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PATHOLOGY

Spontaneous orbitofacial neurofibroma in a sow

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ABSTRACT: Neurofibromas are neoplasms derived from nerve bundles and are frequent in humans but not common in animals. This report described the macroscopic, histologic, and immunohistochemistry findings of an orbitofacial neurofibroma in a sow. The sow presented left eyelids with marked expansion, associated with severe ectropion and reddening of both conjunctival mucosae. The mass on the cutting surface was homogeneous, with a light tan, and shiny, measuring 9.0 x 7.0 x 7.0 cm in width, compressing the eyeball. Microscopically, there was paucicellular neoplastic proliferation of elongated cells amid the accentuated myxoid matrix. There were multifocal areas where well-differentiated nervous fascicles and skeletal musculature were seen. Neoplastic cells were immunostained positive for GFAP, neurofilament, S-100, Sox-10, and vimentin. Cytokeratin showed immunolabeling around primitive nerve bundles and desmin around neoformed vessels and muscle bundles within the tumor. Orbitopalpebral and orbitofacial neurofibroma, despite being common in humans, have not been described in domestic pigs and should be considered as a differential diagnosis in eyelid tumors in pigs.
Key words: palpebral tumors, immunohistochemistry, neoplasia, swine pathology, unilateral blindness.

Neurofibroma orbitofacial espontâneo em uma matriz suína

RESUMO: Neurofibromas são neoplasias derivadas dos feixes nervosos e são frequentes em humanos, mas incomuns em espécies animais domésticos. Esse relato tem como objetivo descrever os achados macroscópicos, histológicos e imuno-histoquímicos de um neurofibroma orbitofacial em uma matriz suína. O animal apresentava as pálpebras esquerdas acentuadamente expandidas, associada a severo ectrópio e avermelhamento de ambas as mucosas conjuntivais. A tumoração palpebral, na superfície de corte, era homogênea, levemente acastanhada e brilhante, medindo 9,0 x 7,0 x 7,0 cm, e comprimia o globo ocular. Microscopicamente foi observada uma proliferação neoplásica pobremente celular de células alongadas no meio de uma acentuada quantidade de matriz mixoide. Havia áreas intratumorias multifocais onde fascículos nervosos bem diferenciados e musculatura esquelética foram observados. As células neoplásicas foram positivas na imuno-histoquímica para GFAP, neurofilamento, S-100, Sox-10 e vimentina. Citoqueratina mostrou uma imunomarcação ao redor de feixes nervosos primitivos e desmina ao redor de vasos neoformados e feixes musculares dentro do tumor. O neurofibroma orbitofacial, apesar de comum em humanos, nunca foi descrito em suínos domésticos e deve ser considerado um diagnóstico diferencial para neoplasias palpebrais em suínos.
Palavras-chave: tumores palpebrais, imuno-histoquímica, neoplasia, patologia suína, cegueira unilateral.

INTRODUCTION

Neurofibromas (NF) are peripheral nerve sheath tumors that can present clinically as solitary or multiple masses. These neoplasms are paucicellular and composed of a mixed cell population with a disordered proliferation of epineurium, perineurium, and endoneurium cells (HIGGINS et al., 2016). Grossly, they may display a homogeneous, shiny grayish tan with a smooth or gelatinous consistency and no capsule (ERB et al., 2007). Essentially, NFs are benign in humans, and around 5%–7% of cases can undergo malignant transformation (ERB et al., 2007; HIGGINS et al., 2016). The World Health Organization (WHO) divides NFs into three main categories according to their macroscopic presentation: focal neurofibromas, plexiform neurofibromas, and diffuse neurofibromas, which do not form nodules (OSENDORF & GUTMANN, 2015).

In humans, NFs have a genetic background and are divided into two syndrome types: type 1 neurofibromatosis (NF-1) and type 2 neurofibromatosis (NF-2). Of these, NF-1 is an autosomal dominant hereditary disease (FRIEDMAN, 1999) and presents multifocal neurofibromas in the dermis, subcutaneous tissues, and peripheral nerves of the face, eyelids, head, and limbs (ERB et al., 2007). Despite being a good animal model for neurofibromatosis studies, domestic pigs rarely undergo this disease naturally, in contrast to genetically engineered pigs

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(ISAKSON et al., 2018). In swine, NF has been described as an outbreak at a commercial farm in France (MORVAN et al., 2004) and as a pigmented ventral abdomen plaque (BECKER et al., 2019). Lesions were characterized by white, soft, multifocal lesions within the subcutaneous tissue. This paper describedthe clinical, macroscopic, histological, and immunohistochemical findings for an orbitofacial neurofibroma in a commercially raised pig and its similarities with the human counterpart.

