arachnoideum*, conhecida popularmente como “samambaia” ou “samambaia do campo”, até então não havia sido descrita nessa espécie no Brasil. Na Região Sudeste, a HE bovina é responsável por elevadas perdas econômicas, devidas não apenas aos óbitos, mas também em função da queda de produtividade. No município de São José do Barreiro/SP, alguns produtores de áreas com alta incidência de HE bovina, vêm substituindo os bovinos por búfalos, com base na premissa de que estes seriam mais resistentes à intoxicação. Embora, de acordo com observações iniciais, os búfalos realmente sejam menos sensíveis que os bovinos ao princípio tóxico de *Pteridium* spp., ainda assim, tem-se verificado a ocorrência de casos de hematúria nessa espécie. De acordo com o levantamento inicial, a HE só ocorre em búfalos com idade a partir de seis anos. Ao exame macroscópico, verificou-se a mucosa da bexiga espessa, com múltiplos focos de lesões ulceradas, exofíticas, papiliformes, verrucosas, pedunculadas. Histologicamente, foram observadas alterações neoplásicas e não neoplásicas semelhantes às descritas nos bovinos com HE. Entre as neoplasias foram encontrados papiloma, carcinoma *in situ*, carcinoma urotelial (baixo e alto grau), variantes invertida, microcística e trabecular, carcinoma urotelial com diferenciação divergente (escamosa e glandular), carcinoma de células escamosas, linfangioma, hemangioma e hemangiossarcoma. Ocorreu também coexistência entre neoplasias epiteliais e mesenquimais. Não foram detectadas partículas de papilomavírus bovino pelo teste PCR nas amostras de bexiga analisadas.

TERMOS DE INDEXAÇÃO: Hematúria, búfalos, Brasil, *Pteridium* spp., samambaia, neoplasia de bexiga.

INTRODUCTION

Plants of the genus *Pteridium* are among the most important toxic plants because of their cosmopolitan distribution and high carcinogenic potential for animals and, possibly, humans. *Pteridium esculentum* subsp. *arachnoideum* (*Pteridium arachnoideum* was reclassified as *Pteridium esculentum* subsp. *arachnoideum* (Kaulf) by Thomson 2012) is one of the plants that cause severe economic losses in Brazilian livestock; its common names include “bracken fern” and “field bracken” in the Southeast Region of Brazil. Even though this species is generally known to be invasive by academics that work in this area, only few of the people outside the academic environment know or believe that this is one of the most important toxic plants for cattle (Tokarnia et al. 2012).

In cattle ingestion of *Pteridium* spp. causes one or more of the three different clinic-pathological conditions determined by the radiomimetic action of the plant, which comprise neoplasms of the upper alimentary tract, enzootic hematuria (EH) (Döbereiner et al. 1967, Tokarnia et al. 1969), and hemorrhagic diathesis (Tokarnia et al. 1967), leading to heavy economic losses for cattle ranchers in Brazil (Tokarnia et al. 2012). EH has been described mainly in cattle, whereas reports on EH in buffaloes are scarce. To date, EH in buffaloes has been reported only in Turkey, Formosa, Indonesia, and India (Pamukcu 1955, 1957,

1974, Aydin & Özkul 1995, Özkul & Aydin 1996, Somvanshi et al. 2012, Maiolino et al. 2013, Yücel Tenekeci et al. 2017).

The carcinogenic effect of *Pteridium* spp. is caused by the norsesquiterpene ptaquiloside (PTQ), a toxic principle responsible for its radiomimetic action (Hirono et al. 1984, Hirono 1990). The impact of this radiomimetic action may vary depending on the animal species, time of ingestion, and daily amount ingested (Tokarnia et al. 2012). A recent study showed that bracken additionally contains illudanes, which could contribute to its toxicity and carcinogenicity, although PTQ is considered the primary carcinogen of this plant (O'Connor et al. 2019). Some authors further suggested the co-participation of bovine papillomavirus type 2 in the pathogenesis of enzootic bovine hematuria (EBH) (Olson et al. 1959, Jarrett et al. 1978, Campo et al. 1992, Wosiacki et al. 2002, Borzacchiello et al. 2003, 2007, Roperto et al. 2005, 2007, 2008a, 2008b, Wosiacki et al. 2005, 2006, Balcos et al. 2008, Pathania et al. 2011, Resendes et al. 2011, Dias et al. 2012).

In cattle, EH is characterized by a clinical course that includes chronic intermittent hematuria, anemia, and progressive weight loss, culminating in death. According to Pamukcu (1957), EH affects cattle and buffaloes aged 2 to 14 years.

At necropsy, the bladder of cattle and buffaloes with EH may exhibit thickening of the wall, firm tumors with a yellowish, irregular surface protruding into the lumen, and/or multiple reddish polypoid formations, sometimes ulcerated and covered by blood clots. In some cases, the bladder may reach a size 6-7 times larger than average, and contain dark red urine and blood clots (Souto et al. 2006, Oliveira 2009, Yücel Tenekeci et al., 2017). Grape-like clusters, comprising broad-based nodules that are firm in consistency and grayish in color, may also be observed. The bladder mucosa may be covered by flat, rough, red to orange-colored elevations, which sometimes form clusters (Tokarnia et al. 1969); cattle may exhibit pale mucous membranes and watery blood (anemia) (Souto et al. 2006).

In the Southeastern Region of Brazil, bovine EH is responsible for high economic losses due to decreased productivity and death. In this region, EH occurs in many areas heavily invaded by *P. esculentum* subsp. *arachnoideum*, and has made many small dairy cattle farms unviable. This situation has led to migration of small producers, who need to sell their properties owing to their inability to earn a living and are forced to settle on the outskirts of nearby cities (Galvão & Peixoto 2012). In São José do Barreiro County, São Paulo, for approximately 15 years, some producers in areas with a high incidence of bovine EH have been replacing cattle with buffaloes based on the assumption that the latter are more resistant to this disease than cattle. In fact, initial observations suggest that buffaloes are more resistant to the toxic principle of *Pteridium* spp. than cattle. The present study reports the occurrence of EH and its epidemiological, clinical, and pathological aspects in buffaloes in Brazil.

MATERIALS AND METHODS

Visits were made to nine of 12 dairy buffalo farms in São José do Barreiro County to characterize the epidemiological and clinic-pathological aspects of intoxication by *Pteridium esculentum* subsp. *arachnoideum*. During the visits, hematuria was observed in two buffaloes from different properties. The herds of these properties consisted of 25 to 50 buffaloes. Five buffaloes with a history of hematuria were identified in the properties visited; they were slaughtered owing to

an unfavorable prognosis. It was possible to monitor the slaughter and collect material from three of those buffaloes.

The clinical history of the buffaloes was obtained by interviewing their buyers and/or sellers on the day of the slaughter. On the same day, visual evaluations of the animals were performed in the waiting pen of the slaughterhouses. Various types of behavior (level of consciousness, posture, locomotion, coat, abdominal shape, respiratory characteristics) were observed according to Feitosa (2014); urine color and body condition score (BCS) were assessed based on a scale of 1 to 5 according to Anitha et al. (2011).

Urinalysis. It was possible to collect an *ante mortem* urine sample from only one of the three female buffaloes. The urine sample was collected in a urinalysis flask during spontaneous urination. The sample was kept under refrigeration, and sent to the Laboratory of Clinical Pathology of the "Instituto de Veterinária" (Veterinary Institute - IV) of "Universidade Federal Rural do Rio de Janeiro (UFRRJ)", Seropédica/RJ. Physical, chemical, and sediment analyses were performed.

Samples. Bladders, ureters, and kidneys of three female buffaloes slaughtered in slaughterhouses in Barra Mansa (Rio de Janeiro), Cruzeiro and Bananal (São Paulo) Counties, were obtained for macroscopic and histopathological studies. The three buffaloes were identified as Buffalo 1, Buffalo 2, and Buffalo 3.

Histopathology. Fragments from the organs were fixed in 10% buffered formalin for histopathological examination and were reviewed by three pathologists. The material was routinely processed, and stained with hematoxylin and eosin (HE). The material was processed at the "Setor de Anatomia Patológica" (Pathology Sector - SAP) of IV-UFRRJ.

The histological classification used this study was based on the classification used for humans by the Armed Forces Institute of Pathology of the United States of America (AFIP), described by Murphy et al. (1994), and in the book Ackerman's Surgical Pathology (Ordóñez & Rosai 1996). In addition, histological variants of invasive urothelial carcinomas were classified according to the World Health Organization (WHO) Morphological Classification for human urothelial tumors (Lopez-Beltran et al. 2004, Cheng & Lopez-Beltran 2016, Humphrey et al. 2016) and the standards used for cattle with EH (França 2002, Peixoto et al. 2003, Carvalho et al. 2006, Roperto et al. 2010). Non-neoplastic lesions, when possible, were categorized according to the standards described by França (2002), Peixoto et al. (2003), Carvalho et al. (2006), Roperto et al. (2010), and Meuten (2017). Urothelial carcinomas were graded into four categories (grades 1 to 4), as proposed by Cheng et al. (2012) for humans, and adapted for domestic animals as low and high grades (Meuten 2017). Pathological staging of tumor invasion levels based on the TNM system was also performed (Cheng & Lopez-Beltran 2016).

Polymerase chain reaction (PCR). One paraffin-embedded bladder sample from each of the three buffaloes, which had been fixed in 10% buffered formalin and routinely processed by the Pathology Sector (SAP) of the IV-UFRRJ, were sent to the Virology Laboratory of the "Departamento de Patologia Clínica Veterinária" (Veterinary Clinical Pathology Department) of "Universidade Federal do Rio Grande do Sul" (UFRGS) for polymerase chain reaction (PCR) analysis. The samples were macerated with a mortar and pestle in 1mL of phosphate-buffered saline (PBS) at a pH of 7.4 and centrifuged at $720 \times g$ for 10 min. Total DNA was isolated from 100µL of the sample using a phenol-chloroform-based protocol (Sambrook & Russel 2001). DNA quantity and quality were assessed by spectrophotometry using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific). Conventional PCR was performed using

the primers FAP 59 (5'-TAACWGTIGGICAYCCWTATT-3') and FAP 64 (5'-CCWATATCWVHCATITCICCATCATC-3') to amplify a relatively conserved fragment of the L1 gene of all known papillomavirus types (Forsslund et al. 1999). This approach may fail to detect novel papillomavirus genomes in papilloma lesions, given the lower base-pairing homology in the 3' region of both primer binding sites (Daudt et al. 2016). The conditions for performing the PCR were as follows: 94°C for 5 min; 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and 72°C for 5 min. The amplicons were submitted to electrophoresis in a 0.5% agarose gel and visualized under an ultraviolet light source.

