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Subcutaneous neurofibroma as a cause of lameness in a warmblood horse: Neurofibroma in a horse

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A neurofibroma was excised from the subcutis on the medial side of the left thigh of a 15-year-old Warmblood gelding, which had shown lameness of the left hind limb. No other source of lameness was found. Two weeks after surgery, the horse was sound at a lameness examination.

Key words: *horse, lameness, neurofibroma, peripheral nerve sheath tumor*

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Neurofibromas (NFs) are benign peripheral nerve sheath tumors (PNSTs) as well as schwannomas, perineural cell tumors, and nerve sheath myxomas [8, 10, 11]. Schwann cells, perineural cells, and fibroblasts are the predominant components of neurofibromas [4, 5]. NFs are more common in human medicine than in veterinary medicine, although their histological features are very similar [9]. Different from human NFs, those in domestic animals mostly occur in subcutaneous tissue and the gastrointestinal tract [9], and this latter localization appears to be a cause of colic in horses [6, 7]. In contrast, a recent case report describes an NF localized in the sixth cervical nerve as a cause of ataxia in a Warmblood horse [2]. NFs have never been reported as a cause of lameness in horses; nevertheless, the following case describes the clinical, ultrasonographic, and histopathological features of an NF causing hind limb lameness in a horse.

A 15-year-old Warmblood gelding was referred to our facility for the excision of a mass in the inner side of its left thigh.

One month previously, the horse had been presented with grade 2/5 hind limb lameness according to the AAEP lameness grading scale (American Association of Equine Practitioners). At the time of the visit, the horse was being normally used for national jump competitions, and until then, no lameness had been shown by the horse.

At a lameness examination, all flexion tests of the limb were negative, and no improvement was seen after perineural nerve block of the entire left hind limb. Even a fetlock joint anesthetic block, tarsometatarsal joint anesthetic block, tibiotarsal joint anesthetic block, and stifle joint anesthetic block produced negative results. All diagnostic analgesia was performed using a local anesthetic (2% mepivacaine solution). The referring veterinarian prescribed seven days of Suxibuzone administered orally (4 mg/kg once a day).

The therapy did not result in any improvement of the lameness, so a second evaluation was carried out, which showed grade 2/5 lameness (AAEP lameness grading scale) as before. During the second lameness examination, an approximately 10 × 6 cm ellipsoidal mass was palpated in the leg on the medial side of the left stifle region, approximately 5 cm caudal to the patella and 5 cm proximal to the stifle joint. The horse showed discomfort and pain at palpation of the mass.

After this finding, the horse was referred to our facility. The evidence for grade 2/5 lameness was assessed, flexion tests and complete lameness examinations were repeated by two veterinary surgeons, and disappearance of the lameness was detected only after perilesional injection of a 2% lidocaine solution near the mass.

The mass appeared mobile and smooth at palpation and was not hot, but it was painful when pulled. Thus, it was decided to surgically remove the mass.

In an ultrasound examination, the mass appeared to be round in shape and capsulated, with its external wall being at a depth of about 2–3 mm from the skin and its capsule thickness being 3 mm. The wall of the mass was hyperechoic, and the content appeared anechoic. In a Doppler examination,

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no blood vessels or signs of new vascularization were found.

An elliptical incision was made in the skin on the distal side of the mass. The subcutaneous tissue was carefully dissected around the neoplasm and easily separated from it. After the mass was completely separated from the subcutaneous tissue, it was removed (Fig. 1). The subcutis was sutured with a 0-polyglycolic acid suture, passive Pen Rose drainage was then applied, and finally the skin was sutured with a 0-monofilament nylon suture; the surgical site was left open without the application of a bandage.

The texture of the excised mass was hard, and a screeching sound was produced when the mass was cut It was then subjected to histological examination.

On gross examination, the resected tumor was greyish in color and had a smooth, oval-shaped surface, well-defined borders, and a pale, fleshy, solid cut surface.