MATERIALS AND METHODS

A sample of an adult (5-year-old) female swine from a commercial batch was submitted by the State Department of Sanitary Inspection of the municipality of Anta Gorda, state of Rio Grande do Sul, to the Veterinary Pathology Department at Universidade Federal do Rio Grande do Sul (UFRGS). The history was requested from the veterinarian in charge. Only the head was sent for macroscopic and histopathological evaluation. Fragments of eyelids, orbital tissues, brain, left and right submandibular lymph nodes, skin, and skeletal muscle were fixed in 10% formalin solution and routinely processed for histology, stained with hematoxylin and eosin (H&E), and evaluated under a light microscope. Additionally, immunohistochemistry (IHC) was performed. Dilution, labeling, clonality, and antigenic recovery of the antibodies used are summarized in table 1. As a chromogen, AEC (3-amino-9-ethyl carbazole, Biocare Medical, Pacheco, California, USA) was used. Primary antibodies were replaced by Universal Negative Control Serum (BioCare Medical, CA, USA) in randomly selected sections of free pathogen control (FPC) as negative controls.

CASE DESCRIPTION

The sow was sent to an abattoir from a commercial farm located at Anta Gorda municipality, state of Rio Grande do Sul, Brazil (lat. 28° 57’ 31’’ South, long. 51° 59’ 45’’ West). The owner reported a small nodule on the lower left eyelid since its acquisition at approximately six months of age. During its stay on the property, no productive and reproductive problems were observed, but the owner noticed a slow-growing nodule that expanded both left eyelids, obliterating the anterior orbital space and compressing the eye (Figure 1). The owner also reported that no similar lesions were seen in the sow’s litters. In the antemortem examination, the female was active and normothermic but presented blindness on the left eye. Both conjunctival mucosae were edematous, with marked reddening and obliterating of the eye (palpebral ptosis). As there were no signs of obvious infectious diseases and the lesion was restricted to the ocular region, the sow was released for slaughter.

The left eyelids showed severe ectropion, characterized by eyelid expansion with exposure of the conjunctiva, which was purple-red and measured 9.0 x 7.0 x 7.0 cm. The left cornea was opaque and adhered to the eyelids’ conjunctiva. Next to the orbit, medially, were similar multifocal nodules measuring between three and five cm in width. These masses were expansive, unencapsulated, and soft; they were homogeneous, light brown, and shiny on the cut surface. The left eye was markedly diminished

Table 1 - Dilution, brand, antibodies clonality used in the immunohistochemistry technique, antigenic recovery method, and positive control.

Antibody/Clonality/Brand	----------Dilution/antigen recovery----------	---------Positive control---------
PanCitokeratin AE1/AE3 Dako®	Ready to use; citrate buffer pH 6.0, 96ºC for 40 min	Canine skin
Desmin D33, Dako®	1:300; Tris EDTA pH 9.0, 2-5-min microwave cycles	SI* feline
Glial Fibrillary Acidic Protein (GFAP), rabbit polyclonal, Dako®	1:200; Tris EDTA pH 9.0 100°C for 10 min	Feline brain
Neurofilament, 2F11, monoclonal, Dako®	1:600; tripsin 0.1% and citrate buffer pH 6.0, 37ºC for 10 min	Mouse brain
S-100, polyclonal, Dako®	Ready to use; citrate buffer pH 6,0, 100ºC for 20 min	Canine melanoma
SOX-10, polyclonal, Dako®	1:200, tripsin 0.1% and citrate buffer pH 6.0 in 37°C for 10 min.	Mouse mammary gland
Vimentin, V9, Zymed®	1:200, citrate buffer pH 6.0, 96ºC for 20 min	Feline fibrosarcoma

*Small intestine.

Figure 1 - Gross aspects of an orbitofacial neurofibroma in a sow. Marked left eye ectropion and multifocal nodules adjacent to the tumor.