RESULTS

Epidemiological data

The epidemiological survey conducted from 2017 to 2019 in the mountainous region known as Sertão da Onça (Jaguar's Backwoods), located in São José do Barreiro County, São Paulo, revealed that all nine properties visited were heavily invaded by *Pteridium esculentum* subsp. *arachnoideum* (Fig.1). According to the accounts of the owners, mowing the pastures throughout the year and planting *Brachiaria* sp. *sin. Urochloa* (signal grass) were common practices. Most of the buffalo dairy farms (8/9) also kept some cattle for milk and beef production. All the milk produced was intended for cheese production, which was sold in the metropolitan region of Rio de Janeiro. Buffalo dairy farming was introduced in the region approximately 15 years ago, based on the assumption that buffaloes are more resistant to poisoning by *P. esculentum* subsp. *arachnoideum* than cattle. Ten of the 12 dairy farmers in the region replaced cattle with buffaloes or introduced buffaloes into dairy farming. All the farmers reported being more satisfied with buffalo husbandry, and stated that buffaloes are more resistant to hematuria than cattle. The farming in the properties was extensive; in some farms (4/9), buffaloes were supplemented with citrus pulp and commercial concentrate in the dry season of the year. These pastures were mainly composed of *P. esculentum* subsp. *arachnoideum* (70-90%) and *Urochloa* sp. (10-30%). Enzootic bovine hematuria (EBH) was a disease that the producers of that region knew well; however, few of them (2/9) believed that it was directly and exclusively associated with ingestion of *Pteridium* sp. More than 50% (6/9) of the farmers believed that EBH was caused by consumption of *Urochloa* sp. or by



Fig.1. Enzootic hematuria in buffaloes in Brazil. Buffalo in pasture invaded by *Pteridium esculentum* subsp. *arachnoideum* in São José do Barreiro County, São Paulo.

the association of both plants. The producers also alleged that EBH cases began to occur after the introduction of *Urochloa decumbens* (var. australiana) in the beginning of the 1970s.

Clinic and pathological findings

During the anamnesis performed in the presence the buyers or sellers of the buffaloes, they were found to have little information about the animals. The buyers or sellers reported that all of the animals had hematuria (Fig.2), and that they were adult dairy cows aged from 6 to 8 years. All three buffaloes had an average level of consciousness (alertness), posture, and locomotion. The coat of the three buffaloes was clean, shiny, and free of ectoparasites. The urine from Buffalo 1 was red in color. The body condition scores (BCSs) were 3.5, 2.5, and 3.0, respectively, for Buffaloes 1, 2, and 3.

At urinalysis, the urine of Buffalo 1 was characterized by a reddish turbid aspect, a specific density of 1.034, an alkaline pH (9.0), and an intense amount of blood and proteins. At the sedimentoscopy, the number of red blood cells per field was uncountable, and reading of the other parameters was impaired owing to intense hematuria.

During slaughter, samples of bladder, ureters, and kidneys of the three buffaloes were collected.

Macroscopy

The macroscopic changes in the bladders of Buffaloes 1 and 2 were similar, and were characterized by a shiny, irregular,



Fig.2. Enzootic hematuria in buffaloes in Brazil. Buffalo with reddish urine (enzootic hematuria).

pleated, and diffusely edematous, thick mucosa with a soft to gelatinous consistency. The proliferative lesions were multifocally disseminated, 0.3cm to 2cm in diameter, firm to elastic, circumscribed, sessile with smooth or arboriform surfaces, and pedunculated. The color of the lesions ranged from whitish to brownish red. There were few foci of erosions, petechiae, and ecchymoses with intravesical clots (Fig.3). The bladder of Buffalo 3 had, in addition to the changes already described for Buffaloes 1 and 2, an ulcerated, proliferative, expansile (transmural) lesion of 14cm in diameter. The contour and surface of this lesion were markedly irregular with areas ranging from gray to dark red. In addition to blood clots, a large quantity of a greenish fibrin suppurative exudate was attached to this lesion.

In addition, there was formation of a plaque with a white surface and well-defined irregular contour, similar to leukoplakia, measuring approximately 1.5cm x 2.0cm in another area of the bladder. The same buffalo exhibited suppurative pyelitis with dilatation of the calyces. The ureters had moderately thickened walls. The left ureter was moderately dilated with serosanguineous content, multifocal mucosal suffusions, and a circumscribed, slightly elevated, brownish lesion located in its cranial segment. The renal parenchyma had a slightly irregular surface with pale areas and multifocal ecchymoses. Discrete whitish linear areas were observed on the cut surface; they were radiated, sometimes coalescent, and irregularly distributed in the medulla. During sample collection, a gravid uterus at a gestational stage of approximately 90 days was observed in all three buffaloes, according to fetal morphometry (Abdel-Raouf & El-Naggar 1969).

Microscopy

The changes found in the bladders of the three buffaloes were divided into non-neoplastic (Table 1) and neoplastic lesions (Table 2). The non-neoplastic lesions were subdivided into dysplasia, metaplasia (Fig.4), hyperplasia, inflammation



Fig.3. Enzootic hematuria in buffaloes in Brazil. Bladder from Buffalo 1 after fixation. The mucosa is bright, markedly and irregularly pleated, diffusely edematous/thick, with a soft to gelatinous consistency. The proliferative lesions were multifocally disseminated, 0.3cm to 2cm in diameter, firm to elastic, circumscribed, sessile with smooth or arboriform surfaces, and pedunculated.

(Fig.5), and non-neoplastic vascular lesions (Fig.6-7), hemorrhage, fibrosis or fibroplasia, and other alterations. Neoplastic lesions included urothelial carcinomas of all grades (1 to 4), and equivalent low and high grades. Most invasive neoplasms were at the T1 stage in all buffaloes, i.e. they invaded only the lamina propria (connective tissue) underlying the epithelium. Only Buffalo 3 exhibited carcinoma at the T2b stage (with invasion of the external musculature). Nearly all invasive tumors were grade 3 or 4 (high grade); very rarely, they were grade 2 (low grade). The histological variants of invasive urothelial carcinomas in the samples analyzed were inverted (Fig.8), trabecular (Fig.9), microcystic (Fig.10). A urothelial carcinoma with divergent, squamous (Fig.11), and

glandular (apocrine - Fig.12) differentiations was observed in the bladders of Buffalo 1 and Buffalo 3. Squamous cell carcinoma *in situ* (Fig.13) was observed in Buffaloes 1 and 2, often near the invasive areas. Squamous cell carcinoma (Fig.14) was also present in only one of the samples, and it had areas with T1 staging (Table 2). All buffaloes had papillomas and hemangiomas (the cavernous type was more frequent than the capillary type); in Buffalo 1, a cavernous hemangioma that converged with a high-grade papillary urothelial carcinoma was present (Fig.15). Hemangiosarcomas were found in all buffaloes; Buffalo 1 exhibited an incipient hemangiosarcoma characterized by structurally well-formed vessels, but with high endothelial atypia (Fig.16). Buffalo 3 showed the highest

Table 1. Non-neoplastic changes in the bladder of buffaloes with enzootic hematuria

Animals	Urothelial atypia and dysplasia	Metaplasia	Urothelial Hyperplasia	Cystitis	Others	Vascular proliferation	Vascular change	Hemorrhage	Fibrosis or fibroplasia
Buffalo 1 SAP 35208	Reactive atypia; dysplasia I, II, III	Squamous	Flat; papillary; Brunn's nests	Follicular; polypoid; ulcerative; glandular	Amyloid; myxoid stromal differentiation	Atypical with architecture of granulation tissue	Myxoid degeneration (muscular artery)	+	+
Buffalo 2 SAP 35240	Reactive atypia; dysplasia II, III	Squamous; intestinal	Flat; papillary; Brunn's nests	Follicular; polypoid; ulcerative; cystic; glandular; intestinal	Myxoid stromal differentiation	Atypical with architecture of granulation tissue	Myxoid degeneration (muscular artery); hyalinization	+	+
Buffalo 3 SAP 35241	Reactive atypia; dysplasia II	Squamous; intestinal; squamous with severe paraqueratosis	Flat; papillary; Brunn's nests	Follicular; polypoid; cystic; glandular; ulcerative with chronic active inflammation and bacterial colonization	Myxoid stromal differentiation; lymphagiectasia; focal leukoplakia	Atypical with architecture of granulation tissue	Myxoid degeneration (muscular artery); hyalinization	+	+

SAP = "Setor de Anatomia Patológica", UFRRJ; + Present; Grade of dysplasia: I = low, II = moderate, III = high.

Table 2. Neoplastic lesions in the bladder of buffaloes with enzootic hematuria

Animals	Epithelial tumors							Vascular tumors			
	Papilloma	CIS	Urothelial carcinoma (low grade)		Urothelial carcinoma (high grade)		Variants of urothelial carcinoma	Squamous cell carcinoma	Hemangioma	Lymphangioma	Hemangiosarcoma
			Grade 1	Grade 2	Grade 3	Grade 4					
Buffalo 1 SAP 35208	+	+	+Ta	+Ta, T1	+Ta, T1	+T1	Microcystic; Divergent differentiation squamous and glandular (apocrine and intestinal) T1; inverted	-	+ that converged with a high-grade papillary urothelial carcinoma	-	+ incipient
Buffalo 2 SAP 35240	+	-	+Ta	+Ta	+T1	+T1	Microcystic	-	+	-	+
Buffalo 3 SAP 35241	+	-	-	+Ta	+T1	+T1, T2b	Trabecular* T1; microcystic; Divergent differentiation squamous T2b.	+ T1	+	+	+

CIS = carcinoma *in situ*; + Present, - absent; * Trabecular: histological variant of urothelial carcinoma, not included in the WHO classification; T = invasion: Ta = papillary urothelial carcinoma not invasive, T1 = invaded only the lamina propria, T2 = with invasion of the detrusor muscle, T2a = internal layer, T2b = external layer.

number of hemangiosarcomas, which were larger and more invasive (stage T2b) with well-differentiated, epithelioid (Fig.17), and poorly differentiated areas. Due to the diversity and coexistence of more than one histopathological alteration in the same bladder, the microscopic findings are listed in Table 1 and 2 to better visualize and understand the lesions found in each buffalo.