Several samples of the mass were fixed in 10% buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin (HE) and Masson's trichrome (for collagen). Immunohistochemical staining of selected sections was also performed with the panel of commercial antibodies shown in Table 1, using a modified avidin-biotin-peroxidase technique (LAB Vision). A positive control was present on each slide.

Histopathological examination revealed a well-demarcated, expansile, densely cellular neoplasm rimmed by compressed fibrous connective tissue, consisting of irregularly woven bundles of spindle cells immersed in a stroma with thick collagen bundles (more evident with Masson's trichrome staining). Inside the neoplasm, it was possible to observe an area composed of numerous tactile-like structures, which were composed of stacks of 5–10 spindle-shaped neoplastic cells surrounded by a perineurial cell capsule (Fig. 2). At the periphery, the presence of nerve bundles and subcutaneous adipose tissue was observed.

At higher magnification, the cells were elongated, 10–15 μm in diameter, and had indistinct borders and poor eosinophilic cytoplasm. They had wavy, tapering, central nuclei with coarse chromatin and an inapparent nucleolus. Anisocytosis and anisokaryosis were mild. Mitotic figures were present at less than one mitosis per ten 400× fields. The morphological diagnosis was suggestive of benign tumors of mesenchymal origin (like fibromas or soft tissue PNSTs).

Immunohistochemically, strong S-100 protein immunoreactivity was observed in the cytoplasm and nuclei of 80% of the spindle cells (Fig. 2). Isolated small nerve fibers were occasionally positive for neurofilament protein (NFP). All cellular components also showed diffuse cytoplasmic immunopositivity for vimentin, while desmin, glial fibrillary acidic protein (GFAP), and smooth muscle actin were negative. Immunohistochemical findings were coherent with a definitive diagnosis of benign tumor of neural origin

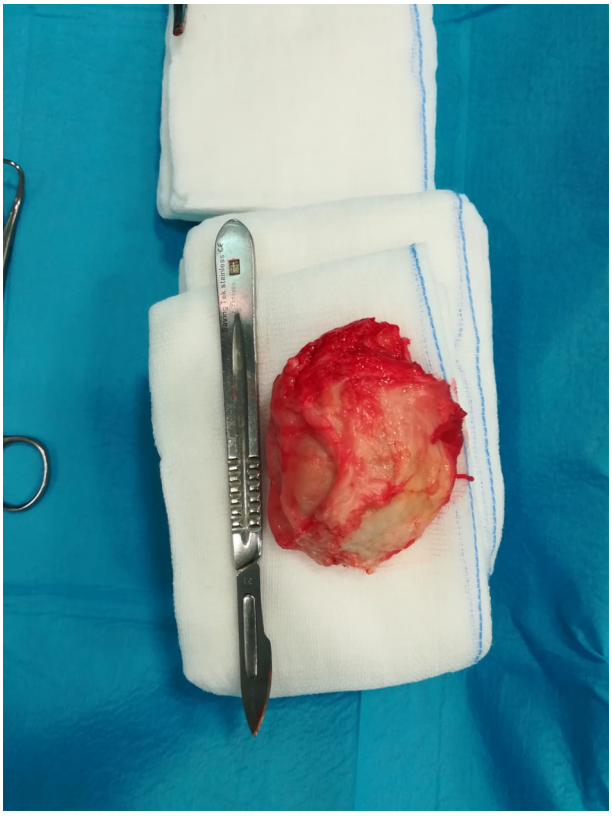

Fig. 1. Mass excised and taken for histopathological examination.

(PNST), with histopathological findings of localized collagenous neurofibroma.

Six days after surgery, the horse was discharged from the clinic. A follow-up examination was planned for ten days after discharge to remove the surgical suture and re-evaluate lameness, during which the horse appeared to be sound.

A six-month follow-up examination showed no signs of lameness relapse.

