

# Pathomorphological and Immunohistochemical Studies of Tumours in the Urinary Bladders of Water Buffalo in Marmara, the Central and Western Black Sea Region of Turkey <sup>[1]</sup>

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<sup>[1]</sup> This research was funded by The Scientific and Technological Research Council of Turkey (Project number 1100943) and presented as an oral presentation at the 32<sup>nd</sup> World Veterinary Congress, 13-17 September 2015, İstanbul, Turkey

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Article Code: KVFD-2016-16414 Received: 26.07.2016 Accepted: 30.11.2016 Published Online: 30.11.2016

## Citation of This Article

Yücel Tenekeci G, Öztekin M, Bozkurt MF, Sepici Dinçel A, Özkul İA: 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, 2017. DOI: 10.9775/kvfd.2016.16414

## Abstract

In this study, non-neoplastic and neoplastic lesions in the urinary bladders of water buffalo were determined in the Marmara, the central and western part of the Black Sea region of Turkey. In this context, water buffalo with lesions in the urinary bladder were obtained from the private and public slaughterhouses of several provinces. The lesions were evaluated pathologically and immunohistochemically. In addition, species of bracken ferns were identified in these provinces. Histopathologically, 39 of the collected tissue samples were diagnosed as neoplasia. These neoplasms were classified and graded according to The World Health Organization Classification of Tumours published in 2004. In this context, papilloma (16 cases), papillary urothelial neoplasm of low malignant potential (PUNLMP) (7 cases), low-grade papillary carcinoma (13 cases), high-grade papillary carcinoma (2 cases), and low-grade papillary carcinoma with hemangiosarcoma (1 case) were detected in samples diagnosed as neoplasia. In the collected bracken ferns, *Athyrium filix-foemina* (L.) Roth; *Dryopteris dilatata* (Hoffm.) Gary; *Dryopteris filix-mas* (L.) Schott; *Polystichum aculeatum* (L.) Roth; *Polystichum setiferum* (Forsk.) Woynar; *Polystichum woronowii* Fomin, *Polypodium vulgare* L. and *Pteridium aquilinum* (L.) Kuhn spp. were identified. As a result, it has been understood that the consumption of identified bracken ferns leads more frequently to epithelial neoplasms.

**Keywords:** Bracken fern, Chronic enzootic haematuria, Neoplasia, Tumour, *Pteridium spp*, Water buffalo

## Türkiye’de Marmara, Orta ve Batı Karadeniz Bölgelerinde Bulunan Mandaların İdrar Keselerinde Rastlanılan Tümörlerin Patomorfolojik ve İmmunohistokimyasal Yönden İncelenmesi

## Özet

Çalışmada, Marmara, Orta ve Batı Karadeniz Bölgelerinde bulunan mandaların idrar keselerinde saptanan neoplazik ve neoplazik olmayan bulgular değerlendirildi. Orta, Batı Karadeniz ve Marmara Bölgelerindeki bazı illerde bulunan özel ve kamu kurumu mezbahalarında kesilen mandaların idrar keselerinde rastlanılan lezyonlar patolojik ve immunohistokimyasal yönden incelendi. Ayrıca hastalığın bulunduğu bölgelerdeki hayvanların otladıkları yerlerde doğal olarak yetişen eğrelti otları toplanarak tür teşhisi yapıldı. Çalışma sonucunda toplanan idrar keselerinin histolojik incelenmesinde; otuzdokuz adet neoplazik görüldü. Bu neoplazilerin tanısı Dünya Sağlık Teşkilatı’nın 2004 yılındaki tümör sınıflamasına göre yapıldı. Histolojik incelemelerde; otuzdokuz adet neoplazik idrar keselerinin onaltı adedi papillom; yedi adedi düşük malignite potansiyelli papiller ürotelyal neoplazi, onüç adedi düşük dereceli papiller karsinom, iki tanesi yüksek dereceli papiller karsinom ve bir tane ise hemangiosarkom görülmüştür. Düşük dereceli papiller karsinom görülen idrar keselerinden bir tanesinde aynı zamanda hemangiosarkoma da rastlandı. Çalışma sonucunda toplanan eğrelti otlarında ise; *Athyrium filix-foemina* (L.) Roth; *Dryopteris dilatata* (Hoffm.) Gary; *Dryopteris filix-mas* (L.) Schott; *Polystichum aculeatum* (L.) Roth; *Polystichum setiferum* (Forsk.) Woynar; *Polystichum woronowii* Fomin; *Polypodium vulgare* L. ve *Pteridium aquilinum* (L.) Kuhn türleri saptandı. Sonuç olarak, manda idrar keselerinde türleri tayin edilen eğrelti otlarının tüketilmesiyle daha çok epitelyal neoplazilerin tetiklendiği anlaşılmıştır.