(atrophied), with the firm sclera and lens absence summarizing in a single ocular cavity (phthisis bulbi) (Figure 2A). No enlarged lymph nodes and/or lesions suggestive of metastasis were observed.

In the eyelid histology, there was a paucicellular neoplastic proliferation of elongated cells that were not delimited and not encapsulated (Figure 2B). The cells were loosely arranged and supported by a marked myxoid matrix and rarely by a collagenous matrix. Interspersing the tumor were occasional well-differentiated nerve fascicles (Figure 2C), adipocytes, and skeletal muscles. Cells were fusiform to stellate, with a scanty, eosinophilic cytoplasm and indistinct cytoplasmic borders. The nuclei were elongated and oval, with speckled chromatin and inconspicuous nucleoli. Cells showed mild anisocytosis and anisokaryosis, with one mitotic figure in 2.37 mm². Amid the tumor, a focal area of tumor malignancy was also observed, characterized by cellular density with marked pleomorphism, multinucleated cells, macrokaryosis, aberrant mitoses, and lymphocyte emperipolesis by neoplastic cells, associated with intratumoral hemorrhage and a discrete, focally extensive lymphocytic inflammatory infiltrate with 13 mitotic figures in 2,37 mm². Table 2 summarizes the immunohistochemistry results.

In the conjunctival mucosa epithelium, *rete pegs* formation was present, associated with a moderately diffuse inflammatory infiltrate of lymphocytes, macrophages, and rare eosinophils, in addition to discrete multifocal thrombosis. The left eye presented with eye globe disruption, associated with marked extensive scleral and corneal fibrosis. The eye chambers were markedly reduced in size and collapsed, forming a single chamber. The cornea was thickened, with diffuse areas of moderate fibrosis and neovascularization. Moderate multifocal acanthosis of the cornea and wrinkling of Descemet's membrane were noted. The iris showed moderate diffuse fibrous connective tissue proliferation and adhered to the cornea (anterior synechiae), associated with iridocorneal angle obliteration. In the posterior part of the eyeball, all layers of the retina were lost and replaced by scar tissue.