Neurofibromas are reported to be infrequent in veterinary medicine compared with human medicine. These tumors have been mostly documented in dogs and cattle and have been documented less frequently in horses and birds [1, 10]. With reference to horses, only six horses have been reported in the literature to have had one or more NFs [2, 3, 6, 7, 9].

The histological and immunohistochemical characteristics of our case were consistent with PNST, and the detected compresence of Schwann cells (S-100 positive), perineurial cells, small axonal structures (NFP positive), and fibroblasts was also coherent with a definitive diagnosis of NF. Indeed, the relatively loose arrangement of the spindle cells and presence of thick collagen fibrils and a subset of cells that failed to label with Schwann cell markers, as well as the presence of isolated nerve fibers observed multifocally

Table 1. Results and antibodies used for immunohistochemical characterization of the equine neurofibroma

Antibody specificity	Supplier	Dilution	Results
Vimentin*	Dako, Dakopatts, Glostrup, Denmark	1:40	+ (100%)
S-100±	Novocastra, Newcastle upon Tyne, U.K.	1:40	+ (80%)
Neurofilament protein (NFP)*	Dako	1:200	+ (occasional)
Glial fibrillary acidic protein (GFAP)*	Dako	1:8,000	−
Smooth muscle actin*	Dako	1:100	−
Desmin*	Dako	1:100	−

*Monoclonal antibody. ±, polyclonal antibody; +, positive; −, negative.

