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Urinary Bladder Lesions in Bovine Enzootic Haematuria

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Summary

In cattle, bracken fern chronic toxicity is characterized by the presence of multiple tumours in the bladder (bovine enzootic haematuria). From October 1999 to March 2003, 433 urinary bladders with macroscopical lesions were collected in the slaughterhouse of São Miguel Island (Azores, Portugal), an endemic area where *Pteridium aquilinum* infestation in pastures is high. Bladder lesions were divided into three main categories (inflammatory lesions, non-neoplastic epithelial abnormalities and tumours) and described in detail. In some cases, neoplastic growth was confined to a single site, but in most cases multiple tumours developed within the same bladder. Epithelial tumours alone were present in 51·2% of the affected bladders, mesenchymal tumours alone in 17·4%, and both epithelial and mesenchymal tumours in the remaining 31·4%. The large number of tumours examined (870) revealed new categories not yet included in other veterinary classification systems, namely, inverted papilloma, papillary neoplasm of apparent low malignant potential, and haemangioendothelioma.

Keywords: bovine enzootic haematuria; bracken fern; cattle; tumour; urinary bladder

Introduction

Naturally occurring urinary bladder neoplasms are common in dogs, accounting for 0.5–1% of all canine neoplasms (Maxie, 1993; Meuten, 2002; Meuten *et al.*, 2004), and generally thought to be rare in other domestic and laboratory animals (Yoshikawa *et al.*, 1981), except in cattle. In certain parts of the world, the prevalence of bovine bladder neoplasia is extremely high, being associated with the chronic ingestion of bracken fern (mainly *Pteridium aquilinum*) (Maxie, 1993; Ozkul and Aydin, 1996; Smith, 1997; Meuten, 2002; Borzacchiello *et al.*, 2003a; Meuten *et al.*, 2004).

Bracken fern is among the five commonest plants in the world and is the only higher plant known to cause cancer naturally in animals (Evans *et al.*, 1972; Smith *et al.*, 1988; Smith, 1997). It has a considerable number of toxic components, one of which is ptaquiloside (PT), a glycoside sesquiterpenoid capable of inducing clastogenesis in cell cultures and also with mutagenic and carcinogenic activity (Smith, 1997; Alonso-Amelot and Avendano, 2002). PT is the main bracken carcinogen and is carcinogenic in experimental animals (Pamukcu *et al.*, 1978; Hirono *et al.*, 1987; Shahin *et al.*, 1998a). PT is eliminated in the urine, inducing bladder tumours in cattle, and also in the milk, with potential risks to human health (Evans *et al.*, 1972; Hirono *et al.*, 1987; Shahin *et al.*, 1998b, 1999).

In cattle, bracken fern chronic toxicity causes multiple tumours in the bladder wall and haemorrhages in the bladder mucosa, giving rise to so-called bovine enzootic haematuria (BEH) (Pamukcu *et al.*, 1976; Meuten *et al.*, 2004). This disease, which is associated with haematuria, leucopenia, anaemia, and reduced haemoglobin, has been reproduced experimentally in bracken-fed cattle (Pamukcu *et al.*, 1967; Prakash *et al.*, 1996). Prakash *et al.* (1996) showed that, in samples of ileum and urinary bladder, the codon 61 of *h-ras* carried a specific mutation. DNA changes were recently identified by Sardon *et al.* (2005) in bladder lesions of cattle exposed to bracken fern, H-ras expression being significantly increased in chronic cystitis and tumours.

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Infection by bovine papillomavirus (BPV-2) was demonstrated by Campo *et al.* (1992) in bladder tumours of cattle exposed to bracken fern. BPV-2 was isolated from normal urothelium and also from naturally occurring and experimentally induced tumours, the virus being capable of remaining latent in some tissues, including urinary bladder (Campo, 1999). Immunosuppression was sufficient to lead to premalignant lesions, but the mutagens present in bracken were responsible for their progression to neoplasia (Campo *et al.*, 1992; Campo, 1999; Borzacchiello *et al.*, 2003b). Upon cancer development, BPV-2 would seem to undergo significant changes, expressing the viral oncoprotein E5 and modifying telomerase activity (Borzacchiello *et al.*, 2003b). A similar synergism between papillomavirus and bracken fern was also postulated in bovine and human gastrointestinal tumours, this time associating BPV-4 and quercetin, a mutagenic flavonoid also present in bracken fern (Campo *et al.*, 1999).

São Miguel Island, in the Azores, is an endemic area where *P. aquilinum* grows in pastures. An epidemiological study between 1997 and 1999 indicated an association between BEH and the level of infestation of pastures with *P. aquilinum* (Pinto *et al.*, 2000, 2001). PT was detected in high concentrations in *P. aquilinum* from São Miguel Island, where, in 1999, 21% of the dairy farms had at least one animal with clinical signs or tumour lesions associated with BEH (Pinto *et al.*, 2001). Moreover, *ca* 10% of the adult cows at present slaughtered on this island are rejected because of urinary bladder tumours (Pinto *et al.*, 2004); due to the high incidence of this disease, urinary bladders are systematically opened in all animals slaughtered.

A new WHO classification system for tumours of the urinary system of domestic animals was recently published (Meuten *et al.*, 2004). Although extensive, this classification was incomplete in respect of BEH lesions. This was not surprising in view of the heterogeneity of inflammatory lesions, epithelial abnormalities and tumours found in BEH. The aim of the present study was therefore to perform an extensive and detailed histological analysis of the bladder lesions that arise in BEH.