**Anahtar sözcükler:** Eğrelti otu, Kronik enzootik hematüri, Manda, Neoplazi, Tümör, *Pteridium spp*.



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## INTRODUCTION

Enzootic haematuria syndrome has occurred in cattle and water buffalo after they have grazed for an extended period of time on the natural plant cover of an area that is infested with bracken fern. The bracken ferns contain toxic chemicals. The syndrome is primarily encountered in Brazil, China, Italy, Japan, Portugal, Turkey, and New Zealand [1-9]. Haematuria is a clinical finding of chronic enzootic haematuria appearing sporadically and attracting attention permanently. Anaemia and weight loss are also amongst the common clinical findings [2,8]. The long process of digestion triggers the syndrome in cattle and buffalo over the age of two [5,10,11]. It has been determined that different types of ferns, e.g. *Pteridium aquilinum*, *Pteridium esculentum*, *Cheilantes sieberi*, *Cheilantes farinosa*, *Christella dentata*, *Polystichum squarrosus*, *Dryopteris juxtaposita*, etc. are found in the areas where the syndrome is encountered [12-14]. In the flora of Turkey are found 16 families, 24 genera, and 79 taxa (species and sub-species) of ferns. The ferns thrive in humid and shady areas, in forest underbrush, alongside streams, and in rock fissures. The ferns grow in every region of Turkey, especially in the Black Sea region [15,16].

Ferns which build up the largest part of sporophytes live mostly on land and are autotrophs. However, they need water for insemination. They are standing out mostly with their green leaf like structures which are great sporophyte mostly pinnate leaves. Green leaved like sporophytes are doing photosynthesis and carry bags producing spor and providing proliferation [17]. It has been documented that bracken ferns contain ptaquiloside and quercetin. It is also suggested that the ferns can have carcinogenic, mutagenic, and clastogenic effects [1,11]. Among them, bracken fern comprises predominantly major toxin of ptaquiloside [18]. This toxin cause errors in DNA synthesis (especially, H-ras expression) and activation of proto-oncogenes which are controlled under cell division mechanism [4,19,20]. Because disruption of signalling cascades in the cells, this situation creates any changes in morphological diversity and biological behaviour of the cells and consequently leads to unbalanced tissue microenvironment [21,22].

Cystitis, epithelial hyperplasia, and hydropic degeneration are mentioned as non-neoplastic findings of the syndrome. Neoplastic findings of mesenchymal origin (hemangioma, hemangiosarcoma, fibroma, and myxoma) and epithelial origin (papilloma and transitional cell carcinoma) are reported [2,5,6].

In this study, it was aimed to reveal neoplastic and non-neoplastic lesions in urinary bladder of water buffaloes and to show types of bracken fern, which found naturally in the habitat of Marmara, the Central and Western Black Sea Regions of Turkey, and to proven the possible relationship between urinary bladder tumours and this herb.

## MATERIAL and METHODS

The urinary bladders of water buffalo aged three through eight years and of both genders were collected from slaughterhouses in İstanbul, Adapazarı, Bolu, and Samsun in which chronic enzootic haematuria was previously detected.

### **Pathomorphological and Immunohistochemical Examinations**

In total, 163 urinary bladders with lesions were evaluated. The bladders were fixed in 10% neutral formalin and evaluated macroscopically in the Department of Pathology, Faculty of Veterinary Medicine, at the University of Ankara. Portions of lesions were taken from the samples, subjected to routine tissue processing, and embedded in paraffin. Tissue sections were cut at a thickness of 5-6 µm and then stained with a specific haematoxylin and eosin.

