Localized, Plexiform, Diffuse, and Other Variants of Neurofibroma in 12 Dogs, 2 Horses, and a Chicken

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Abstract. In humans, neurofibroma and schwannoma are distinct entities within the group of benign peripheral nerve sheath tumors. In the veterinary literature, these tumors are often classified together simply as benign peripheral nerve sheath tumors, and diagnostic criteria for their subclassification are not well established. We describe peripheral nerve sheath tumors with microscopic, immunohistologic, and ultrastructural features similar to those in subtypes of human neurofibroma in 12 dogs, 2 horses, and 1 chicken. Dogs were of different breeds and were aged 2 months to 15 years. The canine tumors were located in the skin, peripheral nerve, tongue, and large intestine. The 2 horses were 11 and 12 years old. The equine tumors were located in the subcutis of the neck and axilla. The chicken was a mature white Leghorn chicken with an ocular neoplasm. Neurofibromas of this study had localized, plexiform, diffuse and combined plexiform and diffuse growth patterns, and microscopic features similar to those in classic, collagenous, cellular, myxoid, and pigmented neurofibromas of humans. One diffuse neurofibroma contained areas of schwannian differentiation (hybrid neurofibroma-schwannoma). Two plexiform neurofibromas occurred together with diffuse ganglioneuromatosis in the large intestine of young dogs, as has also been reported in humans. This investigation shows the existence of identical subtypes of neurofibroma in animals and humans and identifies similarities in tumor location and patient age between animals and humans. This report will allow a more discriminating classification of benign peripheral nerve sheath tumors and probably has a bearing on epidemiology, pathogenesis and prognosis.

Key words: Chickens; diffuse neurofibroma; dogs; horses; localized neurofibroma; plexiform neurofibroma; neurofibroma variants.

Benign peripheral nerve sheath tumors (PNST) in humans include schwannomas (neurilemmomas) and neurofibromas, as well as perineurial cell tumors, nerve sheath myxomas (neurothekeomas), and a few other, quite rare, entities. Schwannomas and neurofibromas, which are the most common benign PNSTs, are distinguished by certain microscopic features, supported by immunohistochemistry and electron microscopy. Important features of schwannomas include the concurrent presence of highly and poorly cellular areas of fusiform neoplastic Schwann cells in a stroma that is either collagenous and scant, or is myxoid and abundant (designated Antoni A areas and Antoni B areas, respectively). Other features are nuclear palisading, the formation of Verocay bodies, and hyalinized microvessels. Nerve fibers are absent within the tumor but are often present at the tumor margin, and immunoreactivity for S100 protein, a Schwann cell marker, is strong and diffuse. Although schwannomas are entirely formed of neoplastic Schwann cells, neurofibromas are a mixture of Schwann cells, perineurial cells, and fibroblasts. Evidence indicates that the Schwann cell is the primary neoplastic cell of neurofibroma. In contrast to the microscopic features of schwannoma, neurofibromas are composed of very slender, elongated cells with characteristic buckled and/or wavy nuclei in a fibromyxoid stroma with thin, wire-like collagen fibers. Within the mass, nerve fibers can be identified; S100 immunoreactivity is restricted to a subpopulation of the tumor cells, because this marker only labels the neoplastic Schwann cells but not the remaining tumor cells. Schwannomas and neurofibromas are further subclassified according to their growth patterns (schwannoma: localized or plexiform; neurofibroma: localized, plexiform, or diffuse) and histo-
pathologic features, e.g., classic, collagenous, cellular, and pigmented. A localized schwannoma or neurofibroma is a single, well-demarcated, expansile tumor, whereas a plexiform variant is composed of multiple nodular masses. Diffuse neurofibroma has infiltrative growth, but other features of malignancy are absent.

In humans, the recognition of tumors as schwannoma and neurofibroma and their variants is important for patient management and prognosis. In animals, PNSTs are most often reported in dogs and cattle, infrequently in cats and horses, and rarely in other species, such as goats, pigs, and birds. Spontaneous PNSTs were also described in laboratory animals. The classification of PNSTs in the veterinary literature is inconsistent and confusing. For example, adult cattle often develop multiple PNSTs, with a predilection for the autonomic nervous system. This condition has been referred to as neurofibromatosis, perhaps because the masses can be multiple, although the tumors are microscopically similar to human schwannomas. Certain investigators differentiate 3 histologic patterns of schwannoma: neurofibroma, neurilemmoma, and plexiform.