In the ureters of Buffalo 3, there was marked transmural edema, inflammatory infiltrate and fibroblastic proliferation, in addition to multifocal erosion of the urothelium, diffuse, discrete-to-moderate lymphoplasmocytic and macrophagic infiltrates with rare eosinophils. The ureters of Buffalo 1 did not showed relevant changes. It was not possible to collect and evaluate the ureters of Buffalo 2. In the kidneys of Buffaloes 1 and 2, anisokaryosis of the collecting duct cells near the

pelvis, interstitial mucinous deposits near the pelvis, and swelling of groups of collecting duct cells were evident. In the kidneys of Buffalo 3, there was mild-to-moderate segmental glomerulonephritis, necrosis, degeneration, and hyperplasia with atypia of tubular cells (tubular regeneration), mainly of the collecting ducts, in which there was a lining of large and atypical cells with marked megakaryosis and atypical (tripolar) mitotic figures (Fig.18). In the pelvis, the interstitium was found to have a moderately edematous aspect; there was intima-media myxoid differentiation in the muscular artery,

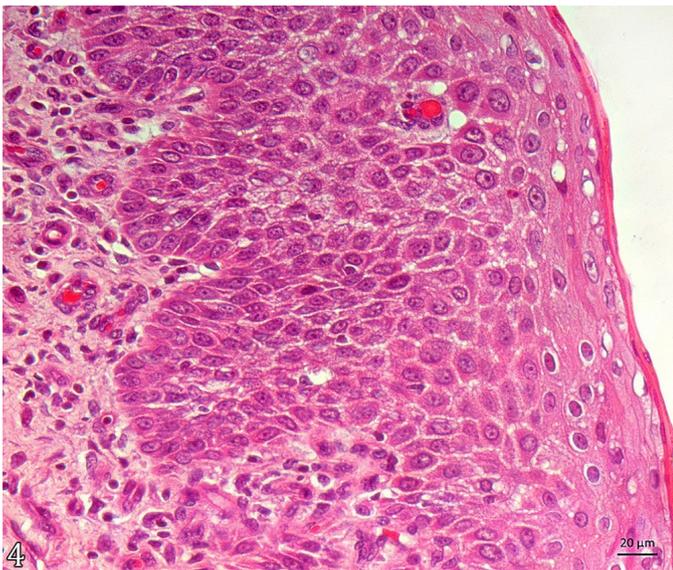


Fig.4. Squamous metaplasia of the transitional epithelium. HE, obj.40x.

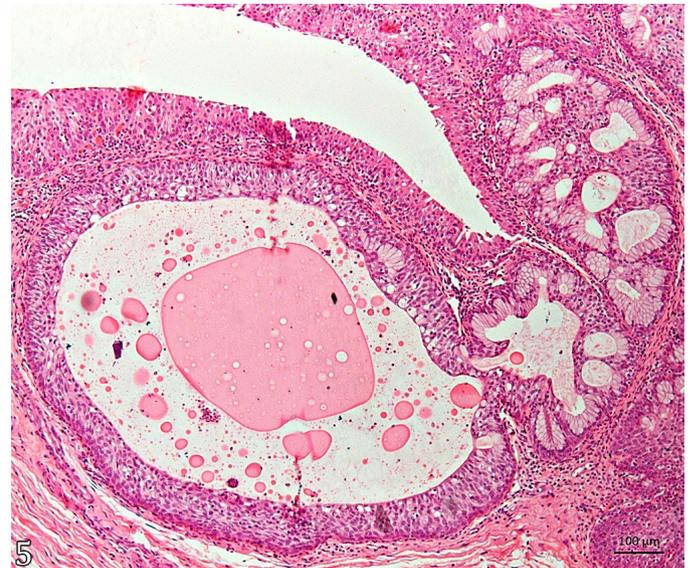


Fig.5. Cystic and glandular cystitis with intestinal differentiation: Brunns' nests with the presence of moderately dilated lumen by eosinophilic and globular material (cystic cystitis) and concomitant areas of glandular differentiation with columnar epithelium and calyciform cells (glandular cystitis). In the surrounding stroma, there is a mild lymphocytic inflammatory infiltrate. HE, obj.10x.

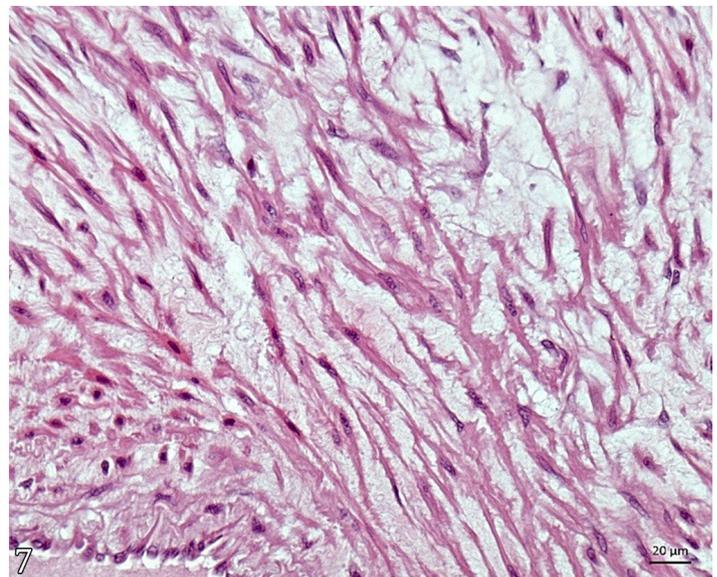
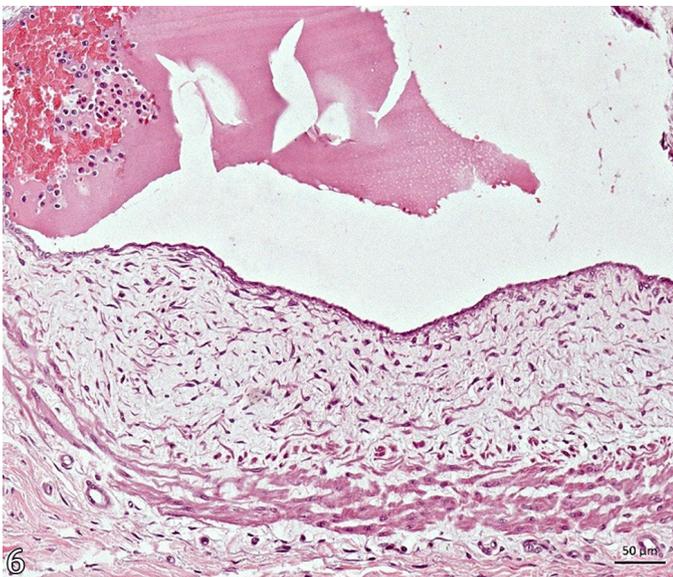


Fig.6-7. (6-7) Intima-media vascular (arterial) myxoid degeneration: the tunicas intima and media of the muscular arteries exhibit myxoid stroma and sparse spindle-shaped cells, some still exhibiting characteristics of leiomyocytes, whereas others have a stellate appearance and some nuclear atypia. (6) HE, obj.20x. (7) HE, obj.40x.

papillary hyperplasia-type epithelial papilliform proliferation, and areas of degeneration and necrosis of the urothelium with neutrocytic infiltrates.

PCR

The result of the test for bovine papillomavirus detection in the pooled bladder samples of the three buffaloes was negative.

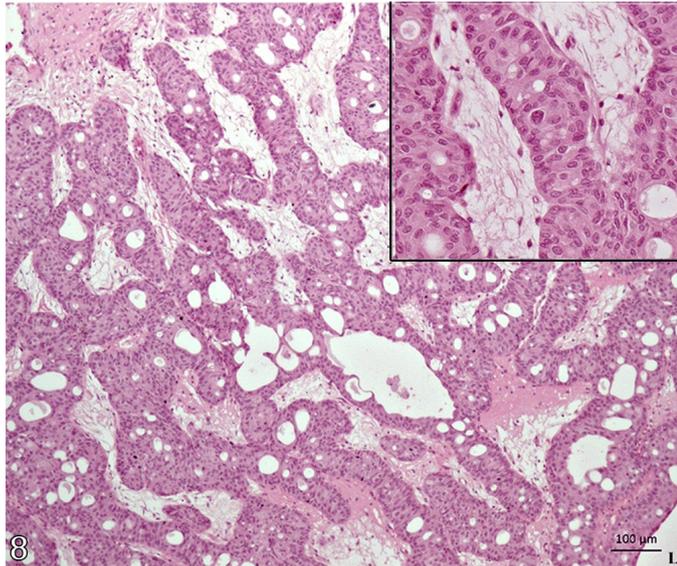


Fig.8. High-grade urothelial carcinoma with intraepithelial cysts resembling a glandular lumen: the neoplastic cells proliferate in contiguity with the lining of the urothelium and project into the lamina propria (variants inverted and microcystic); there is loss of maturation and the orderly arrangement of normal urothelium, besides moderate anisokaryosis (detail). HE, obj.10x.

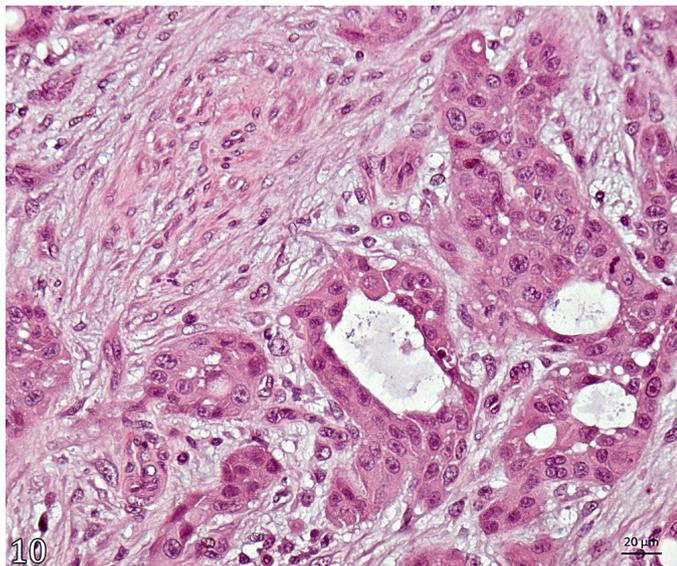


Fig.10. Microcystic urothelial carcinoma with different presentations: lumen with slightly eosinophilic material lined by two layers of low cubic to cylindrical epithelial cells, with cyst formation. HE, obj.40x.