For immunohistochemistry, the streptavidin- biotin-peroxidase complex (GBI SPlink HRP Broad Bulk Kit, D01-110) method was used. All sections were cut by microtome and adhered to positively charged slides All slides were deparaffinized, hydrated, and put into citrate buffer pH 6.0 for antigen retrieval. An 800 watt microwave pressure cooker was run for 20 min. The primary antibodies used for urothelium were Lifespan (LS-C40107/5F161) and mouse monoclonal anti-Uroplakin III antibody (1/10 dilution, 60 min at RT) and for endothelium Santa Cruz (SC-1506R) and rabbit polyclonal CD31 antibody (1/400 dilution, 60 min at RT). Then, biotinized secondary antibody and streptavidin-peroxidase were dropped onto the tissue sections. After this process, sections were visualised with 3-amino-9-ethylcarbazole (AEC, C01-12, GBI) chromogen. The background was coloured with Gill's (I) Hematoxylin. Slides were covered with aqueous mounting medium.

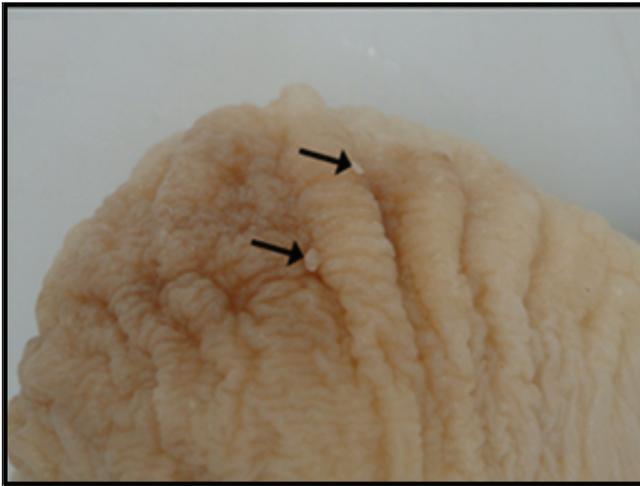
### **Drying Collected Plant Samples and Performing Species Identification**

Samples of bracken fern were collected from pastured areas in which chronic enzootic haematuria cases had been previously detected during field studies.

## RESULTS

### **Macroscopic Results**

Diffuse and petechial hemorrhages were observed in some of the urinary bladders. Varying sizes of the white foci were seen on the surface of the bladder, some of which had the appearance of cauliflower (*Fig. 1*). In one case, the urinary bladder was filled with blood. The bladder reached a size 6-7 times larger than normal. It had a very thin wall and a dark blackish color.