DISCUSSION

Based on clinical, macroscopic, histological, and immunohistochemical findings,

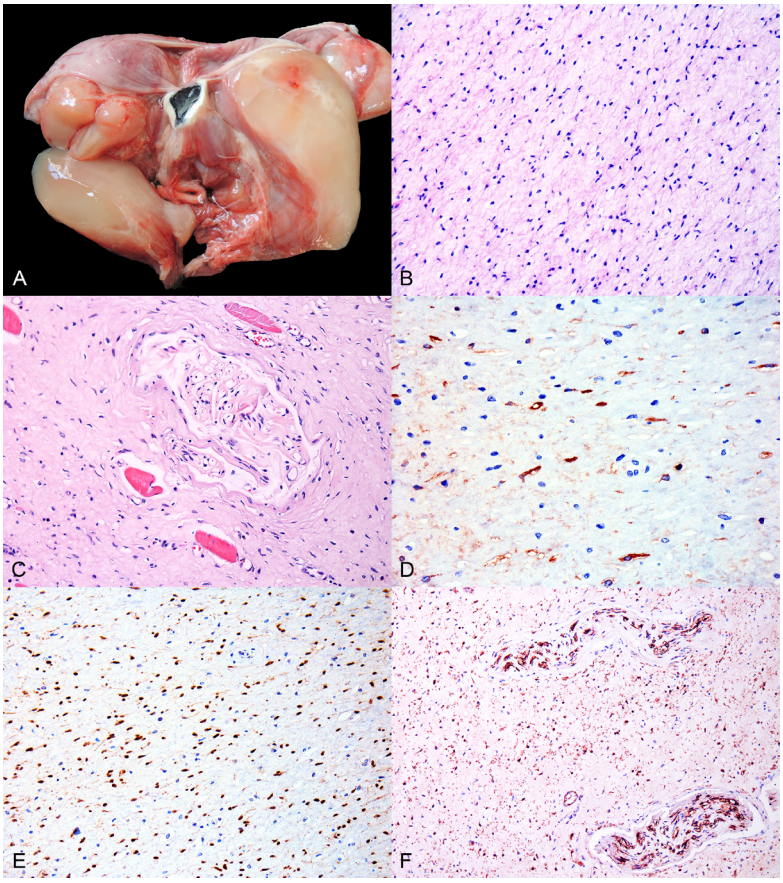

Figure 2 - Gross, histological, and immunohistochemical (IHC) aspects of an orbitofacial neurofibroma in a sow. (A) Cut surface showing a multilobulated, homogeneous, light brown, and shiny tumor. Note that the eyeball is collapsed by the tumor, associated with marked fibrosis of the sclera, absence of lens, and vitreous humor (phitiasis bulbi); (B) Elongated cells with paucicellular neoplastic proliferation embedded in myxomatous matrix. HE, 200x; (C) Nerve bundles, muscle differentiation areas, and arterioles interspersed with neoplastic cells embedded in myxoid matrix. HE, 200X; (D) Moderate multifocal immunolabeling in cytoplasmic extensions. IHC anti-neurofilament, AEC Romulin, 400x; (E) Marked multifocal nuclear immunolabeling. IHC anti-Sox10, AEC Romulin, 200x; (F) Marked multifocal cytoplasmic immunolabeling in neoplastic cells and within the neoformed nerve bundle. Anti-S100 IHC, AEC Romulin, 100x.

an orbitofacial neurofibroma diagnosis was made. In pigs, this neoplasm has been described as an outbreak in breeding and finishing animals (MORVAN et al., 2004), and the clinical signs vary according to the location and size of the lesion; however, generally, there are no deleterious effects in affected animals (MORVAN et al., 2004; DAMMANN et al., 2020). The pig in the present study lived for more than five years with neurofibroma without any damage, only facial disfiguration as seen in humans. In recent months, it impaired a pig's vision with eyeball compression and an insidious clinical evolution, as reported for humans (CHAUDHRY et al., 2012).

Orbitofacial NFs are common in humans (ERB et al., 2007; CHAUDHRY et al., 2012; ANTÔNIO et al., 2013) and have been reported in birds and cattle. This is the first case of orbitofacial NF in a pig. In this report, there were facial disfigurement, eyelid swelling, eyelid ptosis, and loss of vision, associated with eyeball atrophy secondary to tumor expansion, as seen in humans with orbitofacial/orbitopalpebral NFs (ERB et al., 2007; CHAUNDRY et al., 2012). The cases described in animals within the literature did not determine an

Table 2 - Immunolabeling characterization of an orbitofacial neurofibroma in a sow.

Antibody	----------Immunolabeling features----------
Citokeratin	Immunolabeling around primitive nerve bundles
Desmin	Intracytoplasmic immunolabeling in newly formed muscle cells and around vessels within the tumor
GFAP	Multifocal cytoplasmic immunolabeling of neoplastic cells
Neurofilament	Moderate multifocal immunolabeling in cytoplasmic neoplastic cell extensions (Figure 2D).
SOX-10	Marked diffuse nuclear immunolabeling in neoplastic cells (Figure 2E).
S-100	Marked diffuse cytoplasmic and nuclear immunolabeling of neoplastic cells (Figure 2F).
Vimentin	Marked multifocal cytoplasmic staining in neoplastic cells.

etiology or cause for lesion development (MORVAN et al., 2004). In the present case, only one animal in the flock was affected, and litter with lesions was not reported, suggesting a spontaneous case.

The microscopic findings were consistent with those described for human and animal NFs (SARTIN et al., 1994; ERB et al., 2007; SCHÖNIGER & SUMMERS, 2009; HIGGINS et al., 2016), with all components of the nerve bundle being present within the neoplasm. However, contrasting to what has been reported for humans, in this case, there was no mast cell infiltration (ERB et al., 2007; CHAUNDRY et al., 2012). In veterinary medicine, NFs are within a large group of designated peripheral nerve sheet tumors (e.g., nerve sheet tumors) since all those entities (such as SHSs and perineuromas) present a distinct morphology but a similar biological behavior.