Case reports in animals often use the generic designation “nerve sheath tumor.” In recent years, authors of some veterinary texts have advocated the term benign PNST instead of schwannoma or neurofibroma, because i) distinct histologic criteria for the classification of benign PNSTs as schwannoma or neurofibroma have not been established in the veterinary literature, ii) it seems that all benign PNSTs have similar clinical behavior, iii) the existence of true neurofibromas in domestic animals is questionable, and iv) diseases similar to human neurofibromatosis 1 (NF1, von Recklinghausen’s disease) and neurofibromatosis 2 (NF2) have not been recognized in domestic animals. Patients with NF1 develop multiple neurofibromas, which often undergo malignant transformation and additional neoplastic and non-neoplastic lesions in multiple organs. In contrast, NF2 is characterized by bilateral vestibular schwannoma and possible additional tumors of the central nervous system and/or cutaneous schwannomas.

This report is a study of spontaneous peripheral nerve sheath tumors that illustrates the existence of neurofibroma and several subtypes in dogs, horses, and a chicken like those tumors that occur in humans. Furthermore, this study showed that similarities between neurofibroma in animals and humans are not restricted to microscopic features but also exist in regard to tumor location and patient age.

Materials and Methods

Clinical cases

All cases in this study were collected between 1979 and 2005 (Table 1). They include excisional biopsy specimens submitted as formalin-fixed specimens to the Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, NY, USA (case Nos. 1–3, 5–7, and 10–14) and cases sent to one investigator (BAS) for consultation (case Nos. 4, 8, 9, and 15). Nos. 7, 11, and 13 were reviewed by C. Fletcher at Harvard Medical School.

Histopathology and immunohistochemistry

Submitted formalin-fixed biopsy specimens (case Nos. 1–3, 5–7, and 10–14) were processed routinely, embedded in paraffin, sectioned, and mounted on glass slides. HE stained sections were examined for all cases. Case Nos. 2, 5, 6, and 10–14 were also stained with a Masson trichrome stain for the identification of collagen. A Fontana-Masson stain was applied to case No. 7 for the identification of melanin pigment.

Immunohistochemistry was performed in the Autostainer Plus (Dakocytomation, Carpenteria, CA, USA) by the streptavidin-biotin immunoperoxidase technique, with 3,3′-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. Tumors were immunostained for S100 (Dakocytomation, no pretreatment) for the detection of neoplastic Schwann cells (case Nos. 1–3, 5–7, 10–15) and neurofilament (Dakocytomation, no pretreatment) for the verification of intratumoral nerve fibers (case Nos. 1–3, 5–7, 10–14). In negative controls, the primary antibody was replaced by nonimmune serum. Appropriate positive controls were used.

Electron microscopy

Electron microscopy was performed on one canine plexiform neurofibroma (case No. 6). Formalin-fixed tissue was further fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate, washed 3 times in cacodylate buffer, and then postfixed in 2% osmium tetroxide. After further washing, the tissue was progressively dehydrated in graded ethanol solutions, incubated in 100% acetone, and embedded in epon araldite plastic. The area of interest was selected from 1-μm-thick sections, stained with toluidine blue. Ultrathin sections were contrasted with lead citrate and uranyl acetate, and were examined by using a Philips 300 transmission electron microscope (Philips, New York, NY).

Results

Signalment, clinical history, and diagnosis

Signalment, clinical history, and diagnoses are provided in Table 1. Neurofibromas were identified in 12 dogs, 2 horses, and 1 chicken. Seven dogs were purebred (7 different breeds); one was mixed breed; in 4, the breed was not specified. Ages ranged from 2 months to 15 years. The dogs comprised 5 females, 5 males, and 2 of unknown
sex. The canine neurofibromas were located in the skin (dermis and/or subcutis, \( n = 6 \)), peripheral nerve (1), tongue (3), and large intestine (2). One horse was an 11-year-old male Warmblood; the other was a 12-year-old male Morgan. One equine tumor was in the subcutis of the neck; the others (both in 1 horse) were in the axillary subcutis. The chicken was an adult White Leghorn chicken of unknown sex with ocular and periocular neurofibroma.