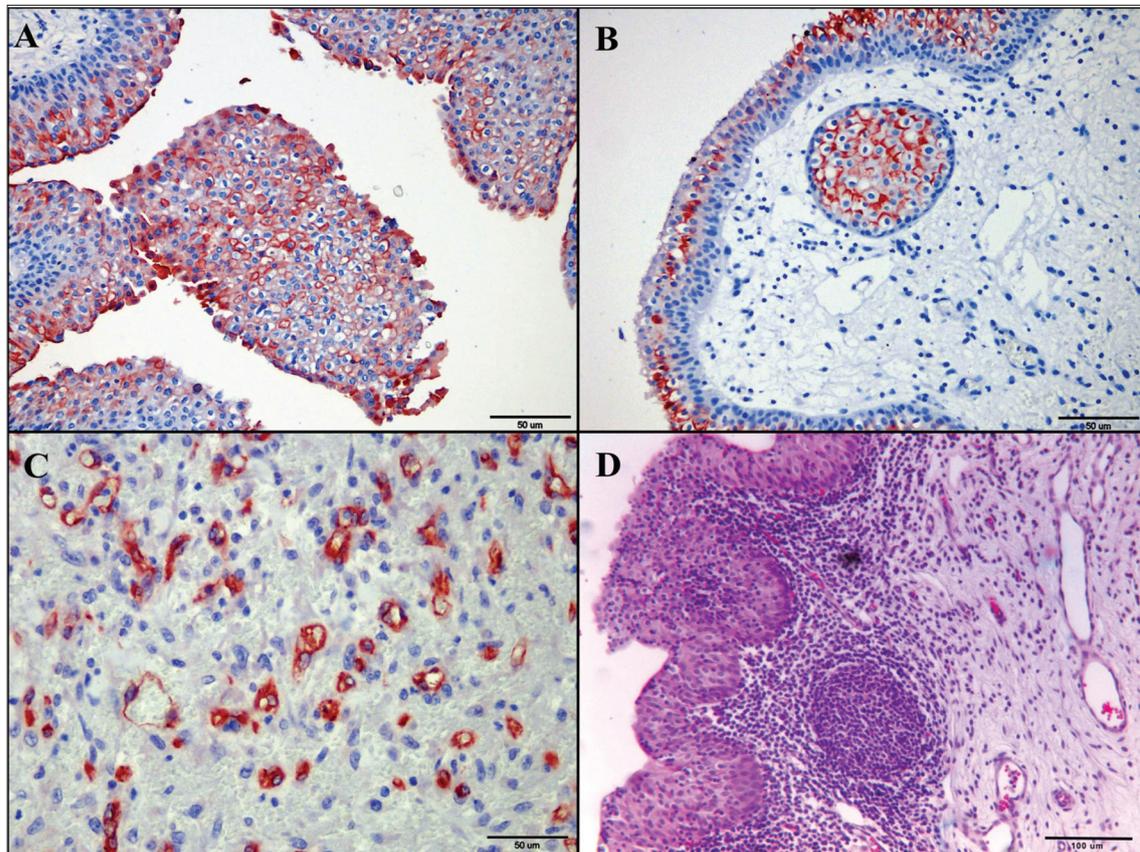


**Fig 1.** White papillomatous foci on the surface of the bladder (arrows)

In some cases, mononuclear cell infiltrations composed of lymphocytes and plasma cells were seen on the epithelium of the bladders.

In 39 cases, neoplasia was observed. These neoplasms were diagnosed according to The World Health Organization Classification of Tumours published in 2004. According to this classification, 43.8% of all tumours were papilloma; 17.9% were papillary urothelial neoplasms of low malignant potential (PUNLMP); 33.3% were low-grade papillary carcinoma; 5.1% were high-grade papillary carcinoma; and 2.5% were hemangiosarcoma. In one urinary bladder, low-grade papillary carcinoma and also hemangiosarcoma was observed (Table 1).

An immunohistochemical technique was used to support the diagnosis of possible neoplasms. Immuno-



**Fig 2.** A- Positive staining with UPIII of Papilloma, B- Positive staining with UPIII of Brunn's nest, C- Positive staining with CD31 of hemangiosarcoma, D- Follicular cystitis

### **Histopathological and Immunohistochemical Results**

In histopathology, chronic cystitis was observed in 67 urinary bladders. 74.6% of these bladders with cystitis had lesions like aggregate lymph follicle in propria mucosa. These were diagnosed as follicular cystitis. They also were associated with severe inflammation (Table 1), (Fig. 2-D).

histochemically, positive staining by UPIII was observed at the cytoplasm of the umbrella and intermediate cells. However, the umbrella cells reacted more than the intermediate cells (Fig. 2-A). The cells located in the center of the Brunn's nest stained stronger than the peripheral cells (Fig. 2-B). In one case, positive staining by CD31 was observed at the cytoplasm of the tumour endothelial cells (Fig. 2-C).