Materials and Methods

From October 1999 to March 2003, a total of 41 363 Friesian cows (after calving, and > 2 years of age) were slaughtered in the abattoir of São Miguel Island. Rejection due to bladder lesions occurred in 5638 (13·6%), from which 433 were selected, at random, for the present study. Their ages ranged from 2 to 16 years (mean, 7). Up to 90% of the animals rejected due to bladder lesions had shown haematuria at or before slaughter.

Because many bladders exhibited multiple lesions, multiple samples were collected from large and heterogeneous tumours. In all, 1337 tissue fragments were selected for histopathological examination. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3–5 μm) stained with haematoxylin and eosin (HE) were examined and classified independently by two of the authors (T.C. and M.C.P.). When the diagnosis differed, sections were re-examined to achieve a consensus.

Histological typing of bladder tumours followed, as far as possible, WHO established criteria for the diagnosis of urinary bladder tumours in domestic animals (Meuten *et al.*, 2004). However, the WHO classification system for human urinary bladder tumours (Mostofi *et al.*, 1999; Eble *et al.*, 2004) was used for lesions not included in the corresponding classification for domestic animals. New categories were proposed when no matching description was found in either of the WHO systems.

Results

Bladder lesions were subdivided into three major different categories, inflammatory lesions, non-neoplastic epithelial abnormalities, and tumours.

Of the 433 urinary bladders collected 373 showed neoplasia (Fig. 1); 55 showed inflammatory lesions or non-neoplastic epithelial abnormalities (NNEA), or both, NNEA being present in 26 bladders within this group; and the remaining five showed no changes whatsoever. In 107 cases in which neoplasms were diagnosed, various types of NNEA were also identified in the same organ.

Bladders were considered normal when the mucosa was lined by several (3–7) layers of normal transitional (urothelial) cells, including superficial, intermediate and basal cells, exhibiting characteristic polarity, with palisading of the basal cells. Under the epithelium lay the lamina propria, displaying an interrupted layer of smooth muscle, consisting of the muscularis mucosae, overlying the muscularis propria (the large smooth muscle bundles of the bladder wall).

Inflammatory Lesions

The inflammatory lesions most frequently observed were polypoid and follicular cystitis, both of which deformed the bladder wall profile. Polypoid cystitis was diagnosed when an oedematous lamina propria, often inflamed, gave rise to elongated mucosal folds covered by normal urothelium (Fig. 2a). Follicular cystitis was diagnosed when lymphoid follicles were seen in the lamina propria. Occasionally, reactive atypia was seen,

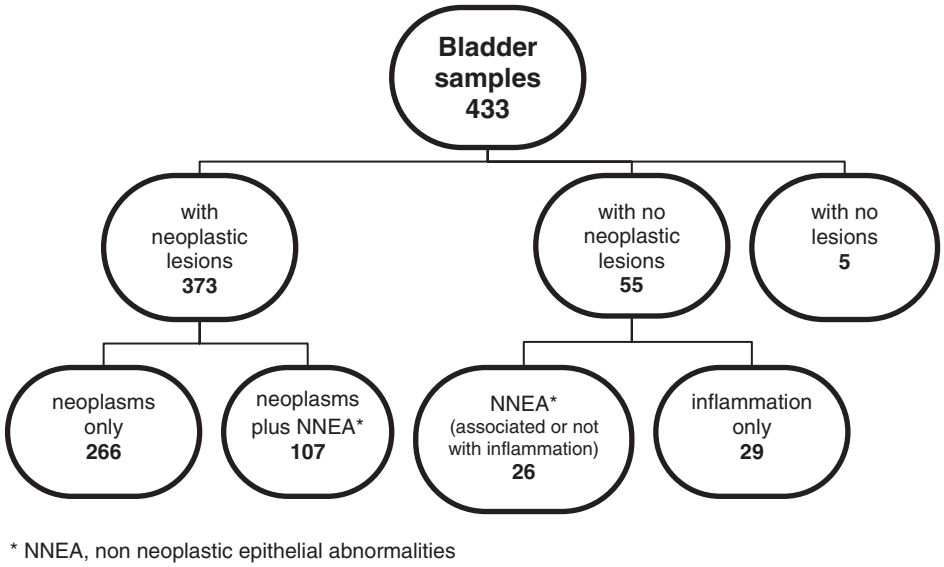

Fig. 1. Types of histopathological lesion present in bladder samples.