### Table 1. Signalment, clinical history, and variants of neurofibroma in 12 dogs, 2 horses, and a bird.*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Animal</th>
<th>Clinical History</th>
<th>Diagnosis</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Canine, fs, 4–7 y</td>
<td>Firm subcutaneous mass at the dorsal nasal area: 12.7 cm</td>
<td>Mixed plexiform–diffuse classic NF</td>
<td>S100 + (80%), neurofilament +</td>
</tr>
<tr>
<td>2</td>
<td>Canine, f, 11 y</td>
<td>Mass on ventral aspect of tongue</td>
<td>Diffuse classic NF</td>
<td>S100 + (80%), neurofilament +</td>
</tr>
<tr>
<td>3</td>
<td>Canine, Great Dane, m, 2 y</td>
<td>Large cystic mass in dermis and subcutis on left side of the neck</td>
<td>Diffuse classic NF with tactile-like structures</td>
<td>S100 + (50%), neurofilament +</td>
</tr>
<tr>
<td>4</td>
<td>Canine, Lhasa Apso, f, 14 mo</td>
<td>Tenesmus, prolapsed rectum at 4 mo of age, 2 cm mass in rectum</td>
<td>Plexiform classic NF with diffuse ganglioneuromatosis</td>
<td>S100 NA, neurofilament NA</td>
</tr>
<tr>
<td>5</td>
<td>Canine, Dalmatian, m, 8 y</td>
<td>Firm mobile multinodular subcutaneous mass at left lower periorbital area: 2.5 × 0.7 cm</td>
<td>Plexiform classic NF</td>
<td>S100 (30%), neurofilament +</td>
</tr>
<tr>
<td>6</td>
<td>Canine, West Highland White Terrier, m, 15 y</td>
<td>Subcutaneous mass in left flank region: 1.5 × 0.75 cm</td>
<td>Plexiform classic NF</td>
<td>S100 (30%), neurofilament +</td>
</tr>
<tr>
<td>7</td>
<td>Canine, Schnauzer, f, 4 y</td>
<td>Subcutaneous whitish mass at left hip: 2 cm in diameter</td>
<td>Diffuse pigmented NF</td>
<td>S100 (70%), neurofilament +</td>
</tr>
<tr>
<td>8</td>
<td>Canine, 7 mo</td>
<td>Chronic vomiting and constipation, mass in colon</td>
<td>Plexiform myxoid NF with diffuse ganglioneuromatosis</td>
<td>S100 NA, NF NA</td>
</tr>
<tr>
<td>9</td>
<td>Canine</td>
<td>Ulnar nerve</td>
<td>Plexiform classic NF</td>
<td>S100 NA, NF NA</td>
</tr>
<tr>
<td>10</td>
<td>Canine, Golden Retriever, m, 11 y</td>
<td>Firm mobile subcutaneous mass over right zygomatic arch: 2.5 × 2 cm</td>
<td>Plexiform classic NF</td>
<td>S100 (60%), neurofilament +</td>
</tr>
<tr>
<td>11</td>
<td>Canine, Scottish Terrier, m, 6 y</td>
<td>Firm lobulated mass on ventral aspect of the tongue: 3 × 1.5 cm</td>
<td>Plexiform classic NF</td>
<td>S100 (90%), neurofilament +</td>
</tr>
<tr>
<td>12</td>
<td>Canine, mixed-breed, f, 4 y</td>
<td>Mass in tongue, recurrence 10 mo after surgical removal</td>
<td>Hybrid diffuse classic NF/schwannoma</td>
<td>S100 (70% in NF; 100% in schwannoma), neurofilament −</td>
</tr>
<tr>
<td>13</td>
<td>Equine, Warmblood, m, 11 y</td>
<td>Two nodular masses in subcutis in the axillary region; each nodule 1.5 cm in diameter</td>
<td>Localized cellular NF</td>
<td>S100 (20%), neurofilament +</td>
</tr>
<tr>
<td>14</td>
<td>Equine, Morgan horse, m, 12 y</td>
<td>Mass in subcutis of the left lateral neck: 12 × 12 cm</td>
<td>Diffuse collagenous NF</td>
<td>S100 (80%), neurofilament −</td>
</tr>
<tr>
<td>15</td>
<td>Chicken, White Leghorn, adult</td>
<td>Enlarged globe with opaque cornea</td>
<td>Diffuse classic NF with tactile-like structures</td>
<td>S100 (60%), neurofilament NA</td>
</tr>
</tbody>
</table>

* fs = female spayed; NF = neurofibroma; (n%) = percentage of immunopositive neoplastic cells; f = female; m = male; NA = stain not available.
Tumor size was indicated for 9 cases; the largest was 12.7 cm long, but most measured less than 3 cm. Interestingly, the tumors with the largest reported size were a canine combined diffuse and plexiform neurofibroma (case No. 1; 12.7 cm in length) and an equine diffuse neurofibroma (case No. 14; 12 × 12 cm). For the remaining 6 cases, no measurements were provided, including one described as “large and cystic.” Tumors of the subcutis and in the tongue were said to be firm; some were reportedly mobile on palpation. Two tumors that proved to be plexiform neurofibromas were described as multinodular. (Nodularity is a hallmark of plexiform tumors.) Local recurrence was reported only for case No. 12, a canine lingual diffuse neurofibroma with areas of schwannian differentiation.