DISCUSSION

The diagnosis of enzootic hematuria (EH) associated with the ingestion of *Pteridium esculentum* subsp. *arachnoideum* in buffaloes was based on the characteristic clinic and pathological picture and reinforced by the epidemiological survey. EH occurs only in areas where there are toxic ferns. However, some research groups believe in the possible participation of bovine papillomavirus (BPV) in the genesis of bladder lesions that cause hematuria (Olson et al. 1959, Brobst & Olson 1965,

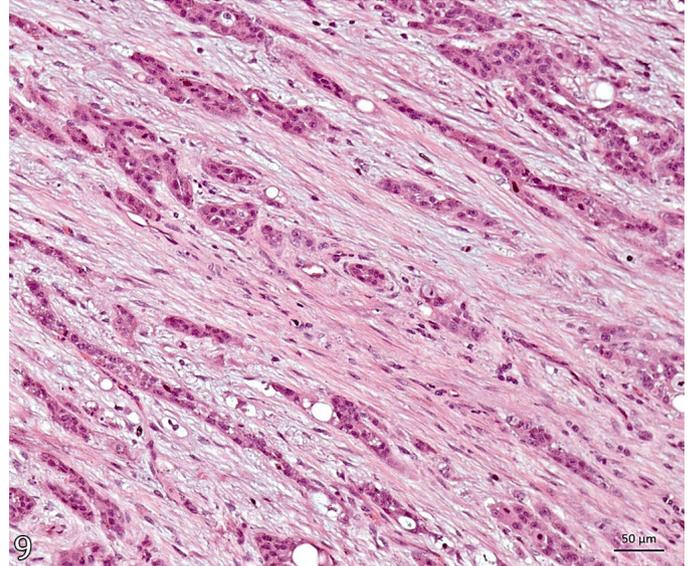


Fig.9. High-grade urothelial carcinoma in a trabecular arrangement (variant trabecular): note the small cells arranged in thin trabeculae infiltrating a densely patterned fibrous stroma (fibers are present in only one direction). HE, obj.20x.

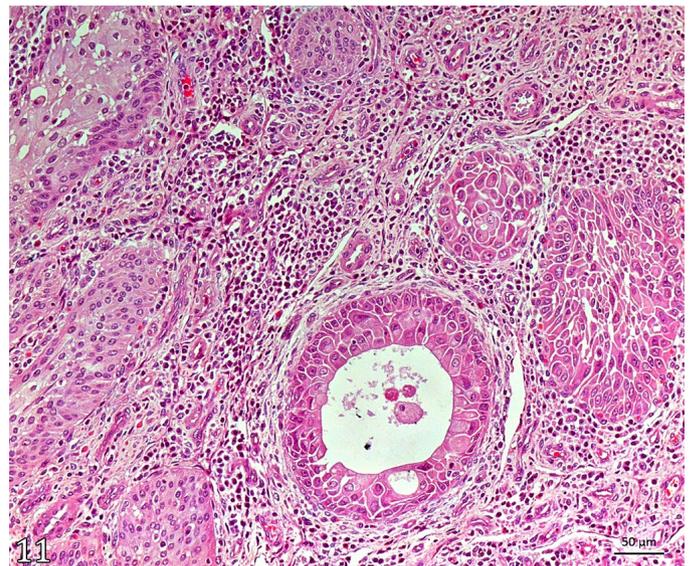


Fig.11. High-grade urothelial carcinoma with squamous differentiation (divergent): amid a conventional urothelial carcinoma, there are solid nests and a microscopic structure composed of polyhedral neoplastic cells, hyper-eosinophilic, and with noticeable intercellular bridges. HE, obj.40x.

Campo et al. 1992, Borzacchiello et al. 2003, 2007, Wosiacki et al. 2006, Balcos et al. 2008, Roperto et al. 2008a, 2008b, Pathania et al. 2011, Resendes et al. 2011, Dias et al. 2012). In addition to the studies conducted in cattle, some papers highlight the hypothesis that BPV plays a role in urothelial

carcinogenesis in buffaloes (Somvanshi 2011, Somvanshi et al. 2012, Maiolino et al. 2013, Roperto et al. 2013).

The most important factor to be considered when performing the differential diagnosis and establishing the diagnosis is that there is no analogous disease that presents

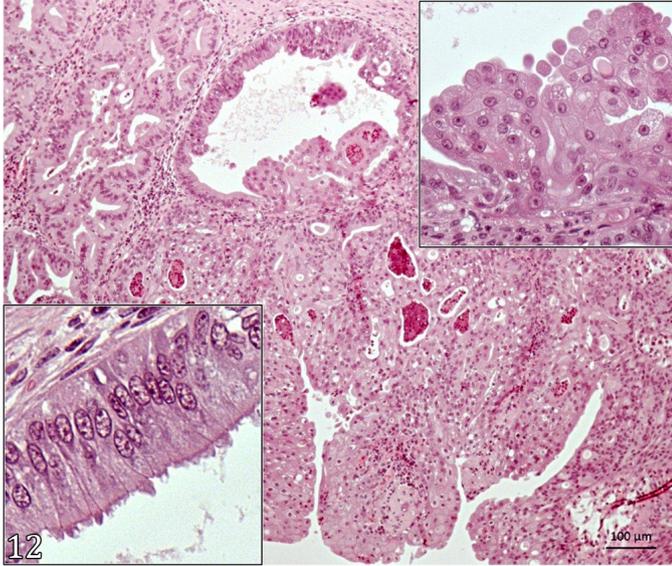


Fig.12. Low-grade urothelial carcinoma with glandular differentiation (divergent): besides the urothelium, multiple areas of glandular differentiation are noted with cylindrical cells lining the lumens, invading and compressing the lamina propria. In the upper highlight, the apical membrane of some neoplastic cells detaches in eosinophilic globular material into the lumen (apocrine differentiation). In the lower highlight, cylindrical cells demonstrate a uniform and hypereosinophilic lining with irregular projections in their apical membrane. HE, obj.10x.

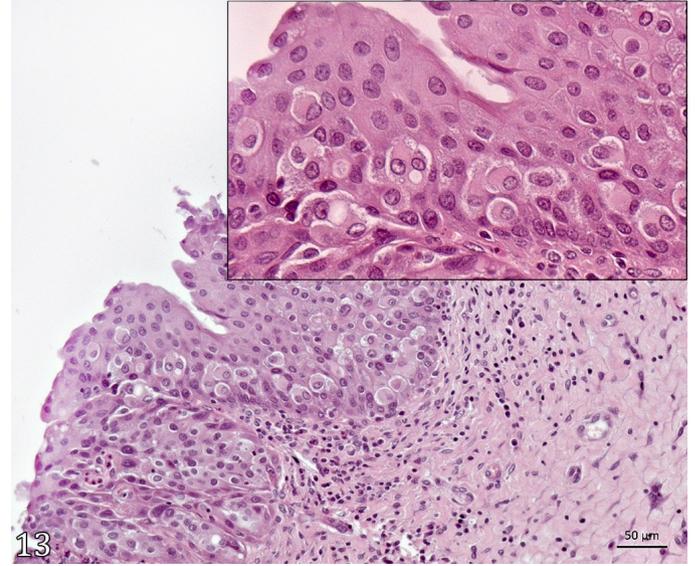


Fig.13. Morphological evidence of progression from dysplasia to carcinoma *in situ*: dysplastic urothelium in the left portion of the image; note the cytological characteristics of malignant cells including hyperchromatic nuclei with an irregular contour and size (anisokaryosis) and evident nucleoli, besides a discrete focus of microinvasion. In detail, note the presence of globular urothelial cells, eventually binucleated, with pagetoid appearance. HE, obj.20x.

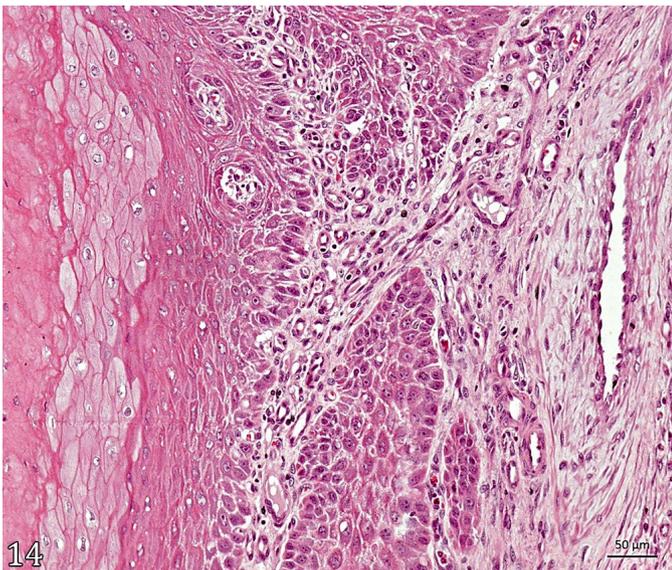


Fig.14. Squamous cell carcinoma: in a region of squamous metaplasia with marked keratinization, the cells that make up the lesion exhibit moderate atypia with several evident nucleoli and projections toward the lamina propria. This area corresponds to a macroscopic lesion similar to leukoplakia. HE, obj.20x.

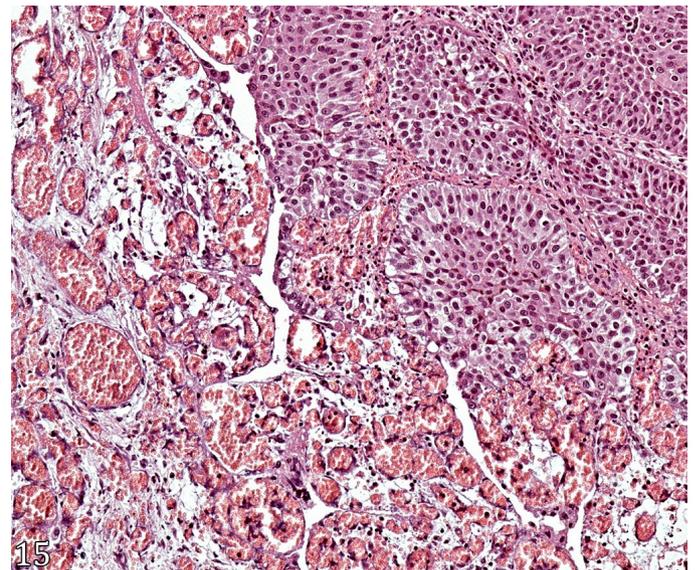


Fig.15. Collision of a cavernous hemangioma with a high-grade urothelial carcinoma: large vascular spaces lined by endothelial cells without atypia and filled by red blood cells that meet the neoplastic urothelium.

with bladder neoplasms and hematuria in an enzootic form as does EH in buffaloes and cattle. The epidemiological data and results of experiments involving the ingestion of plants of the genus *Pteridium* indicate that these plants are the primary cause, or at least the leading cause, of EH. Rosenberger (1965) clinically reproduced EBH in Germany by daily administration of approximately 2kg of *P. aquilinum* hay per day to a cow over approximately eight to nine months. At the same time, Stamatovic et al. (1965) also induced neoplasms in cattle by oral administration of *P. aquilinum*. This prolonged ingestion time coincides with the time required for cattle introduced into pastures invaded by bracken to develop hematuria.