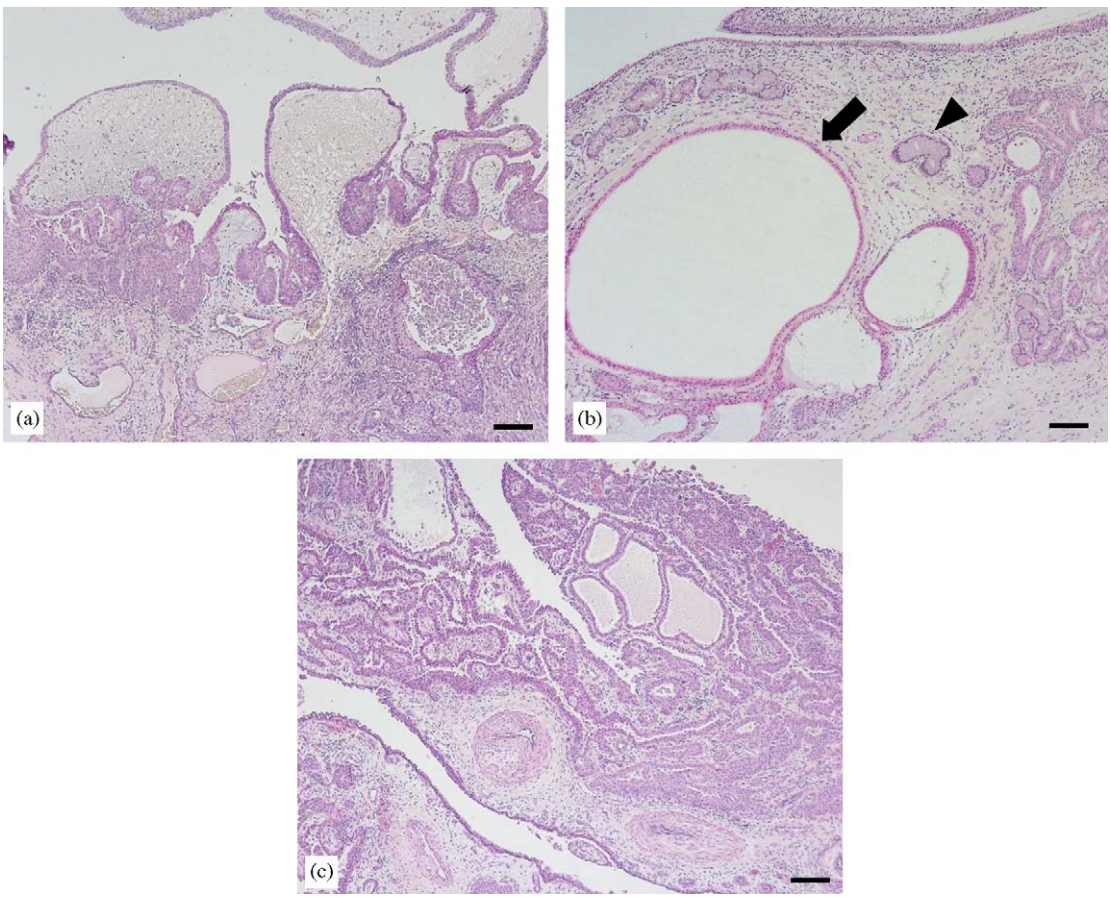

Fig. 2a–c. Non-neoplastic epithelial abnormalities. (a) Polypoid cystitis. (b) Cystitis cystica associated with cystitis glandularis (arrowhead). (c) Nephrogenic adenoma. Bovine bladder. HE. Bar, 150 µm.

with variations in nuclear size and staining of transitional cells.

Non-neoplastic Epithelial Abnormalities

NNEA included hyperplasia, von Brunn's nests, cystitis cystica, glandular metaplasia (cystitis glandularis and intestinal metaplasia) and nephrogenic adenoma. These lesions were often associated with neoplasia. Increased numbers of normal transitional cell layers, either flat or undulated, were described as hyperplasia. Von Brunn nests, which were found frequently, consisted of compact, round aggregates of transitional cells in the lamina propria, with or without connection to the surface epithelium. When these aggregates showed central lumina, the lesion was classified as cystitis cystica (Fig. 2b). Glandular metaplasia was also frequently seen, characterized by mucus-containing epithelial cells of colonic type lining the surface of the bladder or forming glands in the lamina propria. Glandular metaplasia either resembled intestinal mucosa ("intestinal metaplasia") or was associated with cystitis cystica ("cystitis glandularis"). Nephrogenic adenoma, which was found comparatively rarely, usually took the form of a proliferation in the lamina propria of structures similar to renal tubules, lined by cuboidal or low-columnar epithelial cells (Fig. 2c). In all the lesions described above, epithelial cells showed little if any atypia.

Neoplasms

The location of tumours within the bladder wall varied greatly. In some cases (141/373) neoplasia was confined to a single site, but in the majority multiple tumours occurred within the same bladder (Fig. 3a).

The gross appearance of neoplastic lesions varied greatly. Exophytic growths, which varied in diameter from a few millimeters to several centimetres, had an attachment to the bladder wall which was either pedunculated or broad, and a surface which was either smooth or branch-like (Fig. 3b). Depending on