Pathology

Like human neurofibromas, the canine, equine, and avian tumors could be classified according to growth pattern and microscopic subtypes. As for human tumors, we could identify 3 growth patterns: localized, plexiform, and diffuse; one tumor had combined plexiform and diffuse growth patterns. The microscopic subtypes were classic, cellular, collagenous, myxoid, and pigmented neurofibroma. One diffuse neurofibroma contained areas of schwannian differentiation (hybrid neurofibroma/schwannoma). Each of the 3 growth patterns had variable microscopic patterns. Hence, a diffuse tumor might be classic, pigmented, or collagenous (Table 1). 

A. Growth patterns of the canine, equine, and avian neurofibromas. The two localized neurofibromas (No. 13) were in the axillary region of an 11-year-old male Warmblood horse; both tumors were cellular neurofibromas and each formed a well-demarcated, expansile mass, which was rimmed by compressed fibrous connective tissue (Fig. 1). All plexiform neurofibromas were in dogs and located in the subcutis (Nos. 5, 6, and 10), the ulnar nerve (No. 9), tongue (No. 11), and the large intestine (Nos. 4 and 8). They were composed of well-demarcated multinodular tumor masses with centrally located nerve fiber bundles surrounded by neoplastic nerve sheath tissue (Figs. 2, 3). The concentrically proliferating cells expanded the subperineural space and somewhat disrupted the central nerve fascicles. In the large intestine, these nodular masses involved the deeper submucosa, tunica muscularis, and serosa, and, in dog No. 8, the mesentery. Interestingly, both intestinal plexiform neurofibromas (Nos. 4 and 8) occurred with diffuse ganglioneuromatosis in the wall of the colon in young dogs (Fig. 3). Diffuse ganglioneuromatosis was characterized by expansion of the submucosa and tunica muscularis by the neoplastic proliferation of ganglion cells and Schwann cells (Figs. 4, 5). In case No. 4, the plexiform neurofibroma and the ganglioneuromatosis formed distinct masses (Fig. 4), whereas in No. 8, some nodular tumor masses in the mesentery contained a mixture of neoplastic nerve sheath tissue and neoplastic ganglion cells. Diffuse neurofibroma was diagnosed in 3 dogs, 1 horse, and the chicken. The canine tumors were in the skin (Nos. 3 and 7) and tongue (No. 2). The equine tumor occurred in the subcutis (No. 14). The avian neoplasm was intra- and periocular (No. 15). Diffuse neurofibromas were poorly demarcated, infiltrative neoplasms that invaded the cutaneous trunci muscle (Nos. 3, 7, and 14) and the intrinsic tongue muscle (No. 2; Fig. 5). In the globe of the chicken (No. 15), neoplastic cells diffusely expanded the uvea and the sclera, and infiltrated the periocular soft tissue, with the formation of a tumor mass lateral and posterior to the globe. The presence of the tumor within the sclera was associated with osteolysis, fragmentation, and remodeling of scleral ossicles. Two diffuse neurofibromas (Nos. 3 and 15) contained tactile-like structures (sudomotorian corpuscles), which were composed of stacks of 5–10 spindle-shaped neoplastic cells surrounded by a perineurial cell capsule (Figs. 6, 7). The plexiform and diffuse neurofibroma (No. 1) was in the subcutis of a dog. It was composed of plexiform neurofibroma surrounded by neurofibromatous tissue, which infiltrated the cutaneous trunci muscle.

B. Microscopic subtypes of the canine, equine, and avian neurofibromas. All tumors (Nos. 1–15) had microscopic features diagnostic for human neurofibroma. They were composed of elongated neoplastic cells with indistinct cell borders. Each neoplastic cell had a single hyperchromatic wavy, buckled, or tapering nucleus, and a small-to-moderate amount of eosinophilic cytoplasm. Neoplastic cells had only mild anisocytosis, anisokaryosis, and cellular pleomorphism. The mitotic rate was very low, with less than 1 mitotic figure per ten 40× fields. Tumors had a variable cellular density and a variable amount of collagenous myxomatous stroma (Figs. 8–11). The classic neurofibromas (Nos. 1–6, 9–12, and 15) had a moderate cellular density and fibromyxoid stroma with ropy collagen fibers (Fig. 8). Mild multifocal lymphocytic infiltration was observed in tumor Nos. 1 and 2. In contrast to the classic pattern, a collagenous
Fig. 1. Subcutis; horse No. 13. Localized neurofibroma. This well-demarcated expansile tumor is rimmed by compressed fibrous connective tissue. HE.