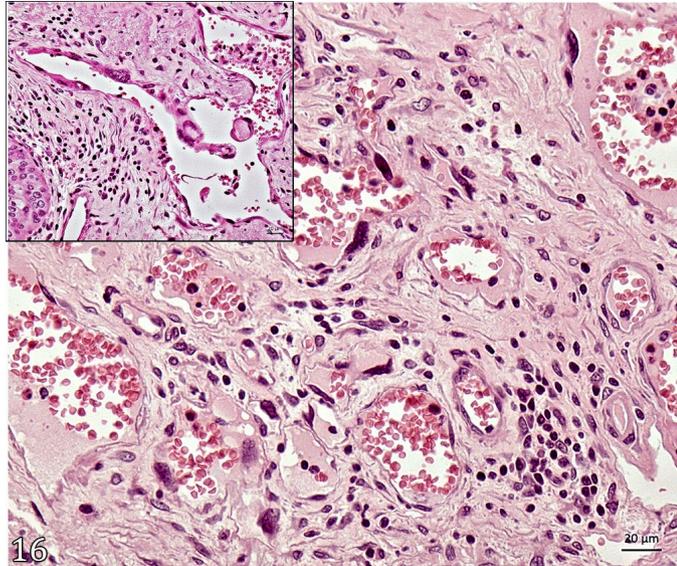


Fig.16. Incipient hemangiosarcoma: structurally well-formed vessels resembling venules in the edematous stroma, often lined by endothelial cells with high nuclear atypia (large and convoluted nuclei and multinucleation). HE, obj.40x.

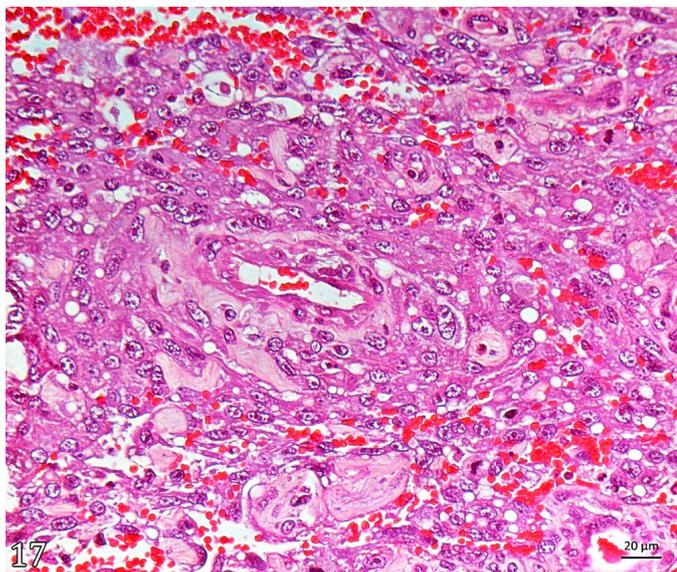


Fig.17. Poorly differentiated epithelioid hemangiosarcoma: predominantly solid neoplastic proliferation, small vascular lumens, abundant eosinophilic cytoplasm, and moderate nuclear pleomorphism. HE, obj.40x.

Epidemiologically, there is another robust evidence of the close association between ingestion of *Pteridium* spp. and EH. For example, hematuria caused by neoplastic or hyperplastic lesions in the bladder only occurs when animals graze in areas with fern in the fields. In Brazil, *P. esculentum* subsp. *arachnoideum* has a wide distribution and occurs mainly in mountainous, cold, and high rainfall regions with acidic and well-drained soils (Tokarnia et al. 2012); such edaphic characteristics are present in the region studied. In another study in São José do Barreiro County, São Paulo, it was observed that 75% of cows in properties were affected by EH (França et al., 2002). The buffaloes affected by EH in this study were aged between 6 and 8 years, which agrees with the age range of higher occurrence of bladder tumors in cattle and buffaloes affected by this disease (Pamukcu 1957, Yücel Tenekeci et al. 2017). Even though the three buffaloes affected in this study were female, EH occurs equally in males and females (Pamukcu 1963).

An opposing hypothesis to explain the development of EH is that previous infection with BPV is necessary for cattle to develop the characteristic bladder lesions. However, this claim is inconsistent in view of the fact that a large number of cattle with EH are BPV negative, and also considering the persistent failure to induce neoplasms and/or hyperplastic processes in cattle bladders since the study by Olson et al. (1959) - Döbereiner et al. (1966), for example, failed to induce these lesions by inoculation with BPV. The argument that some plants of the genus *Pteridium* may induce, by themselves, the formation of neoplasms is also extremely solid when considering the numerous experiments performed with plants of this genus in the most diverse animal species, such as guinea pigs (Evans 1968), quails (Maeda 1975), rats (Price & Pamukcu 1968), and mice (Hirono et al. 1970). All these experimental studies indicate that BPV infection is not required for EH to occur in buffaloes or cattle. However, since the hypothesis that BPV causes EH is still considered today,

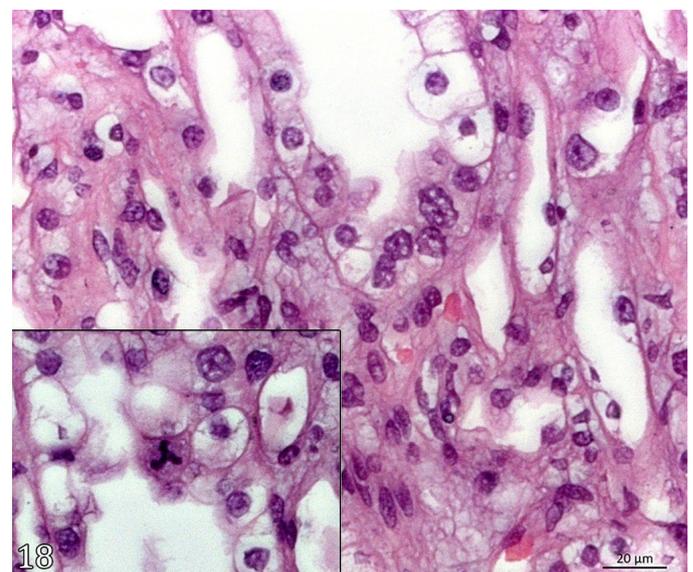


Fig.18. Degeneration and atypia of the epithelium of collecting ducts. The collecting ducts exhibit swollen epithelial cells, partly with moderate anisokaryosis and tripolar mitosis. HE, obj.40x.

bladder samples from the three buffaloes studied were sent to a laboratory to detect this virus by PCR, and the result was negative. However, the possibility of a false-negative result cannot be ruled out because formalin fixation may decrease the possibility of BPV detection in tissue samples (De Alcântara et al. 2015). Under ideal conditions, the possible presence of BPV should be tested using *in situ* hybridization (ISH), to correlate the location and distribution of this virus in tissues with cytomorphological alterations (Yagui et al. 2006), and/or using metagenomic analysis, in order to analyze the infection status and coinfections between different types of papillomaviruses, survey the viral genetic diversity, investigate whether these factors contribute to epidemiological differences in the development of neoplastic lesions (Hu et al. 2020, Sauthier et al. 2021), and even check the possibility of presence of new viruses (Pastrana et al. 2018). Moreover, considering the evidence provided by the works mentioned above, epidemiological data, and the clinical and pathological findings presented here, everything indicates that the possible presence of BPV in the bladder of buffaloes and cattle with EH would have no significance in the pathogenesis of the disease.

Weakness, progressive weight loss, and isolation from the herd have been described in cattle clinically affected by EH (Souto et al. 2006, Rai et al. 2017); however, none of the buffaloes in the present study had a BCS below 2.5kg (lean). Buffalo 3 was still within the BSC range considered ideal for good reproductive and productive performance in buffaloes (Anitha et al. 2011). Nevertheless, hematuria, a characteristic clinical sign of EH (Dickinson 1940, Pamukcu et al. 1976), was observed in all the buffaloes. Urinalysis of Buffalo 1 revealed macrohematuria and proteinuria, as seen in cattle with EH (Pamukcu et al. 1967, Falbo et al. 2005, Sánchez-Villalobos et al. 2004, 2006). The urine was alkaline, with a pH (9.0) out of the physiological range for the species, which is between 7.43 and 7.62 according to Mohapatra et al. (2017).

The macroscopic lesions in the bladders observed in the present study are similar to those described in cattle and buffaloes. In addition, Buffalo 3 exhibited pyelonephritis with dilatation of the renal pelvis, a lesion interpreted as secondary.

Histologically, there is almost a perfect correspondence between the neoplastic processes observed in cattle and those described in the bladder of humans (Peixoto et al. 2003); the same occurred with buffaloes in the present study. According to Maiolino et al. (2013), the morphological criteria suggested for the classification of urothelial tumors in cattle (Roperto et al. 2010) can also be used for buffaloes. We also observed, in the buffaloes, non-neoplastic processes similar to those observed in the urinary tract of humans exposed to radiotherapy (Suresh et al. 1993), just as previously described in bovines (Oliveira et al. 2011).

The non-neoplastic lesions observed in the present study included moderate degree of dysplasia, hyperplastic processes in the form of Brunn's nests, squamous metaplasia, myxoid stromal differentiation, cystic, glandular, follicular, and polypoid cystitis, fibrosis, diffuse-to-multiple lymphoplasmacytic infiltrates, and prominent lymphocytic foci around blood vessels or neoplastic proliferation; these findings are similar to those described in several other studies in cattle and buffaloes with EH (Aydin & Özkul 1995, França 2002, Peixoto et al. 2003, Carvalho et al. 2006, Oliveira 2009, Oliveira et al. 2011, Somvanshi et al. 2012, Yücel Tenekeci et al. 2017).

Urothelial carcinomas with divergent differentiation were identified as of those exhibiting areas of squamous or glandular epithelium derived from conventional urothelial carcinoma. The divergent squamous variant was the most frequent; it was observed in several foci in two buffaloes, with infiltration up to the external muscular layer in one of them. On the other hand, the divergent glandular variant was seen in a single animal; it had only one focus, and invaded the lamina propria. In humans, the squamous variant also occurs at a higher frequency (21%) than the glandular variant (6%) (Cheng & Lopez-Beltran 2016).