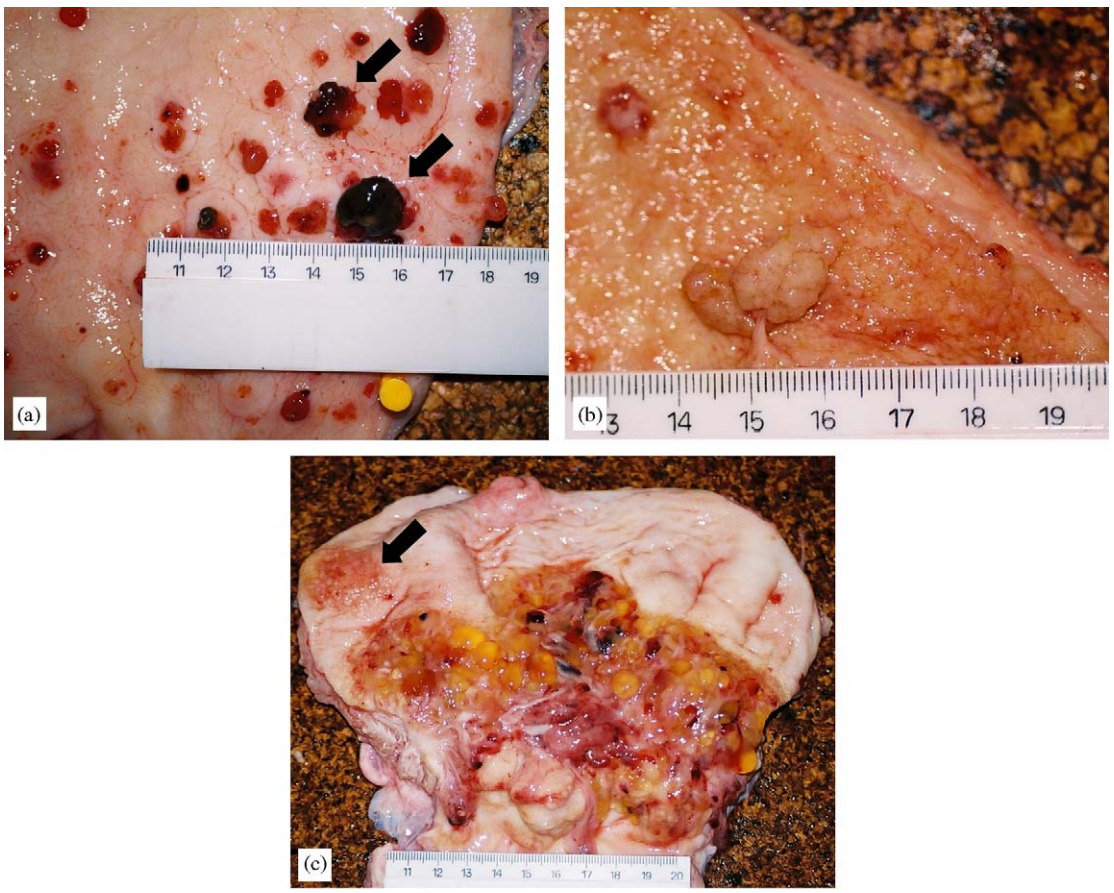

Fig. 3a–c. Macroscopical lesions. (a) Bladder exhibiting multiple tumours, two of them corresponding to exophytic haemangiosarcomas. (b) Bladder exhibiting a papillary neoplasm. (c) Bladder exhibiting an endophytic neoplasm (transitional cell carcinoma) with infiltration of the muscularis propria. The lesion occupies a large portion of the bladder surface. In an adjacent area non-neoplastic epithelial abnormalities, namely cystitis cystica, von Brunn nests and urothelial hyperplasia, were also diagnosed. Scales, mm.

vascularization, these tumours were either pale or haemorrhagic. Endophytic growths took the form of space-occupying lesions of the bladder wall, varying from discrete undulations of the mucosa with moderate change of colour, to deeply infiltrating growths that sometimes covered extensive areas (Fig. 3c). With few exceptions, the macroscopical appearance gave no clear indication of histological pattern.

As expected, a considerable variety of neoplastic lesions was observed. Tumours occurred either as a single type within the same bladder (regardless of the number of tumour masses) or as a combination of different types (Table 1). Of these 870 tumours, 52% were malignant and 42% benign, the remaining 6% being accounted for by epithelial papillary neoplasms of apparently low malignant potential (PNALMP) (Table 2).

Epithelial neoplasms. These were more common (62%) than mesenchymal tumours (38%) (Table 2). Of the benign epithelial tumours, papillomas were the most common (84/870); these exophytic lesions had a delicate fibrovascular stroma forming papillary fronds covered with transitional epithelium which was indistinguishable from the normal urothelium, both in the number of cell layers and in their polarity. A variant not previously described in domestic animals but regularly observed in the present study was the inverted papilloma, a tumour with characteristics of a transitional cell papilloma in which the papillary projections showed an endophytic growth pattern, with invaginations extending into the stroma (Fig. 4). Areas of inverted growth were also frequently identified within papillomas and also transitional cell carcinomas.

Adenomas (8/870) were cauliflower-shaped or pedunculated and characterized by glandular structures that showed few if any signs of cellular atypia.

PNALMP (55/ 870) were similar to the typical papilloma, with minimal variation in the architectural and nuclear features, yet showing increased cellular proliferation, exceeding six cell layers in thickness (Fig. 5). Signs of cellular atypia were rare or non-existent. These

Table 2
Histopathological types and numbers of the bladder neoplasms

Details of tumours	*n*	Percentage (%)
Epithelial	**539**	**62.0**
Benign		
Papilloma	84	9.6
Adenoma	8	0.9
Papillary neoplasm of apparent low malignant potential (PNALMP)	55	6.3
Malignant		
Transitional cell carcinoma	359	41.4
Adenocarcinoma	20	2.3
Squamous cell carcinoma	13	1.5
Mesenchymal	**331**	**38.0**
Benign		
Haemangioma	256	29.5
Fibroma	18	2.0
Malignant		
Haemangioendothelioma	14	1.6
Haemangiosarcoma	43	4.9
Total number of tumours identified	870	100

Table 1
Details and numbers of bladder tumours in the present study and in three previous studies

