Intratesticular benign peripheral nerve sheath tumour in a ferret (*Mustela putorius furo*)

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A domestic ferret was submitted for sterilisation because of right testis enlargement. Oestradiol and cortisol concentrations were within normal physiological ranges, but testosterone was below and progesterone above normal. Microscopically, the right testis, with the exception of a small part of the epididymis, was replaced with neoplastic tissue. The tumour was composed of streams and bundles of closely packed spindle to ovoid cells forming whorls around collagen and capillaries, and separated by a collagenous matrix. In some areas, cells were loosely arranged and separated by a pale myxomatous matrix. The left testis showed atrophy. The majority of neoplastic cells expressed vimentin and S-100 protein, while expression of collagen IV was moderate and there was no expression of glial fibrillary acid protein. On the basis of macroscopical and histopathological findings, and supported by immunohistochemical reactivity, the diagnosis of benign peripheral nerve sheath tumour was made. This is the first report of benign peripheral nerve sheath tumour in ferret testis.

INTRODUCTION

Peripheral nerve sheath tumours (PNSTs) are neoplasms arising from Schwann cells, perineural cells and intraneural fibroblasts. On the basis of morphological features and assessment of benign versus malignant, they can be subdivided into schwannoma or benign PNST (previously called neurilemoma or neurinoma), neurofibroma or malignant PNST (Schulman and others 2009). Due to their uncertain histiogenesis, PNSTs in veterinary medicine are divided into benign and malignant variants in the most recent edition of the WHO International Histological Classification of Tumours of Domestic Animals (Koestner and others 1999). There are few studies of the incidence of neoplasia in ferrets (Brown 1997, Zeeland and others 2006). The most common tumours reported are insulinoma, lymphoma and adrenocortical neoplasms, followed by skin neoplasias and musculoskeletal neoplasms (Dillberger and Altman 1989). Ovarian stromal tumours are the main neoplasia of the reproductive tract in female ferrets, while neoplasms of the male reproductive system are unusual (Beach and Greenwood 1993). A few studies have described findings of testicular neoplasia in ferrets: interstitial cell adenoma (Meschter 1989), Sertoli cell tumour in cryptorchid testis (Powers and others 2007) and mixed interstitial and Sertoli cell tumour (Batista-Arteaga and others 2011). In the available literature, there is only a single report of PNST in ferret, i.e. neurilemoma on the leg (Brown 1997). We now present the first case of benign PNST in a testicle of a ferret and discuss in detail the reasons for this diagnosis.

CASE HISTORY

A 2·2 kg, male, 6-year-old, moderately obese domestic ferret from Zagreb Zoo was submitted to the Surgery, Orthopaedics and Ophthalmology Clinic, Faculty of Veterinary Medicine, University of Zagreb in October 2010 for sterilisation because of right testis enlargement. During the operation, blood samples were taken for determination of oestradiol, progesterone, testosterone and cortisol concentrations. Ultrasound evaluation of the abdomen was performed before the orchidectomy, which was performed through a scrotal incision with ligation of the spermatic cord, in the standard manner.
The testes were submitted to the Department of Veterinary Pathology for histopathological examination. The left testis was round and about 0.8 cm in diameter (volume 0.27 cm³), while the right testis had a diameter of about 3.5 cm (volume 22.43 cm³). For comparison, the average testicular diameter in sexually mature ferrets is approximately 1.2 cm (Sisk 1990), with an average volume of 0.94 to 1.38 cm³ (Schoemaker and others 2008). On the basis of these reported values, the left testis was slightly below the normal range but the right considerably above. The left testis was moderately firm and showed no macroscopic changes on the external or cut surface. The right testis was firm with a rugged, external surface and was whitish-grey and lobulated on the cut surface. The testes were fixed in 10% neutral buffered formalin, embedded in paraffin and processed routinely. Sections were stained by haematoxylin and eosin (H&E).

Immunohistochemistry was performed for exact tumour classification. Primary antibodies used were specific for the following antigens: vimentin (VIM) (Clone V9, Dakocytomation, No. M0725, diluted 1:200), S-100 protein (S-100) (DakoCyto- mation, No. Z0311, diluted 1:400), glial fibrillary acid protein (GFAP) (clone 6F2, DakoCyto- mation, No. M0761, diluted 1:300) and collagen IV (col IV) (clone CIV 22, DakoCyto- mation, No. M0785, diluted 1:25). Antigen retrieval for S-100 was not performed and for VIM was performed at room temperature with proteinase K (DakoCyto- mation, Code S3004) for 5 min. Antigen retrieval for col IV was performed with ethylene diaminetetraacetic acid (EDTA) buffer, pH 9 (DakoCyto- mation, Code S2367) for 3×5 min, and for GFAP with citrate buffer pH 6 (DakoCyto- mation, Code S2031) for 20 min in a Dako PT Link module. Endogenous peroxidase activity was blocked by 5-min treatment with hydrogen peroxide (Dako No. S2023). Dako REAL EnVision Detection System, Peroxidase/DAB, Rabbit/Mouse (No. K5007) was used to detect the antibodies against VIM, S-100, col IV and GFAP.

Standard electro-luminescence serum hormone testing showed an oestradiol concentration of 5.00 ng/L, progesterone of 18.6 nmol/L, testosterone of 0.087 nmol/L and cortisol of 40.8 nmol/L. Ultrasound examination of all abdominal organs, prostate and both adrenal glands (left=7.6 mm length, right=7.8 mm length) appeared normal [reference values reported by Neu- wirth and others (1997) left=8.6 ±1.2 mm; right=8.9 ±1.6 mm].