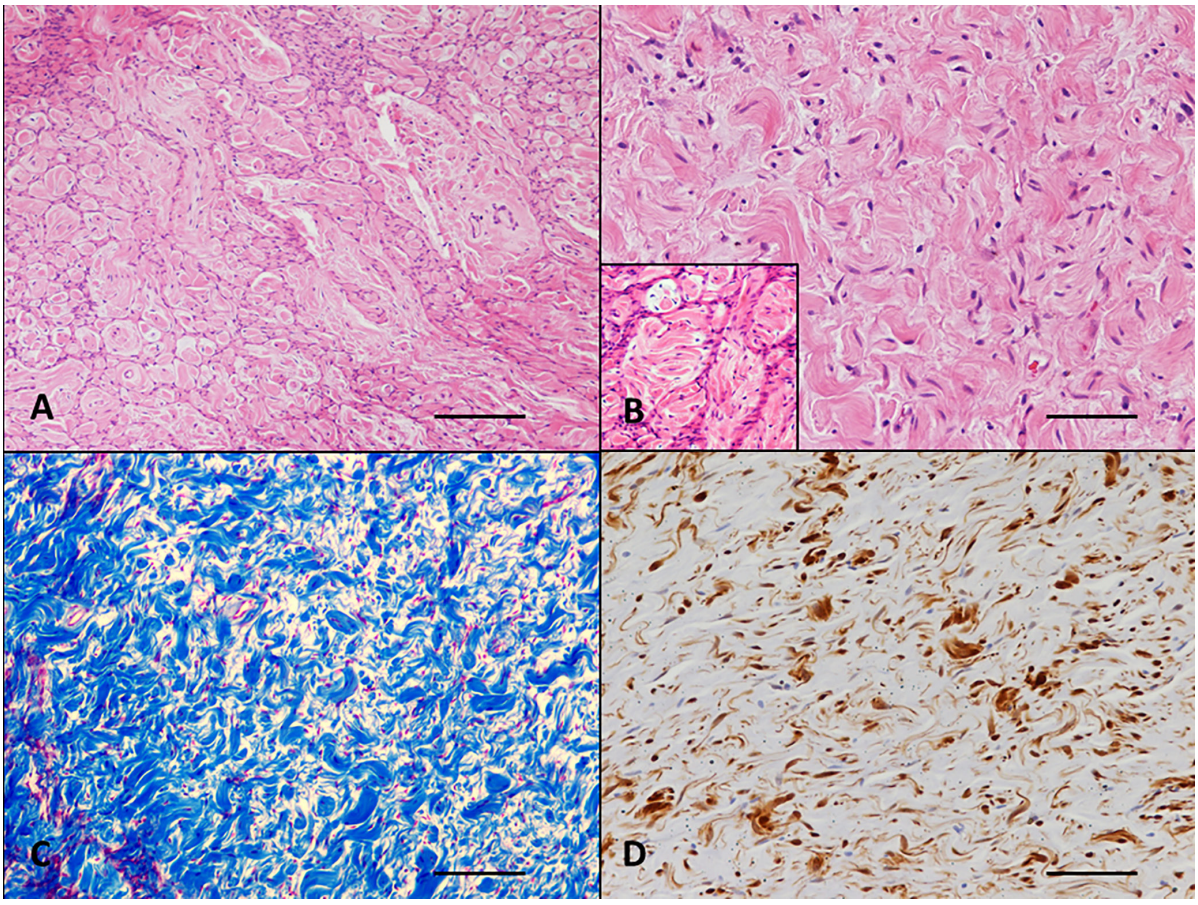

Fig. 2. Horse, collagenous neurofibroma. (A) The tumor is composed of streams and whorls of thin, wavy spindle cells with thick collagen bundles. Haematoxylin-eosin (HE). Bar, 500 μm. (B) The stroma contains thick collagen bundles. HE. Bar, 100 μm. Inset: tactile-like structures were also observed. HE. (C) Thick collagen bundles are more evident with Masson's Trichrome stain. Bar, 500 μm. (D) The results of immunohistochemistry show that S100 is expressed by a subpopulation of the spindle neoplastic cells. Bar, 500 μm.

between neoplastic cells and the absence of nuclear palisading and Verocay bodies and Antoni A and Antoni B areas, could differentiate this tumor from a schwannoma [5, 9].

According to Schöninger and Summers [9], neurofibromas in different animal species (the dog, horse, and chicken), like those described in humans, are distinguished by growth patterns and histological subtypes.

Regarding the growth pattern, given the presence of a thick fibrous capsule that circumscribed the neoplasm, our case seems to correspond to the localized type. As for the histological subtype, the collagenous pattern, a distinctive subtype of NF (reported in the horse in the subcutis of the left lateral neck) [9] characterized by the presence of abundant, thick bands of collagen tissue between spindle cells,

was also well recognizable in our case.

An area of the tumor also contained tactile-like structures that resembled Wagner-Meissner bodies, also called pseudomeissnerian corpuscles, commonly identified in human diffuse neurofibroma and previously reported in animals (one dog and one chicken) [9].

Based on the poor literature concerning NFs in horses, it may be supposed that no breed, sex, or age predisposition is present, but the majority of the horses affected by NFs described in the literature are Warmblood, as in the case in this report.

The NF has not been described to have a definite appearance; it can develop as a single uniform large mass or as multiple, well-circumscribed nodules.

The most frequent localization of NFs has been reported to be the subcutaneous tissue, although gastrointestinal and cervical nerve NFs have also been reported [2, 6, 9].

Gastrointestinal NFs are reported to involve both the large and small intestinal tracts. Normally, they are not a primary cause of colic but are an occasional finding during colic surgery or at the slaughterhouse. Only one report describes a case of a large NF affecting the small colon, obstructing the lumen, and causing an impaction, with subsequent colic symptoms [7].

When affecting the subcutaneous tissue, NFs have been reported to be localized in the axillary region and on the neck [9].

In this case, the particular localization of the mass probably caused a mass effect, with subsequent compression of some structures on the medial side of the stifle region, such as the medial saphenous nerve, the distal portion of the sartorius muscle, and distal portion of the gracilis muscle.

In the present report, an abnormal tissue localization was not described . Nevertheless, a neurofibroma was reported for the first time to be painful and to subsequently cause lameness, probably due to its particular localization in the medial part of the stifle joint.

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