	Present study				
Details of tumours	n	*%*	*Ozkul and Aydin (1996)*	*Pamucku et al. (1976)*	*Xu et al. (1989)*
Epithelial in pure form	**191**	**51.2**	**35.3%**	**35.2%**	**36.7%**
Benign	31	8.3	24%	17%	NI
Malignant	160	42.9	4%	16.5%	NI
Epithelial in combination with mesenchymal	**117**	**31.4**	**25.4%**	**53.9%**	**17.5%**
Benign	19	5.1	19%	9.3%	NI
Malignant	98	26.3	4%	44.6%	NI
Mesenchymal in pure form	**65**	**17.4**	**46.6%**	**9.3%**	**45.4%**
Benign	48	12.9	43.2%	5.7%	NI
Malignant	17	4.6	3.4%	3.6%	NI
Total number of bladders with tumours	373		815	139	354
Total number of tumours identified	870		1063	NI	NI

For the purpose of constructing Table 1, PNALMP (papillary neoplasms of apparent low malignant potential) were regarded as benign. NI, not indicated.

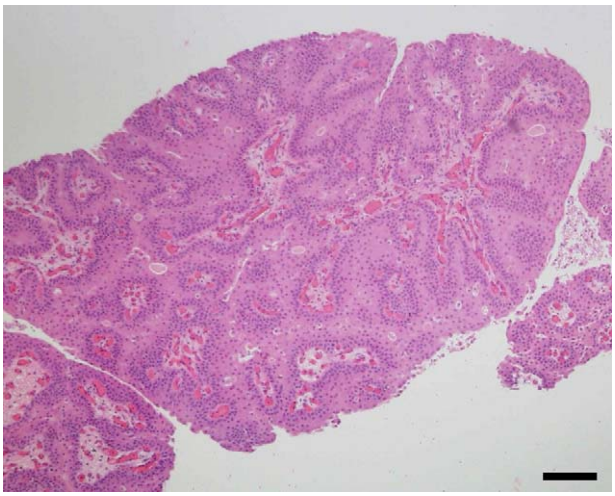

Fig. 4. Inverted papilloma. Bovine bladder. HE. Bar, 150 μm.

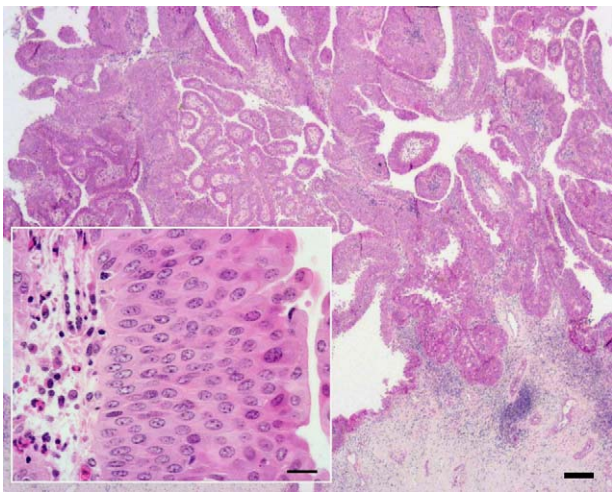

Fig. 5. Papillary neoplasm of apparent low malignant potential. Bovine bladder. HE. Bar, 200 μm; inset bar, 20 μm.

neoplasms, although sharing features common to papilloma and to transitional cell carcinoma, could not confidently be placed in either of these two categories.

Transitional cell carcinoma (TCC) was by far the most common lesion found in bovine urinary bladders (359/870), consisting entirely, partly or focally of anaplastic transitional epithelium. TCCs were subclassified according to several criteria. In terms of growth pattern, lesions were either papillary, invasive, a combination of both, or non-papillary and non-infiltrating. The degree of invasiveness was evaluated with regard to the presence or absence of neoplastic cells within the lamina propria, submucosa or muscularis propria, on which criterion tumours were assigned to one of the three following categories: Ta, with no infiltration (48.7%); T1, infiltration confined to the lamina propria, including the muscularis mucosae (46%); T2, infiltration reaching the muscularis propria (5.3%). Different histological variants or patterns of TCCs were identified, namely micropapillary, nested and microcystic. "Micropapillary" referred to the formation of ramified branches distributed in the tumour stroma, assuming a papillary growth pattern (Fig. 6a). The "nested" variant consisted of deep infiltrations into the lamina propria of small rounded cell aggregates, more numerous and smaller in size than Von Brunn nests, showing mild nuclear atypia (Fig. 6b). The "microcystic" pattern corresponded to transitional cell growths that formed cysts with various shapes; the epithelium lining these cysts had various numbers of cell layers (Fig. 6c). Non-papillary and non-infiltrating TCC (carcinoma *in situ*) was occasionally identified in the vicinity of other tumour lesions but never by itself (Fig. 6d).

When tumoural growth followed a glandular pattern with signs of malignancy, adenocarcinoma was diagnosed (20/870). Occasionally, these lesions showed areas of signet-ring cell proliferation (Fig. 7).

Squamous cell carcinomas (13/870) were characterized by extensive areas of squamous differentiation with keratin pearl formation. Such tumours were frequently large and exuberant, occupying most of the bladder surface and displaying an infiltrating growth pattern, frequently reaching the muscularis propria.