Fig. 2. Subcutis; dog No. 6. Plexiform neurofibroma. This variant is composed of multiple distinct nodular masses. Each mass contains a centrally located nerve (asterisk) surrounded by neoplastic nerve sheath tissue. Inset: Subgross image of the plexiform neurofibroma. The nodule indicated by the arrow is depicted in the main figure. HE.

Fig. 3. Colon; dog No. 4. Plexiform neurofibroma. In addition to plexiform neurofibroma (arrows), diffuse ganglioneuromatosis (asterisks) is apparent. HE.

Fig. 4. Colon; dog No. 4. Diffuse ganglioneuromatosis (enlargement of Fig. 3) is characterized by the neoplastic proliferation of ganglion cells within a schwannian background. HE.
neurofibroma (No. 14) was identified by abundant, thick collagen bundles in a mildly myxomatous matrix (Fig. 9), whereas cellular neurofibromas (No. 13) contained areas of increased cell density (Fig. 10) that could potentially be confused with a malignant neoplasm. Myxoid neurofibroma was seen in one intestinal tumor (No. 8, Fig. 11), with abundant myxoid matrix. Melanin pigment within a variable proportion of the proliferating cells is the hallmark of pigmented neurofibroma. This subtype was seen in one neurofibroma with a diffuse growth pattern (No. 7). Approximately 10% of the neoplastic cells contained cytoplasmic melanin pigment (Fig. 12). The presence of melanin pigment was confirmed by a Fontana-Masson stain (Fig. 12, inset). The tumor had mild multifocal lymphocytic infiltration. Finally, one classic neurofibroma (No. 12) contained areas of schwannian differentiation (designated in humans as hybrid neurofibroma/schwannoma). As in humans, this case was largely neurofibromatous, with multifocal areas of schwannian differentiation that resembled Antoni A areas. In these cell-rich areas, neoplastic spindle-shaped neoplastic cells were arranged in parallel bundles, with the formation of nuclear palisades and Verocay bodies (Fig. 13).

**Immunohistochemistry**

In all neurofibroma subtypes, immunoreactivity for the Schwann cell marker S100 (Nos. 1–3, 5–7, and 10–14) was positive in a subpopulation of the neoplastic cells, which ranged from 30 to 80% in the examined tumors. The immunosignal ranged from weak to moderate (Fig. 14) to intense (Fig. 15). In the diffuse neurofibromas (Nos. 2, 3, and 7), the neoplastic cells forming the tactile-like corpuscles had strong S100 expression, whereas the perineurial capsule was S100-immunonegative (Fig. 15). In the hybrid neurofibroma/schwannoma (No. 12), the tumor cells in the areas of schwannian differentiation were strongly immunopositive for S100 (Fig. 16), with a mosaic of S100-positive and negative cells in the neurofibromatous areas. The S100 immunoreactivity was mainly intracytoplasmic and, in some neoplastic cells, also intranuclear (Figs. 14–16).

**Electron microscopy**

Ultrastructural examination, which was performed on one canine plexiform neurofibroma (No. 6), confirmed the presence of intratumoral Schwann cells and perineurial cells. Neoplastic Schwann cells had irregular, undulating nuclear contours and were identified by their continuous basal lamina and branched cytoplasmic processes (Fig. 18). Perineurial cells were elongated with bipolar cytoplasmic processes, a discontinuous basal lamina and numerous pinocytotic vesicles in the subplasmalemmal cytoplasm. They were arranged in alternating layers with extracellular collagen (Figs. 19, 20).

**Discussion**

In humans, neurofibroma and schwannoma are well-characterized, distinct entities within the category of benign PNSTs. However, in veterinary medicine, the generic diagnosis of benign PNST is often used because clear diagnostic criteria for the distinction between neurofibroma and schwannoma in animals have not been established, and all benign PNSTs are believed to have a similar prognosis. In this study, we asked whether tumors that would satisfy the criteria of neurofibroma in humans occur in animals? The cases reported herein are offered to answer this question. Our findings show the existence of the same growth patterns and several of the microscopic variants of human neurofibroma in selected animal cases.