The present neoplasm was composed of a poorly organized cellular proliferation loosely arranged and embedded in a myxoid matrix, in contrast to perineuromas (LEUNG et al., 2019), where proliferations are denser, and whorls and reticular patterns are seen (HORNICK & FLETCHER, 2005). This proliferation was monomorphic, excluding any hybrid patterns present in hybrid peripheral nerve sheet tumors (HPNST) (MICHAL et al., 2017). A malignant area was noted within the tumor; this finding can be present in 1%–5% of NFs in humans with neurofibromatosis type 1 (ANTÔNIO et al., 2013; CHAUDHRY et al., 2012; ERB et al., 2007), reinforcing the NF diagnosis since schwannomas (SCHs) and other peripheral nerve sheath tumors rarely undergo malignant changes (HIGGINS et al., 2016).

All the differential diagnostics for NF are derived from the neural crest and are S-100 positive, but in SCHs, there is no proliferation of muscle bundles, as observed in the present study. In humans, immunostaining is variable (KAWAHARA et al., 1988), and in bovines, it is positive but with weak immunolabeling (DAMMAN et al., 2020). In swine, genetically engineered mini-pigs, and hybrid pigs, there is GFAP-positive immunolabeling (ISAKSON et al., 2018; BECKER et al., 2019), as shown in the present case. There was positive immunolabeling for neurofilament, SOX-10, S-100, and vimentin. This can be related to the fact that NFs are composed of epineurium, perineurium, and endoneurium formed by many cell lineages derived from the neural crest and mesenchymal origin (ERB et al., 2008; HIGGINS et al., 2016; DAMMAN et al., 2020). The PanCitokeratin was positive around nerve bundles, as seen in a pigmented neurofibroma (BECKER et al., 2019). The Sox-10 is a more specific marker for neurofibroma in humans (KARAMCHANDANI et al., 2012) and is presented as a reliable marker in this case due to its immunolabeling; it is considered a promising marker for this neoplasm in swine.

Orbitofacial neurofibroma can spontaneously occur in pigs and shares many similarities with NFs in humans, such as clinical presentation, anatomic location, gross and histological characteristics, and immunolabeling. This neoplasm; although, uncommon, should be included in the differential diagnosis of eyelid and/or orbital lesions in swine.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

DECLARATION

We, the authors of the article entitled "Spontaneous orbitofacial neurofibroma in a sow", declare, for all due purposes, that this project has not been submitted for evaluation to the Ethics Committee of the University /Research Institute "Comissão de Ética no Uso de Animal - CEUA - UFRGS", but we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal - CONCEA "http://www.mct.gov.br/index.php/content/view/310553.html" if it involves animals.

Thus, the authors assume full responsibility for the presented data and are available for possible questions as required by the competent authorities.

REFERENCES

ANTÔNIO, J. R. et al. Neurofibromatosis: chronological history and current issues. **Anais Brasileiros de Dermatologia**, v.88, n.3, p.329-34, 2013. Available from: <https://doi.org/10.1590/abd1806-4841.20132125>. Accessed: Sep. 21, 2022. doi: 10.1590/abd1806-4841.20132125.

BECKER, K. et al. Cutaneous pigmented neurofibroma in a pig – Morphology and Immunohistochemical Profile. **Journal of Comparative Pathology**, v.168, p.25-29, 2019. Available from: <https://doi.org/10.1016/j.jcpa.2019.03.002>. Accessed: Sep. 19, 2022. doi: 10.1016/j.jcpa.2019.03.002.

CHAUDHRY, I. A. et al. Orbitofacial neurofibromatosis: clinical characteristics and treatment outcome. **Eye**, v.26, n.4, p.583-592, 2012. Available from: <https://doi.org/10.1038/eye.2011.336>. Accessed: Sep. 21, 2022. doi: 10.1038/eye.2011.336.

DAMMANN, I. et al. Unexpected high frequency of neurofibroma in the celiac ganglion of German cattle. **Veterinary Research**, v.51, n.1, p.1-13, 2020. Available from: <https://doi.org/10.1186/s13567-020-00800-1>. Accessed: Sep. 30, 2022. doi: 10.1186/s13567-020-00800-1.

ERB, M. H. et al. Orbitotemporal neurofibromatosis: classification and treatment. **Orbit**, v.26, n.4, p.223–228, 2007. Available from: <https://doi.org/10.1186/s13567-020-00800-1>. Accessed: Sep. 19, 2022. doi: 10.1080/01676830600987227.