Mesenchymal neoplasms. These showed limited variation, being predominantly of vascular origin. Haemangioma was diagnosed on the basis of the same criteria as those used for the diagnosis of these lesions in other organs and tissues. Cavernous or capillary, this tumour was the second most common neoplasm of bovine urinary bladder (256/870), consisting of a multitude of vessels with well differentiated capillary-type endothelium, arising in well-defined areas of the lamina propria. The growth pattern was either endophytic or exophytic, the latter term referring to polyps whose lamina propria was occupied by the haemangioma itself.

Haemangiosarcoma (43/870) was diagnosed in cases in which the tumour mass was composed of blood vessels, those still recognizable as such being either small and rare or large and anastomising, highly infiltrating, with both solid and cystic areas, and with the constant presence of cellular atypia.

Haemangioendothelioma (14/870) represented an intermediate category between haemangioma and haemangiosarcoma, consisting of some areas of solid growth and others in which vessel formation was obvious (Fig. 8). Cellular atypia was rare and infiltrating capability was apparent in discrete foci.

The fibroma, which was the only non-vascular mesenchymal tumour observed (18/870), was character-

ized by a well-circumscribed, non-encapsulated proliferation of fibroblasts, showing few if any signs of atypia, and rich in intercellular stroma (Fig. 9). The macroscopical appearance was always that of small (2–4 mm diameter), well-demarcated white nodules on the bladder surface.

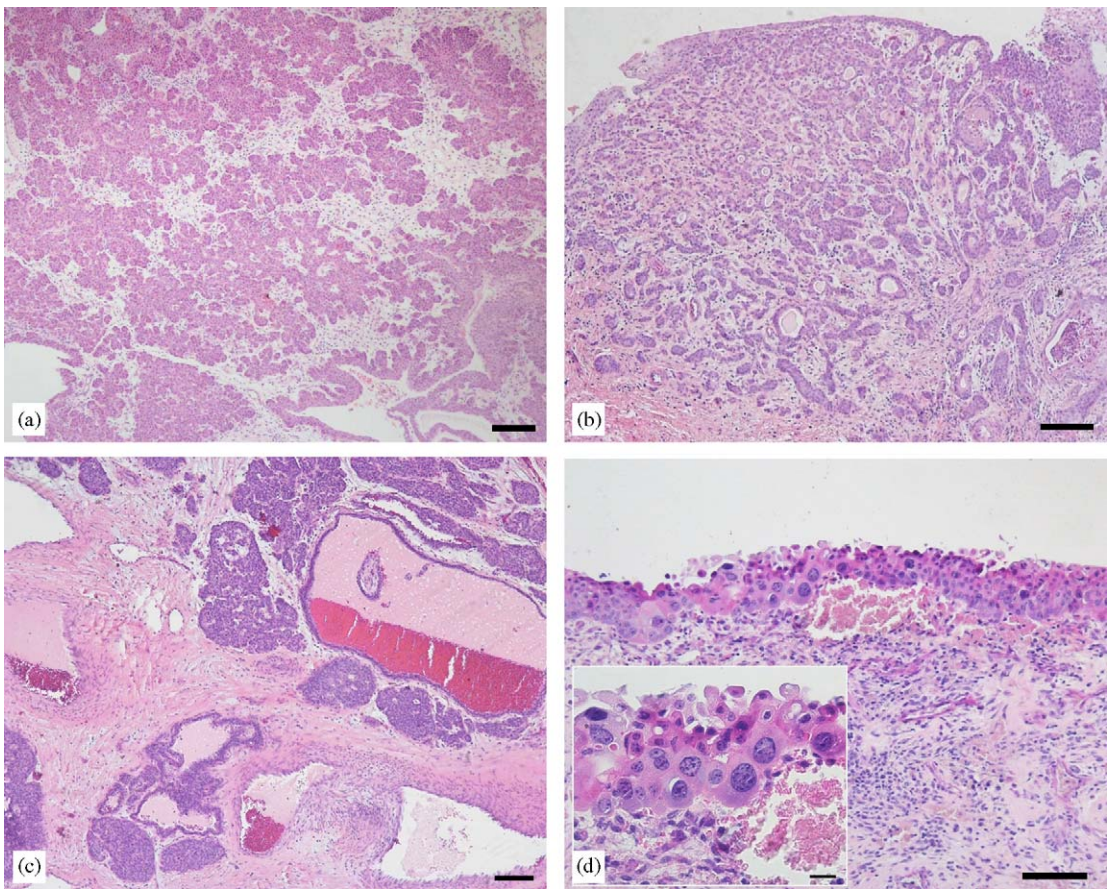

Fig. 6a–d. Transitional cell carcinoma of the urinary bladder. Different histological variants were identified, namely (a) micropapillary, (b) nested variant, and (c) microcystic. (d) Carcinoma *in situ*. Bovine bladder. HE. Bar, 150 µm (a, b, and c). Bar, 100 µm; inset bar, 20 µm (d).