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**Fig. 5.** Haired skin; dog No. 3. Diffuse neurofibroma. The tumor infiltrates the cutaneous trunci muscle (asterisks). Inset: Subgross image of the diffuse neurofibroma, which forms a widely infiltrative tumor mass within the dermis and subcutis. HE.

**Fig. 6.** Dog No. 3. Diffuse neurofibroma. The tumor contains numerous tactile-like structures (asterisks). HE.

**Fig. 7.** Globe; chicken No. 15. Diffuse neurofibroma. This tumor has numerous tactile-like structures (asterisks). HE.
Fig. 8. Mass in tongue; dog No. 12. Classic neurofibroma. The elongated delicate neoplastic cells have wavy, buckled, or tapering nuclei and stroma with ropy collagen fibers intimately associated with the neoplastic cells. HE. Inset: Masson’s trichrome stain.

Fig. 9. Subcutis; horse No. 13. Collagenous neurofibroma. The tumor stroma contains thick collagen bundles (asterisks). HE. Inset: Masson’s trichrome stain.

Fig. 10. Subcutis; horse No. 14. Cellular neurofibroma. The neoplastic cells form dense bundles separated by scanty collagenous stroma. Notice bland nuclei lacking atypia. HE. Inset: Masson’s trichrome stain.

Fig. 11. Colon; dog No. 8. Myxoid neurofibroma. The tumor has low cellularity. Neoplastic cells are embedded in abundant myxoid matrix (asterisks). HE.

Fig. 12. Subcutis; dog No. 7. Pigmented neurofibroma. HE. About 10% of the neoplastic cells contain dark-brown cytoplasmic pigment (arrows), identified as melanin by the Fontana-Masson stain (inset).

Fig. 13. Tongue; dog No. 12. Schwannian differentiation. The diffuse neurofibroma contains multifocal areas of schwannian differentiation (asterisks). HE.
These have not been previously recorded in veterinary literature, because we found no discussion (for example) of diffuse, plexiform, cellular, or collagenous neurofibroma in standard textbooks of veterinary pathology.

The tumors in this study were diagnosed as neurofibroma based on their morphologic features diagnostic for human neurofibroma, i.e., growth pattern (localized, plexiform, and diffuse) and microscopic features (classic, cellular, collagenous, and pigmented), together with the presence of intratumoral nerve fibers and the restriction of the S100 immunostaining to a subpopulation of the neoplastic cells.22,29,42 The diagnosis was further supported by the electron microscopic examination of one canine plexiform neurofibroma, which confirmed the presence of Schwann cells and perineurial cells. To our knowledge, the ultrastructural confirmation of the presence of different cell populations in neurofibromas of animals has not been reported previously.

Among these 15 cases, even examples of complex neurofibroma patterns described in humans, such as a plexiform neurofibroma embedded in a background of diffuse neurofibromatous tissue (No. 1)42 and hybrid neurofibroma/schwannoma were encountered (No. 12). Several diffuse neurofibromas of this study contained tactile-like structures that resembled Wagner-Meissner bodies.33 These structures, sometimes called pseudomeissnerian corpuscles,33 are commonly identified in human diffuse neurofibroma.22,29 In benign PNSTs of animals, comparable tactile-like structures seem to be almost unknown. A schwanna was reported in the cecum of a cow that contained Wagner-Meissner bodies.39 Hyperplasia of Herbst corpuscles was noted in avian neurofibromas.23

A rigorous comparison of the 15 cases we describe here with many previously reported benign PNSTs in animals was problematic because of case definition. Distinct criteria for the classification of benign PNSTs as schwannomas and neurofibromas have not been agreed upon in the veterinary literature.18 Furthermore, some investigators have recognized that veterinary tumors reported as benign PNSTs, schwannomas, or neurofibromas might even represent other types of spindle cell tumors with a similar microscopic appearance.11 Most common in the dog, such cases have patterns that might suggest schwanna (more than neurofibroma) and for which hemangiopericytoma is commonly included in the differential diagnosis.11 Within benign PNSTs in animals, few subtypes have been identified. One study of 17 cases of benign and malignant PNSTs in dogs identified plexiform-multilobular nodular growth in two canine benign PNSTs.4 The existence of canine benign PNSTs with a plexiform architecture was mentioned in a veterinary textbook.11 Benign myxoid PNSTs were recognized as a distinct subtype of canine PNST.11 This tumor has only been observed in the digits of dogs and has microscopic features similar to those of human neurothekeoma.11 The myxoid benign PNST differs from the myxoid plexiform neurofibroma observed in this study because of the lack of a plexiform growth pattern and the absence of centrally located nerve fiber bundles surrounded by neoplastic nerve sheath tissue. In the human literature, myxoid schwannoma and myxoid neurofibroma can be distinguished by the S100 immunoreaction of the neoplastic cells, i.e., uniform in schwannoma and patchy in neurofibroma, and the absence (schwanna) or the presence (neurofibroma) of intratumoral nerve fibers.22,29,42