FRIEDMAN, J. M. Epidemiology of neurofibromatosis Type 1. **American Journal of Medical Genetics**, v.89, p.1-6, 1999. Available from: <https://doi.org/10.1002/(SICI)1096-8628(19990326)89:1%3C1::AID-AJMG3%3E3.0.CO;2-8>. Accessed: Sep. 19, 2022. doi: 10.1002/(SICI)1096-8628(19990326)89:1<1::AID-AJMG3>3.0.CO;2-8.

HIGGINS, R. et al. Tumors of the nervous system. In: MEUTEN, D.J. **Tumors in domestic** animals, 5. ed. Iowa: Wiley Blackwell, 2016. Cap.19, p.834-891.

HORNICK, J. L.; FLETCHER, C. D. Soft tissue perineurioma: clinicopathologic analysis of 81 cases including those with atypical histologic features. **The American Journal of Surgical Pathology**, v.29, n.7, p.845-858, 2005. Available from: <https://doi.org/10.1097/01.pas.0000155166.86409.d2>. Accessed: Sep. 18, 2022. doi: 10.1097/01.pas.0000155166.86409.d2.

ISAKSON, S. H. et al. Genetically engineered minipigs model the major clinical features of human neurofibromatosis type 1. **Communications Biology**, v.158, n.1, p.1-11, 2018. Available from: <https://doi.org/10.1038/s42003-018-0163-y>. Accessed: Sep. 30, 2022. doi: 10.1038/s42003-018-0163-y.

LEUNG, K. C.; KO, T. C. Orbital hybrid peripheral nerve sheath tumors. **Taiwan Journal of Ophthalmology**, v.10, n.3, p.181-183, 2020. Available from: <https://doi.org/10.4103/tjo.tjo_28_20>. Accessed: Sep. 29, 2022. doi: 10.4103/tjo.tjo_28_20.

KARAMCHANDANI, J. R. et al. Sox10 and S100 in the diagnosis of soft-tissue neoplasms. **Applied immunohistochemistry & molecular morphology**, n.20, v.5, p.445-450, 2012. Available from: <https://doi.org/10.1097/pai.0b013e318244ff4b>. Accessed: Sep. 18, 2022. doi: 10.1097/PAI.0b013e318244ff4b.

KAWAHARA, E. et al. Expression of glial fibrillary acidic protein (GFAP) in peripheral nerve sheath tumors. A comparative study of immunoreactivity of GFAP, vimentin, S-100 protein, and neurofilament in 38 schwannomas and 18 neurofibromas. **The American Journal of Surgical Pathology**, v.12, n.2, p.115-120, 1988. Available from: <https://doi.org/10.1097/00000478-198802000-00004>. Accessed: Sep. 18, 2022. doi: 10.1097/00000478-198802000-0000.

MICHAL, M. et al. Hybrid peripheral nerve sheath tumors: A review. **Česko-Slovenská Patologie**, v.53, n.2, p.81-88, 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28597668>. Accessed: Sep. 20, 2022.

MORVAN, H. et al. Neurofibromatose chez le porc: un nouveau syndrome. **Bulletin de l'Académie Vétérinaire de France**, v.157, n.3, p.31-36, 2004. Available from: <http://dx.doi.org/10.4267/2042/47709>. Accessed: Sep. 20, 2022. doi: 10.4267/2042/47709.

OSENDORF, A. P.; GUTMANN, D. H. Neurofibromatosis. In: ROSENBERG, N.; PASCUAL, J. M. **Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease**, 5. ed. Academic Press: London, 2015. Cap.79, p.921-933.

SARTIN, E. A. et al. Characterization of naturally occurring cutaneous neurofibromatosis in Holstein cattle. A disorder resembling neurofibromatosis type 1 in humans. **The American Journal of Pathology**, v.145, n.5, p.1168-1174, 1994. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1887412>. Accessed: Sep. 20, 2022.

SCHÖNIGER, S.; SUMMERS, B. A. Localized, plexiform, diffuse, and other variants of neurofibroma in 12 dogs, 2 horses, and a chicken. **Veterinary Pathology**, v.46, n.5, p.904-915, 2009. Available from: <https://doi.org/10.1354/vp.08-vp-0322-s-fl>. Accessed: Sep. 18, 2022. doi: 10.1354/vp.08-VP-0322-S-FL.