Table 1. Histopathological findings of 67 urinary bladders with chronic enzootic hematuria							
Epithelium of the Urinary Bladder							
Desquamation	Hydropic degeneration	Hyperplasia	Inflammatory cells on the epithelium	Haemorrhagia on the epithelium	Ulcer	Cystitis glandularis	Cystitis cystica
5	58	81	4	1	7	14	2
Epithelium of the Urinary Bladder (Urothelial lesions)				Propria mucosa of Urinary Bladder			
Papilloma	Papillary urothelial neoplasm of low malignant potential (PUNLMP)	Low grade papillary carcinoma	High grade papillar carcinoma	Inflammation	Lymph follicle	Hemorrhagia	Vascularisation
16	7	13	2	65	49	44	64

### Findings Regarding Species Determination

It was observed that the water buffalo did not eat the bracken ferns by design, but consumed them incidentally while grazing on grasslands. The identified species of fern were *Athyrium filix-foemina* (L.) Roth, *Dryopteris dilatata* (Hoffm.) Gary, *Dryopteris filix-mas* (L.) Schott, *Polystichum aculeatum* (L.) Roth, *Polystichum setiferum* (Forsk.) Woyнар, *Polystichum woronowii* Fomin, *Polypodium vulgare* L., *Pteridium aquilinum* (L.) Kuhn.

## DISCUSSION

Enzootic haematuria cases have been described in cattle that graze on fern-covered areas of Turkey [5,6]. However, there are limited studies regarding water buffalo with enzootic haematuria [5,23-26]. In this study, we determined the age of the water buffalo to be between three and eight years old, which refers to the possible age of the chronic enzootic haematuria syndrome. Pamukcu [6], Ozkul and Aydin [5] also studied this syndrome in cattle after a period of life that was an old age.

In our study, 76 cases had chronic cystitis. However, it was not the acute cystitis previously reported by Somvanshi et al. [25] and mentioned in their study. Of that study's subjects, 53.7% had epithelial hyperplasia (flat/papillary) and 32.8% had hydropic degeneration. 8.9% of the total subjects had lymphocyte and plasma cell infiltrations on the epithelium of the urinary bladder. In terms of the chronic cystitis, 74.6% of cases were diagnosed as follicular cystitis which was formed like aggregate lymph follicles in propria mucosa. It was commented that severe inflammation had developed. However, Aydin and Ozkul [27] worked on urinary bladders both of cattle and water buffalo which had non-neoplastic findings and determined only 20% lymphoid cell aggregation in propria mucosa of the urinary bladders. The recent study pointed out the significant aggregate lymph follicles in more subjects than the previous study. Also, 21 of the chronic cystitis cases had haemorrhages that were in accordance with other studies [6,26].

As a result of the histopathological examination of the urinary bladders, we observed 39 neoplasia that were

classified according to The World Health Organization Classification of Tumours published in 2004 [28]. 43.8% of all tumours were papilloma; 17.9% were papillary urothelial neoplasm of low malignant potential (PUNLMP); 33.3% were low-grade papillary carcinoma; 5.1% were high-grade papillar carcinoma; and 2.5% were hemangiosarcoma.

In previous studies, mesenchymal tissue tumours were more frequently reported than epithelial tissue tumours in cattle [2,5,9,24]. However, we observed that epithelial tissue tumours occurred more frequently than mesenchymal tumours in water buffalo. Our data also correspond to others [6,24].

Immunohistochemically, examination of the tumours of water buffalo with chronic enzootic haematuria and pathologically diagnosed as urothelial epithelial cell tumours, just similar to the findings of Carvalho et al. [29]. We also observed that the umbrella cells were intensely stained with UPIII and, unlike the umbrella cells, the intermedial cells were weakly stained. In addition, the cytoplasm of the tumour endothelial cells of the urinary bladders with hemangiosarcoma were stained with CD31, and those results were also in accordance with Carvalho et al. [29].

Many varieties of bracken ferns grow around the world. The types detected in India, *Athyrium*, *Dryopteris*, *Polystichum*, and *Pteridium*, were also found in our working field [14,30]. Some types of bracken ferns, such as *P. aquilinum*, commonly grow across a wide area of Turkey and are the cause of chronic enzootic haematuria.

Water buffalo, unlike cattle which are bred intensively, require extensive breeding. As a result of their need to graze in large pasturelands, water buffalo consume many types of grasses in addition to bracken ferns, which can cause the syndrome called chronic enzootic haematuria. This study will be informative for all researchers and breeders.

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