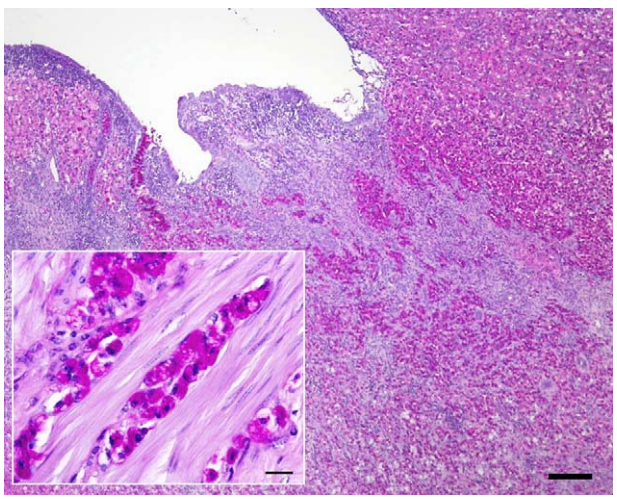

Fig. 7. Adenocarcinoma, signet ring variant, with invasion of the muscularis propria by the neoplastic cells. Bovine bladder. Periodic acid-Schiff. Bar, 150 µm; inset bar, 20 µm.

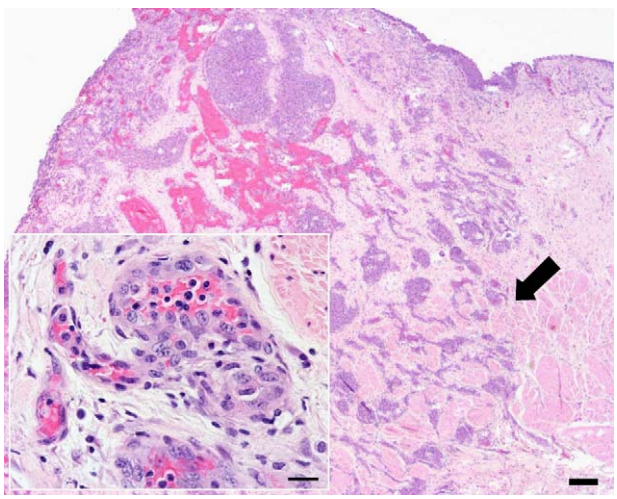

Fig. 8. Haemangioendothelioma, with invasion of the muscularis propria. Bovine bladder. HE. Bar, 200 µm; inset bar, 20 µm.

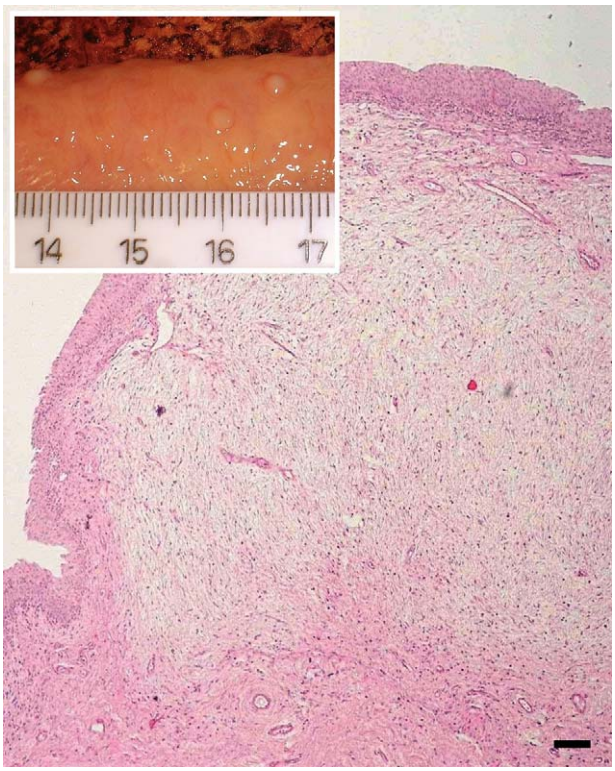

Fig. 9. Fibroma. Bovine bladder. HE. Bar, 200 µm. Inset: gross appearance; scale, mm.

Discussion

This report illustrates the considerable histological variety of urinary bladder lesions in cattle in São Miguel Island, where BEH is endemic.

Bladder tumours associated with BEH are common in other parts of the world, and Table 1 shows the results of previous similar studies (Pamukcu *et al.*, 1976; Xu, 1992; Ozkul and Aydin, 1996). The results of Pamukcu *et al.* (1976) were similar to those described here in respect of epithelial versus mesenchymal tumours, but the earlier reports did not present detailed histological descriptions, making it difficult to compare classification criteria. Nevertheless, the types of tumour described generally resembled those of the present study, except for the non-epithelial tumours. Thus, in our study, other than fibromas, only tumours of vascular origin were identified, whereas fibrosarcomas, leiomyosarcomas, rhabdomyosarcomas and round cell tumours were diagnosed by the three previous studies. The present study also confirmed the findings of others regarding the diagnosis of haemangioendothelioma (Pamukcu *et al.*, 1976; Ozkul and Aydin, 1996; Pires, 1998), a type of vascular tumour not included in WHO classifications (Pamukcu, 1974; Meuten *et al.*, 2004), although mentioned by Pamukcu *et al.* (1976). Carcinosarcoma (Pamukcu *et al.*, 1976; Ozkul and Aydin, 1996) was not identified in our samples. Variation in the results of studies from different parts of the world may be attributed to differences in sample size, species of bracken, and criteria used in the collection of bladder samples at slaughter, as suggested by other authors (Xu, 1992; Ozkul and Aydin, 1996).