In the human medical literature, neurofibromas that contain melanin-laden neoplastic cells are classified as pigmented neurofibromas,22,29,42 but occasionally the term melanotic neurofibroma is used as a synonym.4 Pigmented neurofibromas may be infiltrative (diffuse pigmented neurofibroma) but do not metastasize.42 In contrast, schwannomas that contain neoplastic cells with melanin pigment are classified as melanotic schwannomas. Compared with classic schwannoma, melanotic schwannomas may metastasize and often develop in patients with Carney syndrome (myxomas, endocrine disorders, and abnormalities in pigmentation).35,42

Although pigmented neurofibromas have not been previously described in dogs, a subcutaneous melanotic neurofibroma was reported in a steer.15 Most of the benign neurofibromas in this study affected middle-aged to older dogs, but 3 were diagnosed in young dogs: a diffuse subcutaneous neurofibroma in a 2-month-old dog and intestinal plexiform neurofibroma in 2 dogs aged 7 and 14 months, respectively. Although benign PNSTs have not been previously described in young dogs, they have rarely been reported in young animals of other species: a plexiform schwanna in the subcutis of a 6-month-old pig40 and subcutaneous neurofibromas in 9-week-old chickens infected with subgroup A avian leucosis-sarcomas virus.23 Reported locations of canine benign PNSTs are commonly the skin,8,11,36 although this is the controversial group in which the diagnosis is open to debate, and the spinal and cranial nerves.19,36 To our knowledge, canine lingual and intestinal benign PNSTs have not been reported previously.
Fig. 14. Dog No. 12. Diffuse neurofibroma. S100 is expressed by a subpopulation of the neoplastic cells; arrows indicate some negative cells. *Inset:* A few S100-immunopositive neoplastic cells are depicted at higher magnification. DAB was used as chromogen.

Fig. 15. Dog No. 3. Diffuse neurofibroma. The fusiform cells that form the tactile-like structures have strong and uniform S100 expression (asterisks), whereas the perineurial capsule is S100-immunonegative (arrows). DAB was used as chromogen.

Fig. 16. Dog No. 12 (see Fig. 13). Schwannian differentiation. The neoplastic cells in areas of schwannian differentiation express S100 strongly and uniformly (asterisks). DAB was used as chromogen.
Benign PNSTs were described in horses 2 to 16 years of age. The 3 equine tumors were diagnosed in horses aged 11 and 12 years. Reported locations of equine benign PNSTs are the skin, especially the palpebral subcutis, and the gastric and intestinal walls. The 3 tumors of this study were in the subcutis of the neck and the axillary region (2 tumors in 1 horse). A case report describes the presence of multiple schwannomas and neurofibromas in the small intestine of a horse. The intestinal tumors were separated into neurofibroma and schwannoma based on their S100 immunoreactivity (patchy in neurofibroma and uniform in schwannoma).

Rare cases of neurofibroma were reported in chickens. The tumor in the chicken of this study was briefly mentioned in a review of avian ophthalmology. In humans, neurofibroma can occur as a sporadic tumor or as part of the inherited disease NF1, also known as von Recklinghausen’s disease. Localized neurofibromas are usually solitary tumors in individuals without the inherited disease, whereas diffuse and plexiform neurofibromas are frequently associated with NF1. NF1 is caused by germline mutation in the NF1 tumor suppressor gene, and the clinical presentation of NF1 is heterogenous. Patients usually develop multiple neurofibromas and often have gliomas of the optic nerve, pigmented nodules of the iris (Lisch nodules), and cutaneous hyperpigmented macules (cafe au lait spots). In patients with NF1, malignant transformation of neurofibromas is not uncommon. We assume that the neurofibromas that we report here were sporadic, and we are not aware of cases in animals with lesions in multiple organs that would qualify as NF1. Although the term neurofibromatosis was used for affected cattle, the spectrum seen in the human diseases was lacking. In domestic animals, no association between neurofibromas and an underlying genetic disease and/or signature maturation has been established. Trisomy D2 had been detected in a single feline neurofibroma. Cytogenetic studies on a solitary canine neurofibroma revealed trisomy 2, a derivative chromosome 13, and centric fusion 10/35 and 24/31.