Microscopically, with the exception of a small part of epididymis, the remainder of the right testis was replaced with neoplastic tissue (Fig 1). The tunica albuginea was not penetrated by neoplastic cells. Neoplastic tissue was composed of interlacing streams and bundles of closely packed spindle to ovoid neoplastic cells and separated by small amounts of collagenous matrix. These cells often formed whorls around the collagen and capillaries (Antoni A-like areas) (Fig 2). In some areas, cells were more loosely arranged and separated by a pale amphiphilic, myxomatous matrix (Antoni B-like areas) (Fig 3). Borders of neoplastic cells were variably distinct and the cytoplasm was scant and eosinophilic. Nuclei varied from round to elongate with finely stippled, marginated or condensed chromatin, with one to two poorly distinct nucleoli. Mitotic figures were less than 1/10 per high-power fields. Areas of coagulative necrosis and haemorrhage

FIG 1. Right testis. With the exception of a small part of the epididymis, the rest of the right testis was replaced with neoplastic tissue (H&E, ×10)

FIG 2. Right testis. Antoni A-like areas, neoplastic cells form whorls around collagen and capillaries (H&E, ×10)

FIG 3. Right testis. Antoni B-like areas, neoplastic cells loosely arranged and separated by a pale amphiphilic, myxomatous matrix (H&E, ×10)
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Benign PNSTs are mesenchymal spindle cell tumours and are in 100% of cases positive for VIM (Chijiwa and others 2004, Bergmann and others 2009). S-100 identifies Schwann cells and hence, most PNSTs express this molecule (Chijiwa and others 2004, Bergmann and others 2009). As Col IV is a basement membrane component of Schwann cells, PNSTs can also be positive to this antibody (Gaitero and others 2008). Finally, GFAB, which is a neuronal marker, may be variably expressed (around 67%) in PNSTs (Chijiwa and others 2004, Bergmann and others 2009). Spindle cell tumours which can have similar IHC reactivity are malignant PNSTs, rhabdomyosarcoma and other tumours of vascular origin (Chijiwa and others 2004, Bergmann and others 2009). In our case, typical histological features excluded potential diagnosis of these tumours. Moreover, IHC reactivity to VIM, S-100 and col IV confirmed the diagnosis of benign PNST (Koestner and Higgins 2002, Chan and others 2007, Gaitero and others 2008, Schulman and others 2009).

IHC staining for VIM was strongly positive in cytoplasm (Fig 4) and S-100 was moderately positive in the nuclei and cytoplasm of majority of neoplastic cells (Fig 5). Col IV was moderately extracellularly positive (Fig 6), whereas staining for GFAB was negative in the tumour.

**DISCUSSION**

To our knowledge, this is the second report of a benign PNST in a domestic ferret and first report of this tumour in the testis (Brown 1997). The diagnosis of benign PNST was based on macroscopic and histopathological findings which were identical to those described in the literature (Rothwell and others 1986, Koestner and Higgins 2002, Chijiwa and others 2004, Chan and others 2007, Bergmann and others 2009). As there is no single specific IHC marker that is able to define PNST, a panel of reagents is generally employed. PNSTs are mesenchymal spindle cell tumours and are in 100% of cases positive for VIM (Chijiwa and others 2004, Bergmann and others 2009). S-100 identifies Schwann cells and hence, most PNSTs express this molecule (Chijiwa and others 2004, Bergmann and others 2009). As Col IV is a basement membrane component of Schwann cells, PNSTs can also be positive to this antibody (Gaitero and others 2008). Finally, GFAB, which is a neuronal marker, may be variably expressed (around 67%) in PNSTs (Chijiwa and others 2004, Bergmann and others 2009). Spindle cell tumours which can have similar IHC reactivity are malignant PNSTs, rhabdomyosarcoma and other tumours of vascular origin (Chijiwa and others 2004, Bergmann and others 2009). In our case, typical histological findings excluded potential diagnosis of these tumours. Moreover, IHC reactivity to VIM, S-100 and col IV confirmed the diagnosis of benign PNST (Koestner and Higgins 2002, Chan and others 2007, Gaitero and others 2008, Schulman and others 2009).
Oestriadiol and cortisol concentrations were within the reference ranges of 8 ±3 ng/L (Carroll and Baum 1989) and 53 ±42 nmol/l (Rosenthal and Peterson 1996), respectively. However, the progesterone concentration was approximately twice the normal value (3-1 ±0-42 ng/mL) (Carlson and Rust 1969) and the testosterone concentration was more than 100 times lower than the normal value (9 to 73 nmol/l) (Schoemaker and others 2008).

As PNSTs are not hormone-secreting neoplasms, this raises some questions concerning the altered plasma concentrations of progesterone and testosterone. One explanation could be that progesterone is produced, in male animals, mainly by the adrenal glands and is then converted to testosterone in testes (Eckstein and others 1987, Maitra and Abbas 2005). Elevated progesterone and decreased testosterone levels could be the result of replacement of the testicular tissue of the right testis with neoplastic tissue and left testis atrophy. As a very small amount of functional testicular tissue remained, a very low conversion rate of progesterone to testosterone could be assumed. As a result, testosterone concentrations would drop and progesterone concentration be increased in plasma.

Considering that surgical castration of male ferrets is a common practice to prevent reproduction and to reduce interspecies aggression (Schoemaker and others 2008), it may be difficult to define the prevalence of testicular tumours in ferrets (Batista-Arteaga and others 2011). According to this, our finding of intratesticular benign PNST could contribute to a better understanding of the incidence and clinic significance of testicular neoplasm in ferrets.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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