The histological classification of urinary bladder lesions in BEH took account of diagnostic criteria commonly used for both domestic animals and man. All the lesions described fell into one of three different categories, namely inflammatory lesions, non-neoplastic epithelial abnormalities (NNEA) and neoplasms. Inflammatory lesions, namely polypoid and follicular cystitis, were similar to those described in other species (Maxie, 1993). NNEA corresponded to a category of proliferative epithelial changes of uncertain biological potential, also recognized in human pathology (Mostofi *et al.*, 1999). Such lesions may be part of a continuum that culminates in true neoplasia (Amin and Young, 1997; Oyasu, 2000). According to Oyasu (2000), hyperplasia, initially flat and subsequently exophytic or endophytic, or both, occurs in the rat bladder after exposure to chemical carcinogens; it may be reversible or progressive depending on the degree of exposure. In the present study, NNEA were identified in 133 of the 433 bladders, and only in 26 was no coexisting neoplastic lesion present. In the human bladder, hyperplasia is also frequently associated with neoplasms, the grade of hyperplasia apparently being related to the grade of tumour (Amin and Young, 1997).

Several NNEA described in the present study were also considered in the WHO classification of urinary bladder tumours of domestic animals (Meuten *et al.*, 2004) to be tumour-like lesions, with the exception of nephrogenic adenoma. This lesion, which was rare in the present study, has probably been interpreted differently by other authors. Nephrogenic adenoma is a metaplastic lesion classified by human pathologists as an epithelial abnormality (Mostofi *et al.*, 1999), and has been regarded as a precursor to neoplasia, due to its propensity to recur and its morphological similarity to adenocarcinoma of the bladder (Amin and Young, 1997).

The neoplasms described generally accorded with the categories devised for all animal species (Meuten *et al.*, 2004), but there were some exceptions. Within benign epithelial tumours, inverted papilloma, recognized for the first time in human pathology by Potts and Hirst (1963), and described in typing of human bladder tumours (Mostofi *et al.*, 1999; Eble *et al.*, 2004), has not been recognized previously in veterinary pathology. However, in the authors' opinion, this type of tumour at present deserves to be considered separately from papillomas; future studies may determine whether the inverted growth pattern is associated with

an increased risk of subsequent carcinoma development.

PNLMP, identified in 6% of the bladder tumours, represented a type of papillary neoplasm that shared features with both papilloma and transitional cell carcinoma; it was, however, difficult to place in either categories because the number of cell layers of the urothelium was higher than normal and epithelial atypia was not present. Recently, the WHO classification for human bladder tumours (Epstein *et al.*, 1998; Mostofi *et al.*, 1999; Eble *et al.*, 2004) introduced a new category, the papillary neoplasm of low malignant potential, similar in most aspects to the lesions described in the bovine bladder. Studies on the grading of urothelial carcinoma have also taken account of this type of tumour (Cheng *et al.*, 2000). In BEH, the absence of follow-up studies makes it impossible to be certain whether these neoplasms possess low malignancy; for this reason they were named papillary neoplasms of apparent low malignant potential.

With regard to malignant epithelial tumours, no major differences were noted from other studies (Pamukcu *et al.*, 1976; Xu, 1992; Ozkul and Aydin, 1996). Reactive atypia was occasionally seen in bladders severely affected by inflammation. Also, sporadic cases of nucleomegaly were identified, consistent with the description of cell crowding and loss of polarity typical of carcinoma *in situ* (McKenney *et al.*, 2001). These non-papillary and non-infiltrating tumours, similar to those previously described (Borzacchiello *et al.*, 2001, 2003a), were not included in our analysis, as they were always seen in the transitional epithelium adjacent to other types of tumour, and never as an isolated neoplasm. They were, therefore, interpreted as severe dysplasia, resulting from the highly aggressive tumours in the surroundings.

Evidence suggests that the amount and duration of bracken exposure play a crucial role in the frequency, nature and severity of BEH lesions (Shahin *et al.*, 1998b). Hence, the development of bladder tumours in BEH is probably part of a continuous process, with atypia, dysplasia and carcinoma *in situ* corresponding to distinct successive stages of the same process, with the borderline between these categories sometimes difficult to establish.

Although not included in the WHO classification of urinary bladder tumours in domestic animals (Meuten *et al.*, 2004), haemangioendothelioma has been described by other authors in the context of BEH (Pamukcu *et al.*, 1976; Ozkul and Aydin, 1996; Pires, 1998). In human pathology this neoplasm is recognized in other organs and tissues, falling between haemangiomas and haemangiosarcomas in terms of invasiveness and cellular atypia, and hence corresponding to a low-grade malignant vascular tumour (Calonje and Fletcher, 1995).

The large numbers and types of neoplasms found in bladders collected from cattle affected by BEH at São Miguel Island slaughterhouse emphasizes the need to open the urinary bladders of all cattle from bracken fern-infested areas, even those not exhibiting clinical signs at slaughter. This precaution was also supported by Sardon *et al.* (2005) in respect of cattle exposed to bracken fern in Spain.

In BEH no association between the histological grade of bladder lesions and their biological behaviour can be made, as samples are obtained at slaughter and there is, therefore, no treatment or follow-up. Nevertheless, there is unquestionable merit in the use of animal models for studying the pathogenesis of bladder neoplasms.

A future extension of the present work lies in the identification of the various pathways for the development of each specific type of neoplasm, particularly in the light of increased understanding of the molecular mechanisms that play a role in carcinogenesis.

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