Another histological variant found was urothelial carcinoma with inverted growth (inverted urothelial carcinoma), which exhibits endophytic growth with thick and irregular anastomoses, strands, and trabeculae of urothelial cells with loss of polarity. This variant has significant nuclear pleomorphism, numerous mitotic figures, and architectural abnormalities consistent with high-grade urothelial carcinoma, but the morphology of this lesion may be mistaken for benign inverted papilloma (Cheng & Lopez-Beltran 2016). In addition, the differential diagnosis becomes more difficult because inverted urothelial carcinoma also exhibits endophytic growth in the lamina propria (Guo et al. 2016, Cheng & Lopez-Beltran 2016).

The microcystic variant of urothelial carcinoma referred to in the present paper is characterized by intracellular and intercellular microcysts engulfed by malignant urothelial or squamous cells in the absence of true glands. The lumen is usually empty, even though it may contain granular eosinophilic debris, mucin, or necrotic cells. The cysts may be of variable size, circular or oval, up to 2mm in diameter, and lined by urothelium; they are infiltrative and mimic cystic and glandular cystitis; elongated and irregular branching spaces are usually seen. This variant was added to the WHO classification in 2004, and is considered equivalent to pseudoglandular transitional cell carcinoma (gland-like lumen) by AFIP authors (Murphy et al. 2004, Venyo 2013); in cattle, Peixoto et al. (2003) describes it as a transitional carcinoma with areas of pseudoglandular differentiation.

A trabecular arrangement, not included in the WHO classification, was considered in this study as one of the histological variants of urothelial carcinoma; this type of tumor was also found in the bladders of cattle with EBH (Peixoto et al. 2003). According to França (2002), it is a tumor constituted by urothelial cells that are poorly differentiated, are rounded or polygonal, have circular or bizarre nuclei, are vesicular with evident nucleoli, have an amphophilic or eosinophilic cytoplasm, and are distributed in trabeculae or cords formed by 1-2 or more cell layers. Finally, the presence of many cells with "pagetoid aspect" in the bladder mucosa of a buffalo should be highlighted. A microscopic alteration with this denomination has already been described in the bladders of cattle with EH. However, the morphology demonstrated by photomicrographs may also represent high grade carcinoma *in situ*; additionally, the occurrence of either type of these tumors is not mutually excludent.

Minor variations between the classifications may sometimes reflect only a preference for one term or another (França 2002). All classification systems are hampered by varying subjectivity that affects interobserver reproducibility (Cheng et al. 2012). In considering the classification of human dysplasias, the World Health Organization (WHO) recommends,

since 2004, not to grade them owing to the lack of criteria to distinguish dysplasias from mild-to-moderate and high-grade carcinomas *in situ* (Hodges et al. 2010, Akhtar et al. 2019). However, in this work, the nomenclature standards used for cattle with EH were followed (França 2002, Peixoto et al. 2003). According to the most current classification system, urothelial carcinomas, formerly called transitional cell carcinomas, were graded into four levels (grade 1 to 4), thus incorporating the strengths of both the 1973 and 2004 classification systems. Using numerical data (grades 1 to 4) and categorical schemes (low grade versus high grade) in a single classification allows better stratification for research purposes, and facilitates clinical decision making. In this system, the term “papillary urothelial neoplasm of low malignant potential” was eliminated, and the use of four grade levels for urothelial tumors was suggested instead of three (Cheng et al. 2012, Cheng & Lopez-Beltran 2016).

Another point that should be highlighted is that the development of buffalo husbandry in the region where this outbreak occurred as a consequence of the assumption that these animals would be more resistant than cattle to EH. There is evidence, in the literature, that buffaloes have greater resistance to the toxic effect of some plants, such as *Palicourea juruana* (Oliveira et al. 2004, Bastianetto & Barbosa 2009), which seems to be the case with regard to *Pteridium* spp.. There are reports on the association between bladder neoplasms and the ingestion of *Pteridium* spp. in buffaloes (Pamukcu 1955, 1957, 1974, Aydin & Özkul 1995, Özkul & Aydin 1996, Somvanshi et al. 2012, Maiolino et al. 2013, Yücel Tenekeci et al. 2017). According to Pamukcu (1957), even though there are no comparative studies between cattle and buffaloes with EH, the incidence of this disease in buffaloes is not as high as in cattle.

Further fieldwork is still necessary to raise awareness about EH in buffaloes and cattle among producers, and to provide them with education on the control and prophylaxis of poisoning by *Pteridium* spp. as well as information on the risks of consuming unprocessed milk and milk derivatives from these animals. There is also a need for more detailed academic research on the epidemiology and clinical aspects of this disease in buffaloes.

CONCLUSIONS

Enzootic hematuria (EH) associated with consumption of *Pteridium esculentum* subsp. *arachnoideum* also occurs in buffaloes in Brazil. The neoplastic and non-neoplastic lesions found in this study are similar to those described in cattle with this disease.

No bovine papillomavirus particles were detected by PCR in formalin-fixed bladder samples.

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REFERENCES

Abdel-Raouf M. & El-Naggar M.A. 1969. The biometry of the gravid uterus in Egyptian buffalo cows. *Zbl. Vet. Med. Reihe A* 16(9):838-854. <<https://dx.doi.org/10.1111/j.1439-0442.1969.tb00566.x>> <PMid:4990975>

- Akhtar M., Al-Bozom I.A., Gashir M.B., Taha N.M., Rashid S. & Al-Nabet A.D.M.H. 2019. Urothelial carcinoma *in situ* (CIS): new insights. *Adv. Anat. Pathol.* 26(5):313-319. <<https://dx.doi.org/10.1097/PAP.0000000000000239>> <PMid:31149909>
- Anitha A., Rao K.S., Suresh J., Moorthy P.R.S. & Reddy Y.K. 2011. A body condition score (BCS) system in Murrah buffaloes. *Buffalo Bull* 30(1):79-96.
- Aydin Y. & Özkul I.A. 1995. Nonneoplastic findings of the urinary bladder in cattle and water buffalo in the black sea region of turkey. *Ankara Univ. Vet. Fak. Derg.* 42:189-192. <https://dx.doi.org/10.1501/VETFAK_0000000792>
- Balcos L.G.F., Borzacchiello G., Russo V., Popescu O., Roperto S. & Roperto F. 2008. Association of bovine papillomavirus type-2 and urinary bladder tumours in cattle from Romania. *Res. Vet. Sci.* 85(1):145-148. <<https://dx.doi.org/10.1016/j.rvsc.2007.10.002>> <PMid:18031773>
- Bastianetto E. & Barbosa J.D. 2009. Diferenças fisiológicas entre bubalinos e bovinos: Interferência na produção. *Proceedings of VIII Congresso Brasileiro de Buiatria, Belo Horizonte, MG, p.1-13. (Resumo)*
- Borzacchiello G., Ambrosio V., Roperto S., Poggiali F., Tsirimonakis E., Venuti A., Campo M.S. & Roperto F. 2003. Bovine papillomavirus type 4 in oesophageal papillomas of cattle from the south of Italy. *J. Comp. Pathol.* 128(2/3):203-206. <<https://dx.doi.org/10.1053/jcpa.2002.0626>> <PMid:12634101>
- Borzacchiello G., Russo V., Spoleto C., Roperto S., Balcos L., Rizzo C., Venuti A. & Roperto F. 2007. Bovine papillomavirus type-2 DNA and expression of E5 and E7 oncoproteins in vascular tumours of the urinary bladder in cattle. *Cancer Lett.* 250(1):82-91. <<https://dx.doi.org/10.1016/j.canlet.2006.09.022>> <PMid:17125915>
- Brobst D.F. & Olson C. 1965. Histopathology of urinary bladder tumors induced by bovine cutaneous papilloma agent. *Cancer Res.* 25(1):12-19.
- Campo M.S., Jarrett W.F.H., Barron R., Neil B.W.O. & Smith K.T. 1992. Association of bovine papillomavirus type 2 and bracken fern with bladder cancer in cattle. *Cancer Res.* 52(24):6898-6904. <PMid:1333885>
- Carvalho T., Pinto C. & Peleteiro M.C. 2006. Urinary bladder lesions in bovine enzootic haematuria. *J. Comp. Pathol.* 134(4):336-346. <<https://dx.doi.org/10.1016/j.jcpa.2006.01.001>> <PMid:16714029>
- Cheng L. & Lopez-Beltran A. 2016. Urinary bladder, p.1681-1735. In: Cheng L. & Bostwick D.G. (Eds), *Essentials of Anatomic Pathology*. 4th ed. Springer International Publishing, Switzerland.
- Cheng L., MacLennan G.T. & Lopez-Beltran A. 2012. Histologic grading of urothelial carcinoma: a reappraisal. *Hum. Pathol.* 43(12):2097-2108. <<https://dx.doi.org/10.1016/j.humpath.2012.01.008>> <PMid:22542126>
- Daudt C., Silva F.R.C., Streck A.F., Weber M.N., Mayer F.Q., Cibulski S.P. & Canal C.W. 2016. How many papillomavirus species can go undetected in papilloma lesions? *Sci. Rep.* 6:36480. <<https://dx.doi.org/10.1038/srep36480>> <PMid:27808255>
- De Alcântara B.K., Alfieri A.A., Headley S.A., Rodrigues W.B., Otonel R.A.A., Lunardi M. & Alfieri A.F. 2015. Molecular characterization of bovine Deltapapillomavirus (BPV1, 2, and 13) DNA in equine sarcoids. *Pesq. Vet. Bras.* 35(4):431-436. <<https://dx.doi.org/10.1590/S0100-736X2015000500007>>
- Dias J.D.C., Ignacchiti M.D.C., Giuriato P.G.G., Nunes L.C. & Pereira Jr O.S. 2012. Detecção do papilomavírus bovino tipo 2 em bexigas de bovinos com hematúria enzoótica pela técnica de reação em cadeia de polimerase no sul do Espírito Santo, Brasil. *Revta Bras. Med. Vet.* 34(2):146-151.
- Dickinson C.G. 1940. Haematuria in cattle. *Aust. Vet. J.* 16:224-226.
- Döbereiner J., Olson C., Brown R.R., Price J.M. & Yess N. 1966. Metabolites in urine of cattle with experimental bladder lesions and fed bracken fern. *Pesq. Agrop. Bras.* 1:189-199.
- Döbereiner J., Tokarnia C.H. & Canella C.F.C. 1967. Ocorrência da hematúria enzoótica e de carcinomas epidermóides no trato digestivo superior em bovinos no Brasil. *Pesq. Agropec. Bras.* 2:489-504.
- Evans I.A. 1968. The radiomimetic nature of bracken toxin. *Cancer Res.* 28(11):2252-2261.