In addition to overlapping microscopic features, the canine tumors of this study were similar to human neurofibroma in other ways. Such other similarities include involvement of the tongue (seen in 3 canine cases), the presence of neurofibroma in early life (2 canine juvenile neurofibromas), and concurrent intestinal neurofibroma and ganglioneuroma–diffuse ganglioneuromatosis (2 canine cases). Human neurofibromas that occur in early life (childhood or adolescence) are usually associated with NF1, whereas most sporadic neurofibromas are observed during early adulthood. Although the concurrent presence of intestinal neurofibroma and ganglioneuroma–diffuse ganglioneuromatosis has been most frequently reported in patients with NF1, it was also observed in patients with multiple endocrine neoplasia type IIb and can occur as a rare spontaneous event. In humans, the recognition of the different growth types of neurofibromas has prognostic significance: localized neurofibromas have an excellent prognosis, because they are well-demarcated tumors and excision is usually curative. Plexiform neurofibromas are usually well demarcated, but their surgical removal can be associated with nerve deficits because of the entrapped intratumoral nerve fiber bundles. Because of their infiltrative nature, diffuse neurofibromas may recur, but metastasis has not been reported. It is likely that a similar prognosis can also be applied to the localized, plexiform, and diffuse neurofibromas of this study. This is supported by the fact that recurrence had been reported for the canine hybrid diffuse neurofibroma/schwannoma. An important entity in the differential diagnosis for localized human neurofibroma is benign neurotized melanocytic nevus; with the assistance of C. Fletcher, we identified a few similar cases in horses (Summers, unpublished). Furthermore, localized neurofibroma must be distinguished from traumatic neuroma.

**Fig. 17.** Dog No. 6. Plexiform neurofibroma. The immunostaining for neurofilament confirms the presence of a centrally located nerve (asterisks) concentrically surrounded by neoplastic nerve sheath tissue.

**Fig. 18.** Dog No. 6. Plexiform neurofibroma. Neoplastic Schwann cells are identified by their continuous basal lamina (arrows) and delicate branching cytoplasmic processes (arrowheads). Electron microscopy.

**Fig. 19.** Dog No. 6. Plexiform neurofibroma. Elongated processes of perineurial cells (asterisks) are arranged in alternating layers with extracellular collagen (arrowheads). The area indicated by the box is depicted at higher magnification in **Fig. 20**.

**Fig. 20.** Dog No. 6. Plexiform neurofibroma. Perineurial cells are distinguished by discontinuous basal lamina (arrows) and numerous subplasmalemmal pinocytotic vesicles (arrowheads). Inset: A few subplasmalemmal pinocytotic vesicles are depicted at higher magnification (arrowheads). Electron microscopy.
a proliferative non-neoplastic lesion that develops secondary to a transecting nerve lesion, with poor apposition or loss of the distal nerve fragment. On microscopic examination, traumatic neuroma is composed of numerous small haphazardly arranged clusters of Schwann cells, some perineurial cells, and axonal sprouts.\textsuperscript{11,29,42}

Diffuse neurofibroma must be distinguished from malignant PNST. In addition to the infiltrative growth, malignant PNSTs have increased cellularity, cellular pleomorphism, an increased mitotic rate, areas of necrosis, and/or evidence for metastasis.\textsuperscript{11,19,42,43} The diffuse neurofibromas of this study were infiltrative but lacked other features of malignancy. Cellular neurofibromas are identified by the absence of elevated mitotic activity and nuclear atypia.

In conclusion, this study showed an increased complexity of PNSTs in animals. Neurofibroma and the varieties we illustrated appear to be uncommon and certainly are encountered with a much lower frequency than malignant PNST in the dog (Summers, personal observation), presenting in spinal or the cranial nerves (trigeminal). The true incidence of neurofibroma in animals is unknown; we hope this will become clearer. Further studies are needed to establish whether neurofibromas in domestic animals, similar to those in humans, have common underlying genetic alterations and if some neurofibroma types might be at risk to undergo malignant transformation. Because three of these tumors were observed in young dogs, the possibility of a germline mutation predisposing to the development of neurofibromas in dogs should be explored.

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**References**


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