- Falbo M.K., Reis A.C.F., Balarin M.R.S., Bracarense A.P.F.R.L., Araújo Jr J.P., Okano W. & Sandini I.E. 2005. Alterações hematológicas, bioquímicas, urinárias e histopatológicas na intoxicação natural em bovinos pela samambaia *Pteridium aquilinum* (L.) Kühn. *Semina, Ciênc. Agrárias* 26(4):547-558.
- Feitosa F.L.F. 2014. *Semiologia veterinária: a arte do diagnóstico*. 3ª ed. Roca, São Paulo, p.77-102.
- Forslund O., Antonsson A., Nordin P., Stenquist Bo. & Hansson B.G. 1999. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. *J. Gen. Virol.* 80(Pt 9):2437-2443. <<https://dx.doi.org/10.1099/0022-1317-80-9-2437>> <PMid:10501499>
- França T.N. 2002. Aspectos histopatológicos da hematúria enzoótica bovina no Brasil. Dissertação de Mestrado, Universidade Federal Rural do Rio de Janeiro, Seropédica. 85p.
- França T.N., Tokarnia C.H. & Peixoto P.V. 2002. Enfermidades determinadas pelo princípio radiomimético de *Pteridium aquilinum* (Polypodiaceae). *Pesq. Vet. Bras.* 22(3):85-96. <<https://dx.doi.org/10.1590/S0100-736X2002000300001>>
- Galvão & Peixoto 2012. Dados não publicados (Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ).
- Guo A., Liu A. & Teng X. 2016. The pathology of urinary bladder lesions with an inverted growth pattern. *Chin. J. Cancer Res.* 28(1):107-121. <<https://dx.doi.org/10.3978/j.issn.1000-9604.2016.02.01>> <PMid:27041933>
- Hirono I. 1990. Carcinogenicity of bracken fern and its causal principle, p.233-240. In: Thomson J.A. & Smith R.T. (Eds), *Bracken Biology and Management*. Australian Institute of Agricultural Science, Sydney.
- Hirono I., Aiso S., Yamaji T., Mori H., Yamada K., Niwa H., Ojika M., Wakamatsu K., Kigoshi H., Niiyama K. & Uosaki Y. 1984. Carcinogenicity in rats of ptaquiloside isolated from bracken. *Gan* 75(10):833-836. <https://dx.doi.org/10.20772/cancersci1959.75.10_833> <PMid:6510632>
- Hirono I., Shibuya C., Fushimi K. & Haga M. 1970. Studies on carcinogenic properties of bracken, *Pteridium aquilinum*. *J. Nat. Cancer Inst.* 45(1):179-188. <<https://dx.doi.org/10.1093/jnci/45.1.179>> <PMid:5449212>
- Hodges K.B., Lopez-Beltran A., Davidson D.D., Montironi R. & Cheng L. 2010. Urothelial dysplasia and other flat lesions of the urinary bladder: clinicopathologic and molecular features. *Hum. Pathol.* 41(2):155-162. <<https://dx.doi.org/10.1016/j.humpath.2009.07.002>> <PMid:19762067>
- Hu J., Zhang W., Chauhan S.S., Shi C., Song Y., Zhao Y., Wang Z., Cheng L. & Zhang Y. 2020. Complete genome and phylogenetic analysis of bovine papillomavirus type 15 in Southern Xinjiang dairy cow. *J. Vet. Sci.* 21(6):e73. <<https://dx.doi.org/10.4142/jvs.2020.21.e73>> <PMid:33263226>
- Humphrey P.A., Moch H., Cubilla A.L., Ulbright T.M. & Reuter V.E. 2016. The 2016 WHO classification of tumours of the urinary system and male genital organs. Part B: prostate and bladder tumours. *Eur. Urol.* 70(1):106-119. <<https://dx.doi.org/10.1016/j.eururo.2016.02.028>> <PMid:26996659>
- Jarrett W.F.H., McNeil P.E., Grimshaw W.T.R., Selman I.E. & McIntyre W.I.M. 1978. High incidence area of cattle cancer with possible interaction between an environmental carcinogen and a papilloma virus. *Nature* 274(5668):215-217. <<https://dx.doi.org/10.1038/274215a0>> <PMid:210386>
- Lopez-Beltran A., Bassi P., Pavone-Macaluso M. & Montironi R. 2004. Handling and pathology reporting of specimens with carcinoma of the urinary bladder, ureter, and renal pelvis. *Eur. Urol.* 45(3):257-266. <<https://dx.doi.org/10.1016/j.eururo.2003.09.018>> <PMid:15036668>
- Maeda T. 1975. Fundamental studies on the etiology of Haematuria vesicalis bovis. I. The induction of carcinomas and haemorrhages of the urinary bladder in Guinea pigs by feeding bracken fern (*Pteridium aquilinum*). *Bull. Fac. Agric., Tottori Univ.* 27:79-80.
- Maiolino P., Özkul A., Sepici-Dincel A., Roperto F., Yücel G., Russo V., Urraro C., Lucà R., Riccardi M.G., Martano M., Borzacchiello G., Esposito I. & Roperto S. 2013. Bovine papillomavirus type 2 infection and microscopic patterns of urothelial tumors of the urinary bladder in water buffaloes. *Biomed Res. Int.* 13:937918. <<https://dx.doi.org/10.1155/2013/937918>> <PMid:23762866>
- Meuten D.J. 2017. *Tumors in Domestic Animals*. 5th ed. Wiley-Blackwell, Nova Jersey, p.632-688.
- Mohapatra S.P., Ingole S.D., Bharucha S.V., Nagvekar A.S., Kekan P.M. & Kharde S.D. 2017. Changes in urinary dipstick parameters in cyclic and early pregnant Murrah buffaloes. *J. Pharm. Innov.* 6(7):8-10.
- Murphy W.M., Beckwith J.B. & Farrow G.M. 1994. *AFIP Atlas of Tumor Pathology: tumors of the kidney, bladder, and related urinary structures*. 3rd Series, Fascicle 11. AFIP, Washington, DC. 326p.
- Murphy W.M., Grignon D.J. & Perlan E.J. 2004. *AFIP Atlas of Tumor Pathology: Tumors of the kidney, bladder, and related urinary structures*. Armed Forces Institute of Pathology, Philadelphia. 394p.
- O'Connor P.J., Alonso-Amelot M.E., Roberts S.A. & Povey A.C. 2019. The role of bracken fern illudanes in bracken fern-induced toxicities. *Mutat. Res.* 782:108276. <<https://dx.doi.org/10.1016/j.mrrev.2019.05.001>> <PMid:31843140>
- Oliveira C.M.C., Barbosa J.D., Macedo R.S.C., Brito M.F., Peixoto P.V. & Tokarnia C.H. 2004. Estudo comparativo da toxidez de *Palicourea juruana* (Rubiaceae) para búfalos e bovinos. *Pesq. Vet. Bras.* 24(1):27-30. <<https://dx.doi.org/10.1590/S0100-736X2004000100007>>
- Oliveira L.G., França T.N., Oliveira L.I., Peixoto P.V. & Brito M.F. 2011. Similarities between non-neoplastic urinary bladder lesions in bovine enzootic hematuria and those induced by radiotherapy in humans, p.388-395. In: Riet-Correa F., Pfister J., Schild A.L. & Wierenga T. (Eds), *Poisoning by Plants, Mycotoxins and Related Toxins*. CABI International, Oxfordshire.
- Oliveira L.G.P. 2009. *Novos aspectos patológicos e patogenéticos da hematúria enzoótica bovina*. Dissertação de Mestrado, Universidade Federal Rural do Rio de Janeiro, Seropédica. 133p.
- Olson C., Pamukcu A.M., Brobst D.F., Satter E.J. & Price J.M. 1959. A urinary bladder tumor unduced by a bovine cutaneous papillomatosis. *Am. J. Pathol.* 35:672.
- Ordóñez N.G. & Rosai J. 1996. Urinary tract: kidney, renal pelvis, and ureter, bladder and male urethra, p.1059-220. In: Rosai J. (Ed.) *Ackerman's Surgical Pathology*. Vol.1. 8th ed. Mosby, St. Louis.
- Özkul I.A. & Aydin Y. 1996. Tumours of the urinary bladder in cattle and water buffalo in the Black Sea region of Turkey. *Br. Vet. J.* 152(4):473-475. <[https://dx.doi.org/10.1016/s0007-1935\(96\)80041-8](https://dx.doi.org/10.1016/s0007-1935(96)80041-8)> <PMid:8791855>
- Pamukcu A.M. 1955. Investigations on pathology of enzootic bovine haematuria in Turkey. *Zentbl. Vet. Med.* 2:409-429.
- Pamukcu A.M. 1957. Tumors of the urinary bladder in cattle and water buffalo affected with enzootic bovine hematuria. *Zentralbl. Veterinaarmed.* 4(2):185-197. <<https://dx.doi.org/10.1111/j.1439-0442.1957.tb00464.x>>
- Pamukcu A.M. 1963. Epidemiologic studies on urinary bladder tumors in Turkish cattle. *Ann. N. Y. Acad. Sci.* 108:938-947.
- Pamukcu A.M. 1974. Tumours of the urinary bladder. *Bull. WHO* 50(1/2):43-52. <PMid:4371741>
- Pamukcu A.M., Göksoy S.K. & Price J.M. 1967. Urinary bladder neoplasms induced by feeding bracken fern (*Pteris aquilina*) to cows. *Cancer Res.* 27(1):917-924.
- Pamukcu A.M., Price J.M. & Bryan G.T. 1976. Naturally occurring and bracken-fern-induced bovine urinary bladder tumors: clinical and morphological characteristics. *Vet. Pathol.* 13(2):110-122. <<https://dx.doi.org/10.1177/030098587601300205>> <PMid:945629>
- Pastrana D.V., Peretti A., Welch N.L., Borgogna C., Olivero C., Badolato R., Notarangelo L.D., Gariglio M., FitzGerald P.C., McIntosh C.E., Reeves J., Starrett G.J., Bliskovsky V., Velez D., Brownell I., Yarchoan R., Wyvill K.M., Uldrick T.S., Maldarelli F., Lisco A., Sereti I., Gonzalez C.M., Androphy E.J., McBride A.A., Van Doorslaer K., Garcia F., Dvoretzky I., Liu J.S., Han J., Murphy P.M., McDermott D.H. & Buck C.B. 2018. Metagenomic discovery of 83 new human papillomavirus types in patients with immunodeficiency. *mSphere*

- 3(6):e00645-18. <<https://dx.doi.org/10.1128/mSphereDirect.00645-18>> <PMid:30541782>
- Pathania S., Kumar P., Devi L.G., Kumar D., Dhama K. & Somvanshi R. 2011. Preliminary assessment of binary ethylenimine inactivated and saponized cutaneous warts (BPV-2) therapeutic vaccine for enzootic bovine haematuria in hill cows. *Vaccine* 29(43):7296-7302. <<https://dx.doi.org/10.1016/j.vaccine.2011.07.065>> <PMid:21803104>
- Peixoto P.V., França T.N., Barros C.S.L. & Tokarnia C.H. 2003. Histopathological aspects of bovine enzootic hematuria in Brazil. *Pesq. Vet. Bras.* 23(2):65-81. <<https://dx.doi.org/10.1590/S0100-736X2003000200004>>
- Price J.M. & Pamukcu A.M. 1968. The induction of neoplasms of the urinary bladder of the cow and the small intestine of the rat by feeding bracken fern (*Pteris aquilina*). *Cancer Res.* 28(11):2247-2251. <PMid:5723967>
- Rai S.K., Sharma R., Kumari A., Rasmussen L.H., Patil R.D. & Bhar R. 2017. 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* 138:31-36. <<https://dx.doi.org/10.1016/j.toxicon.2017.08.010>> <PMid:28803058>
- Resendes A.R., Roperto S., Trapani F., Urraro C., Rodrigues A., Roperto F. & Borzacchiello G. 2011. Association of bovine papillomavirus type 2 (BPV-2) and urinary bladder tumours in cattle from the Azores archipelago. *Res. Vet. Sci.* 90(3):526-529. <<https://dx.doi.org/10.1016/j.rvsc.2010.02.001>> <PMid:20189209>
- Roperto S., Ambrosio V., Borzacchiello G., Galati P., Paciello O., Russo V. & Roperto F. 2005. Bovine papillomavirus type-2 (BPV-2) infection and expression of uroplakin IIIb, a novel urothelial cell marker, in urinary bladder tumors of cows. *Vet. Pathol.* 42(6):812-818. <<https://dx.doi.org/10.1354/vp.42-6-812>> <PMid:16301578>
- Roperto S., Borzacchiello G., Brun R., Leonardi L., Maiolino P., Martano M., Paciello O., Papparella S., Restucci B., Russo V., Salvatore G., Urraro C. & Roperto F. 2010. A review of bovine urothelial tumours and tumour-like lesions of the urinary bladder. *J. Comp. Pathol.* 142(2/3):95-108. <<https://dx.doi.org/10.1016/j.jcpa.2009.08.156>> <PMid:19818448>
- Roperto S., Borzacchiello G., Brun R., Perillo A., Russo V., Urraro C. & Roperto F. 2008b. Multiple glomus tumors of the urinary bladder in a cow associated with bovine papillomavirus type 2 (BPV-2) infection. *Vet. Pathol.* 45(1):39-42. <<https://dx.doi.org/10.1354/vp.45-1-39>> <PMid:18192572>
- Roperto S., Borzacchiello G., Casellato R., Galati P., Russo V., Sonnino S. & Roperto F. 2007. Sialic acid and GM3 ganglioside expression in papillomavirus-associated urinary bladder tumours of cattle with chronic enzootic haematuria. *J. Comp. Pathol.* 137(2/3):87-93. <<https://dx.doi.org/10.1016/j.jcpa.2007.03.008>> <PMid:17624364>
- Roperto S., Brun R., Paolini F., Urraro C., Russo V., Borzacchiello G., Pagnini U., Raso C., Rizzo C., Roperto F. & Venuti A. 2008a. Detection of bovine papillomavirus type 2 in the peripheral blood of cattle with urinary bladder tumours: possible biological role. *J. Gen. Virol.* 89(12):3027-3033. <<https://dx.doi.org/10.1099/vir.0.2008/004457-0>> <PMid:19008389>
- Roperto S., Russo V., Ozkul A., Sepici-Dinçel A., Maiolino P., Borzacchiello G., Marcus I., Esposito I., Riccardi M.G. & Roperto F. 2013. Bovine papillomavirus type 2 infects the urinary bladder of water buffalo (*Bubalus bubalis*) and plays a crucial role in bubaline urothelial carcinogenesis. *J. Gen. Virol.* 94(2):403-408. <<https://dx.doi.org/10.1099/vir.0.047662-0>> <PMid:23100367>
- Rosenberger G. 1965. Längere Aufnahme von Adlerfarn (*Pteris aquilina*) - die Ursache der chronischen vesikalen Haematurie des Rindes. *Wiener Tierärztl. Mschr.* 52(5):415-421.
- Sambrook J. & Russel D.W. 2001. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor, New York, p.A8.9-A8.10.
- Sánchez-Villalobos A., Alvarado C.M.A., García-Bracho D., Pino-Ramírez D., Villarroel-Neri R. & Boscán-Ocando J. 2004. Observaciones clínico patológicas en vacas con hematuria enzoótica bovina. *Revta Cient.* 14(4):317-323.
- Sánchez-Villalobos A., Alvarado C.M.A., Villarroel-Neri R., Pino-Ramírez D., García-Bracho D. & Sánchez-Cómbita G. 2006. Validez, seguridad y cociente de verosimilitud de los métodos tiras reactivas para orina y examen microscópico del sedimento urinario en el diagnóstico de hematuria enzoótica bovina. *Revta Cient.* 16(6):604-612.
- Sauthier J.T., Daudt C., Silva F.R.C., Alves C.D.B.T., Mayer F.Q., Bianchi R.M., Driemeier D., Streit R.S.A., Staats C.C., Canal C.W. & Weber M.N. 2021. The genetic diversity of "papillomavirome" in bovine teat papilloma lesions. *Anim. Microbiome* 3(1):51. <<https://dx.doi.org/10.1186/s42523-021-00114-3>> <PMid:34321106>
- Somvanshi R. 2011. Papillomatosis in buffaloes: a less-known disease. *Transbound. Emerg. Dis.* 58(4):327-332. <<https://dx.doi.org/10.1111/j.1865-1682.2011.01211.x>> <PMid:21435195>
- Somvanshi R., Pathania S., Nagarajan N., Pangty K. & Kumar P. 2012. Pathological study of non-neoplastic urinary bladder lesions in cattle and buffaloes: a preliminary report. *Trop. Anim. Health. Prod.* 44(4):855-861. <<https://dx.doi.org/10.1007/s11250-011-9978-y>> <PMid:21935661>
- Souto M.A.M., Kommers G.D., Barros C.S.L., Rech R.R. & Piazer J.V.M. 2006. Neoplasmas da bexiga associados à hematuria enzoótica bovina. *Ciência Rural* 36(5):1647-1650. <<https://dx.doi.org/10.1590/S0103-84782006000500050>>
- Stamatovic S., Bratanovic U. & Sofrenovic D. 1965. Das klinische Bild der durch Fütterung von Adlerfarn (*Pteris aquilina*) experimentell hervorgerufenen Haematuria vesicalis der Rinder. *Wiener Tierärztl. Mschr.* 52(6):589-596.
- Suresh U.R., Smith V.J., Lupton E.W. & Haboubi N.Y. 1993. Radiation disease of the urinary tract: histological features of 18 cases. *Clin. Pathol.* 46(3):228-231. <<https://dx.doi.org/10.1136/jcp.46.3.228>> <PMid:8463415>
- Thomson J.A. 2012. Taxonomic status of diploid southern hemisphere brackens (*Pteridium*: Dennstaedtiaceae). *Telopea* 14:43-48. <<https://dx.doi.org/10.7751/telopea2012007>>
- Tokarnia C.H., Brito M.F., Barbosa J.D., Peixoto P.V. & Döbereiner J. 2012. Plantas Tóxicas do Brasil para Animais de Produção. 2ª ed. Helianthus, Rio de Janeiro, p.349-364.
- Tokarnia C.H., Döbereiner J. & Canella C.F.C. 1967. Ocorrência da intoxicação aguda pela "samambaia" (*Pteridium aquilinum* (L) Kuhn) em bovinos no Brasil. *Pesq. Agropec. Bras.* 2:329-336.
- Tokarnia C.H., Döbereiner J. & Canella C.F.C. 1969. Ocorrência de hematuria enzoótica e de carcinomas epidermóides no trato digestivo superior em bovinos no Brasil. II Estudos complementares. *Pesq. Agropec. Bras.* 4:209-224.
- Venyo A.K.-G. 2013. Microcystic variant of urothelial carcinoma. *Adv. Urol.* 2013:654751. <<https://dx.doi.org/10.1155/2013/654751>> <PMid:24363668>
- Wosiacki S.R., Barreiro M.A.B., Alfieri A.F. & Alfieri A.A. 2005. Semi-nested PCR for detection and typing of bovine Papillomavirus type 2 in urinary bladder and whole blood from cattle with enzootic haematuria. *J. Virol. Methods* 126(1/2):215-219. <<https://dx.doi.org/10.1016/j.jviromet.2005.01.021>> <PMid:15847940>
- Wosiacki S.R., Claus M.P., Alfieri A.F. & Alfieri A.A. 2006. Bovine papillomavirus type 2 detection in the urinary bladder of cattle with chronic enzootic haematuria. *Mem. Inst. Oswaldo Cruz* 101(6):635-638. <<https://dx.doi.org/10.1590/S0074-02762006000600009>>
- Wosiacki S.R., Reis A.C.F., Alfieri A.F. & Alfieri A.A. 2002. Papilomavírus bovino tipo 2 na etiologia da hematuria enzoótica bovina. *Semina, Ciênc. Agrárias* 23(1):121-130. <<https://dx.doi.org/10.5433/1679-0359.2002v23n1p121>>
- Yagui A., Carvalho C., Freitas A.C., Góes L.G.B., Dagli M.L.Z., Birgel Jr. E.H., Beçak W. & Santos R.C.S. 2006. Papillomatosis in cattle: in situ detection of bovine papillomavirus DNA sequences in reproductive tissues. *Braz. J. Morphol. Sci.* 23(3/4):525-529.
- Yücel Tenekeci G., Öztekin M., Bozkurt M.F., Sepici-Dinçel A. & Özkul İ.A. 2017. Pathomorphological and immunohistochemical studies of tumours in the urinary bladders of water buffalo in Marmara, the Central and Western Black Sea Region of Turkey. *Kafkas Univ. Vet. Fak. Derg.* 23(2):269-273. <<https://dx.doi.org/10.9775/kvfd.2016.16414>>